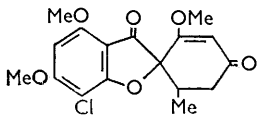
894. *Metabolic Products of* Penicillium patulum.

By W. J. McMaster, A. I. Scott, and S. Trippett.

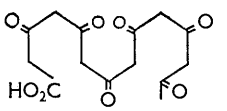
The isolation of five metabolites from cultures of *Penicillium patulum* is described. Two of these are new compounds * for which constitutions (V; R = Me, R′ = H) and (VII) are proposed. The remaining substances have been related to degradation products of griseofulvin.

Certain strains of *Penicillium patulum* produce different related metabolites according to the cultural conditions. For instance, 6-methylsalicylic,[1,2] 6-formylsalicylic,[1] and 3-hydroxyphthalic acid [1] represent blocked or alternate pathways in the genesis of patulin; [2] gentisic acid, gentisyl alcohol, and gentisaldehyde are elaboration products,[3] also corresponding to four units of acetic acid, while the biogenesis of 6-methylsalicylic acid from acetate is authenticated [4] for the related *P. griseofulvum* fermentation; biogenesis of patulin [5] probably proceeds from acetate through 6-methylsalicylic acid, etc.

Griseofulvin (I) has been proved [6] to be formally derived from seven units of acetic acid (*e.g.*, as II). By showing [7] that the spiran system of the griseofulvin molecule may be formed *in vitro* by one-electron oxidative coupling of a benzophenone (III ⟶ IV), we



(I)



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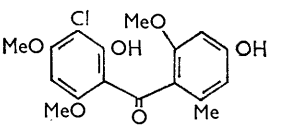

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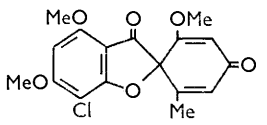

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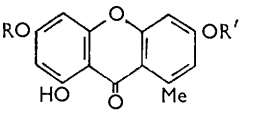

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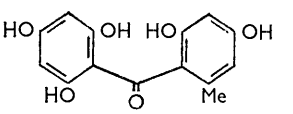


(VI)

recently provided a laboratory analogy for the mechanism of griseofulvin biogenesis suggested by Barton and Cohen.[8] In the hope of isolating further metabolites based on seven acetate units which might provide insight into the exact biogenesis of griseofulvin we have examined the material remaining after extraction of griseofulvin from fermentations of mutant strains of *Penicillium patulum*.

The resin obtained by evaporating the methanol-soluble portion of the mother-liquors was extracted with chloroform and separated into neutral (45%) and acidic fractions. The neutral portion, on chromatography, gave almost equal amounts of griseofulvin [9] (I) and a compound, $C_{17}H_{15}O_6Cl$, $[\alpha]_D$ −23°, whose ultraviolet ($\lambda_{max.}$ 242, 289, 330 mμ) and infrared ($\nu_{max.}$ 1715, 1676 cm.$^{-1}$) bands, together with a mixed m. p. determination, established its identity as dehydrogriseofulvin [7] (IV), $[\alpha]_D$ −26°.

From the fraction (2%) of resin extractable into sodium hydrogen carbonate solution chromatography yielded two further metabolic products. One of these, $C_{15}H_{12}O_5$, contains

* These are respectively identical with griseoxanthone C and griseophenone Y isolated from *P. patulum* by Dr. Rhodes at Glaxo Laboratories Ltd., Sefton Park.

[1] Bassett and Tanenbaum, *Experientia*, 1958, **14**, 38.
[2] Ehrensvärd, *Exp. Cell. Res.*, 1955, *Suppl.* **3**, 102.
[3] Birkinshaw, Bracken, Michael, and Raistrick, *Lancet*, 1943, **245**, 625; Birkinshaw, Bracken, and Raistrick, *Biochem. J.*, 1943, **37**, 726.
[4] Birch, Massy-Westropp, and Moye, *Austral. J. Chem.*, 1955, **8**, 539.
[5] Bu'Lock and Ryan, *Proc. Chem. Soc.*, 1958, 222.
[6] Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360.
[7] Scott, *Proc. Chem. Soc.*, 1958, 195; Day, Nabney, and Scott, *Proc. Chem. Soc.*, 1960, 284.
[8] Barton and Cohen, "Festschrift A. Stoll," Birkhauser, Basle, 1957, p. 117.
[9] Grove, Macmillan, Mulholland, and Rogers, *J.*, 1952, 3949.

