# *Griseofulvin. Part IX.* Isolation of the Bromo-analogue from* Penicillium griseofulvum *and* Penicillium nigricans.

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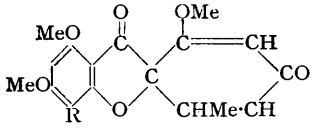
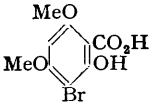
[Reprint Order No. 5285.]

7-Bromo-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione, the bromo-analogue of griseofulvin, has been isolated from the culture filtrates of both *Penicillium griseofulvum* Dierckx and *Penicillium nigricans* (Bainier) Thom, grown on a synthetic medium containing potassium bromide.

When grown on a Czapek–Dox medium, *Penicillium griseofulvum* Dierckx and *P. nigricans* (Bainier) Thom (formerly known as *P. janczewskii* Zal.) produce griseofulvin (I; R = Cl) (Oxford, Raistrick, and Simonart, *Biochem. J.*, 1939, **33**, 240; Brian, Curtis, and Hemming, *Trans. Brit. Mycol. Soc.*, 1949, **32**, 30). The former mould and some strains of the latter also produce dechlorogriseofulvin (I; R = H) under the same conditions (MacMillan, *J.*, 1953, 1697). The bromo-analogue (I; R = Br) has now been isolated from the culture filtrates of these fungi grown on a Czapek–Dox medium in which the potassium chloride was replaced by an equivalent amount of potassium bromide.

(I) 

 (II)

The new metabolic product, $C_{17}H_{17}O_6Br$, was assigned the structure (I; R = Br) by analogy with griseofulvin (Grove, MacMillan, Mulholland, and Rogers, *J.*, 1952, 3977) which it closely resembles in its ultra-violet and infra-red light absorption. Like griseofulvin, it gave 3-methoxy-2 : 5-toluquinone on oxidation with chromic oxide while the substitution pattern of the aromatic ring was confirmed by permanganate oxidation to 3-bromo-2-hydroxy-4 : 6-dimethoxybenzoic acid (II) whose structure was proved by synthesis as follows.

2-Bromo- and 2 : 6-dibromo-3 : 5-dimethoxyphenol were prepared in an unambiguous manner from methyl 4-hydroxy-2 : 6-dimethoxybenzoate by bromination followed by hydrolysis and decarboxylation (cf. Grove, MacMillan, Mulholland, and Zealley, *J.*, 1952, 3967). They were also obtained on a preparative scale by direct bromination of 3 : 5-dimethoxyphenol. Treatment of the 2-bromo-derivative with chloroacetyl chloride and ring closure of the resulting 3-bromo-ω-chloro-2-hydroxy-4 : 6-dimethoxyacetophenone yielded 7-bromo-4 : 6-dimethoxycoumaranone from which the required acid (II) was obtained by oxidation.

7-Bromo-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione (I; R = Br) is the first bromine-containing mould metabolite to be isolated although Clutterbuck, Mukhopadhyay, Oxford, and Raistrick (*Biochem. J.*, 1940, **34**, 664) have demonstrated the ability of *Caldariomyces fumago* Woronichin, strain Ag 92, to convert 40% of the supplied inorganic bromide into an unidentified organic form.

The bromo-compound (I; R = Br) produces a typical griseofulvin-like response into *Botrytis allii* (Brian, *Ann. Bot.*, 1949, **13**, 59). The minimum concentration causing an obvious response is 0·75 μg./ml. compared with 0·1 and 6·25 μg./ml. for griseofulvin and dechlorogriseofulvin respectively.

## Experimental

Microanalyses are by Messrs. W. Brown and A. G. Olney. In chromatography B.D.H. alumina was rendered alkali-free (Prins and Shoppee, *J.*, 1946, 498) and activated for 3 hr. at 250°/15 mm.

*Isolation of 7-Bromo-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione.*—The strain of *P.*

* Part VIII, *J.*, 1954, 429.

*griseofulvum* used in this work (our No. 374) was obtained from Professor Raistrick (L.S.H.T.M. catalogue No. P38) in 1948. The strain (No. 250) of *P. nigricans* which was used produces griseofulvin but not dechlorogriseofulvin on Czapek–Dox medium (MacMillan, *loc. cit.*).

The fungi were cultured by P. J. Curtis, G. L. F. Norris, and G. C. Pick of these laboratories as follows. The Czapek–Dox medium (7·5% glucose) was modified by replacing the potassium chloride by an equivalent amount of potassium bromide; it was made up by dissolving the " AnalaR " glucose in glass-distilled water and passing this solution through a column of B.D.H. " De-acidite F " before adding the " AnalaR " inorganic salts. The medium was dispensed in 1000 ml. portions in earthenware vessels and incubated at 25° for 18—24 days after inoculation. The liquid medium was then separated from the mycelium by filtration and was extracted with chloroform.

(*a*) The brown gum (1·4 g.), obtained from the culture filtrates (24 l.) of *P. nigricans* after 24 days' incubation, was dissolved in benzene (15 ml.), and the solution, after extraction with sodium carbonate, was passed through a column of alumina (7 × 1·5 cm.). Elution with benzene in ultra-violet light gave (i) a colourless band with bright blue fluorescence, recovery yielding an oil (140 mg.) which was retained, and (ii) a pale brown band fluorescing blue; fractional crystallisation of the solid recovered from (ii), from methanol, afforded *7-bromo-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione* (I; R = Br) (62 mg.), m. p. 204—205°, and dechlorogriseofulvin (73 mg.), m. p. and mixed m. p. 179—181°.

From the second half of this batch which was harvested after only 18 days' incubation, the bromo-compound (I; R = Br) (21 mg.) and dechlorogriseofulvin (40 mg.) were obtained from 24 l. of culture filtrate.

(*b*) The brown gum, obtained from the culture filtrates (48 l.) of *P. griseofulvum* after 20 days' incubation, was crystallised from methanol (25 ml.), giving crude dechlorogriseofulvin (3·2 g.