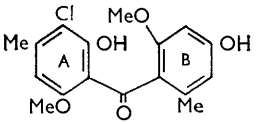
one methoxyl group, gives a red-brown ferric chloride colour, and has ultraviolet and infrared spectra indicating a 1,6-dihydroxyxanthone chromophore (see Table). Since structural elucidation of xanthones is tedious [10] we invoke first a biogenetic argument. The simplest mode of condensation of acetic acid units (as II) to a fully aromatic system leads to the benzophenone (VI) which by oxidation and methylation in unknown sequence would lead to griseofulvin. An alternative is xanthone formation [11] from the ketone (VI), leading to structure (V; R, R′ = H or Me) for our compound. This was confirmed by partial methylation with ethereal diazomethane which gave 1-hydroxy-3,6-dimethoxy-8-methylxanthone (lichexanthone) (V; R = R′ = Me) identical with the natural substance.[1] The structure (V; R = Me, R′ = H) was finally proved by comparison with the 6-methyl isomer [13] prepared by an unambiguous synthesis. The implications of the isolation of this substance from the culture medium will be discussed elsewhere.

Light absorption of xanthones.

Xanthone deriv.	$\lambda_{max.}$ (mμ)	ε	C=O band (in $CHCl_3$) ($cm.^{-1}$)
1-Hydroxy-3,6-dimethoxy [a]	238, 251, 307, 337	33,000, 26,000, 21,400, 10,500	1653
1-Hydroxy-3,6,7-trimethoxy [a]	238, 256, 309, 356	23,500, 32,000, 17,000, 12,300	1647
1-Hydroxy-3,6-dimethoxy-8-methyl [b] (lichexanthone)	242, 254, 306, 340	35,500, 27,000, 22,500, 5600	1648
1,6-Dihydroxyxanthone	247, 269, 305, 355	20,000, 10,000, 12,600, 6400	—
$C_{15}H_{12}O_5$ [b] (V; R = Me, R′ = H)	242, 269, 309, 340	37,000, 9000, 23,000, 7000	1647

[a] Yates and Stout, *J. Amer. Chem. Soc.*, 1958, **80**, 1691. [b] This work.

The second acidic substance, $C_{17}H_{17}O_5Cl$, contains two methoxyl groups, gives a red-brown ferric colour, and is assigned structure (VII) for the following reasons. Ultraviolet ($\lambda_{max.}$ 296 mμ) and infrared [$\nu_{max.}$ 3550 (free OH), 3350 (bonded OH), 1610 (bonded aromatic C=O)] spectra suggested a 2-hydroxybenzophenone system, with a further, free hydroxyl group. Because of the small amount available we next employed a physical method to determine the distribution pattern of the substituents. Dr. Reed and Mr. Wilson of this



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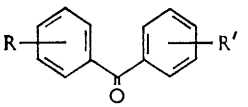

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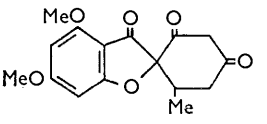
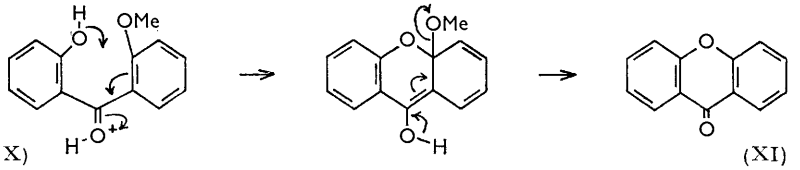


(IX)

Department have shown that the mass spectrum of a benzophenone, *e.g.*, (VIII), has peaks with the following mass assignments: $R{\cdot}C_6H_4{\cdot}CO{\cdot}C_6H_4{\cdot}R'$; $R{\cdot}C_6H_4{\cdot}CO$; $R'{\cdot}C_6H_4{\cdot}CO$; $R{\cdot}C_6H_4$; $R'{\cdot}C_6H_4$. The spectrum of our metabolite had peaks at 336, 199, 171, 165, and 137 in agreement with the values calculated for structure (VII), namely, A·CO·B; A·CO; A; B·CO; B. Thus the relative masses of rings A and B are determined rigorously. We place the methyl group at position 4 in ring A by analogy with the structures of geodin [11] and sulochrin.[14] The alternative position 2 predictable on biogenetic grounds was excluded by the following experiments. Treatment of the metabolite (VII) with base afforded a xanthone whose negative ferric chloride reaction showed the absence of a 1-hydroxyl group. After treatment of this xanthone with acid an intense ferric chloride colour showed that a methoxyl group next to the xanthone carbonyl group had been hydrolysed. [We find that 2-hydroxy-2′-methoxybenzophenones lose methanol readily under very mild basic conditions, yielding xanthones. A mechanism for this was proposed

[10] Roberts, *J.*, 1960, 785.
[11] Cf. Barton and Scott, *J.*, 1958, 1767.
[12] Asahina and Nogami, *Bull. Chem. Soc. Japan*, 1942, **17**, 202.
[13] Grover, Shah, and Shah, *J. Sci. Ind. Res.*, 1956, **15***B*, 629.
[14] Nishikawa, *Acta Phytochim. Japan*, 1939, **11**, 167.

by Barton and Scott; the present case is a further example. In addition to the base-catalysed reaction, we observed that the Zeisel methoxyl determination consistently gave values of exactly one methoxyl group less than those calculated for the benzophenone. This we explain as an acid-catalysed elimination of methanol (X ⟶ XI) which distils before it can be transformed into methyl iodide; in agreement with this, heating the



(X) (XI)

mixture under reflux for an hour before distillation of methyl iodide leads to correct methoxyl values.]

From the fraction obtained by extraction with sodium hydroxide solution were obtained, after chromatography, a further quantity of our xanthone, together with a ketone (III), previously obtained [7] by degradation of griseofulvin and by total synthesis.

Finally, in addition to smaller amounts of our xanthone and the benzophenone (III), the aqueous broth concentrates afforded 4,6-dimethoxy-2′-methylgrisan-3,4′,6′-trione (IX), identified by analysis, etc., and comparison with a sample prepared by hydrolysis of dechlorogriseofulvin.[15]

The isolation of the new metabolites strongly suggests that biogenesis of griseofulvin is based on the sequence, acetate ⟶ benzophenone ⟶ spirodienone, but we reserve comment until our experiments with isotopically labelled intermediates are complete.

Experimental

Ultraviolet spectra were determined for EtOH solutions with a Unicam S.P. 500 spectrophotometer. Infrared spectra were determined by Dr. G. Eglinton and his associates. Neutralised alumina was standardised according to Brockmann's method. Silica gel for chromatography was obtained from Messrs. Hopkin and Williams Ltd. Rotations are for acetone solution. M. p.s were determined on the Kofler block. Microanalyses are by Mr. J. M. L. Cameron and his staff.

Cultural Conditions.—P. patulum was grown according to the specification in B.P. 784,618, and the griseofulvin removed from the medium as described therein.

The resin (25 g.) deposited on concentration of the methanol washings was separated into the following main fractions: (a) Chloroform-soluble: (i) 2% soluble in aqueous sodium hydrogen carbonate; (ii) (50%) soluble in 2N-sodium hydroxide; (iii) neutral (45%); (b) chloroform-insoluble (3%).

3,8-Dihydroxy-6-methoxy-1-methylxanthone (V; R = Me, R′ = H).—The crude solid (500 mg.) obtained by acidification of the hydrogen carbonate extract was chromatographed over silica gel (15 g.) in benzene. Elution with benzene–chloroform (3 : 7) gave the *xanthone* as buff-coloured needles (300 mg.), m. p. 253—255° after repeated crystallisation from methanol, $\nu_{max.}$ (KCl disc) 3300, 1655 cm.$^{-1}$, $\nu_{max.}$ (in $CHCl_3$) 1647 cm.$^{-1}$ (Found: C, 66·15; H, 4·7; OMe, 9·5. $C_{15}H_{12}O_5$ requires C, 66·15; H, 4·45; 1OMe, 11·4%) (for $\lambda_{max.}$ see Table). The compound gave a violet-brown colour with alcoholic ferric chloride. On admixture with a sample of 6-demethyl-lichexanthone a m. p. depression of 25° was observed.

Methylation. This xanthone (65 mg.) was left in ethereal diazomethane overnight at room temperature. Removal of ether *in vacuo* gave a pale yellow solid which after three recrystallisations from methanol gave lichexanthone (1-hydroxy-3,5-dimethoxy-8-methylxanthone) as pale yellow needles, m. p. and mixed m. p. 186—187°, $\nu_{max.}$ (in KCl) 1646, $\nu_{max.}$ (in $CHCl_3$) 1647 cm.$^{-1}$ (Found: C, 66·85; H, 4·7; OMe, 21·1. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·95; 2OMe, 21·7%) (cf. Table). The ultraviolet and infrared spectra of lichexanthone gave identical values.

[15] Macmillan, *J.*, 1953, 1697.

3-*Chloro*-2,4′-*dihydroxy*-6,2′-*dimethoxy*-4,6′-*dimethylbenzophenone* (VII).—The later fractions (benzene–chloroform; 3 : 7) from the above chromatogram, when recrystallised from aqueous methanol, afforded yellow needles (30 mg.), m. p. 181—182°, giving a brown ferric colour and a positive Beilstein test and having $\lambda_{max.}$ 296 mμ (ε 18,000) $\nu_{max.}$ (in Nujol) 3200, 1610, $\nu_{max.}$ (in $CHCl_3$) 3500, 3350, 1610 cm.$^{-1}$ (Found: C, 60·5; H, 5·1; Cl, 10·3; OMe, 17·95. $C_{17}H_{17}O_5Cl$ requires C, 60·7; H, 5·1; Cl, 10·5; 2OMe, 18·4%). The mass spectrum had peaks at 336, 199, 161, 165, and 137 (ε 0·3, 3·3, 0·3, 6·6, and 4·8 respectively).

After this *benzophenone* (10 mg.) had been heated in 2% alcoholic potassium hydroxide solution (20 c.c.) for 2 hr., acidification and isolation in ether gave 4-chloro-6-hydroxy-1-methoxy-3,8-dimethylxanthone, m. p. 199—201° (4 mg.), as pale yellow needles (from methanol), giving no immediate colour with ferric chloride solution, $\lambda_{max.}$ 241, 255 (sh), 310 mμ (ε 37,000, 23,000, and 21,000 respectively), $\nu_{max.}$ (in $CHCl_3$) 3650, 1648 cm.$^{-1}$ [Found: *M* (in mass spectrometer), 304. Calc. for $C_{16}H_{13}ClO_4$: *M*, 304]. This (1 mg.) was heated in a solution of methanol (20 c.c.) containing 6N-sulphuric acid (3 c.c.) on the steam-bath for 1 hr., and the cooled solution was evaporated to 5 c.c. *in vacuo* and extracted with ether; the resultant crude extract gave an immediate intense green colour with ferric chloride solution.

3-*Chloro*-2,4′-*dihydroxy*-2′,4,6-*trimethoxy*-6′-*methylbenzophenone* (III).—Acidification of the sodium hydroxide extract gave a dark red resin (12·4 g.). Chromatography of part (2·6 g.) of this material in benzene–chloroform (2 : 3) over silica gel (80 g.) gave two fractions eluted with this solvent mixture. Fraction (i) was almost pure xanthone (V; R = Me, R′ = H) (30 mg.), m. p. and mixed m. p. 252—254°. Fraction (ii) after several crystallisations from ether–light petroleum (b. p. 40—60°) afforded the benzophenone (III) (800 mg.) as yellow needles, m. p. and mixed m. p. 212—214°, $\lambda_{max.}$ 298 (ε 17,400), $\nu_{max.}$ 3330 and 1615 cm.$^{-1}$ (natural and synthetic material identical), giving a mass spectrum (352, 215, 187, 165, and 137 (ε 0·95, 1·9, 1·9, 0·8, and 0·7 respectively).

(−)-*Dehydrogriseofulvin* (IV).—The neutral fraction (11·0 g.) had m. p. 245—255°, $[\alpha]_D$ +157° (*c* 1·40). Chromatography of this mixture (1·2 g.) in benzene over alumina (grade III) gave griseofulvin (0·5 g.), m. p. and mixed m. p. 220—221°, $[\alpha]_D$ +350° (*c* 1·5). Later fractions eluted with 99 : 1 benzene–ether gave dehydrogriseofulvin (0·4 g.) which after sublimation at 220°/10^{-3} mm. and ten recrystallisations from chloroform–ether had m. p. 270—275° undepressed on admixture with (−)-dehydrogriseofulvin {$[\alpha]_D$ −26°, and $[\alpha]_D$ −23° (*c* 0·5)} (Found: C, 58·30; H, 4·50. Calc. for $C_{17}H_{15}ClO_6$: C, 58·2; H, 4·3%). The natural and synthetic compound had identical light absorption.

Examination of the Aqueous Broth Concentrate from P. patulum.—Concentrated aqueous mother-liquors (3 l.) were brought to pH 12 by addition of sodium hydroxide solution, and solid matter (20 g.) was filtered off. This consisted of resin containing griseofulvin (30%). The material in the filtrate was separated into neutral and acidic fractions. The neutral portion (5 g.) was a 1 : 1 mixture of griseofulvin and dehydrogriseofulvin. The fraction soluble in aqueous sodium hydroxide gave a small amount of a mixture of the benzophenone (III) and the xanthone (V; R = Me, R′ = H).

The sodium hydrogen carbonate extract was acidified and extracted with chloroform which removed a resin (6 g.). Chromatography of this resin on silica gel in benzene afforded compound (IX) (600 mg.), m. p. 245—248° (decomp.) alone or mixed with a sample prepared by hydrolysis of dechlorogriseofulvin; it had $[\alpha]_D$ +420° (*c* 0·25 in 2N-sodium hydroxide) (Found: C, 62·35; H, 5·45. Calc. for $C_{16}H_{16}O_6$: C, 63·2; H, 5·3%). The infrared spectra of the metabolite was identical with that of authentic material.

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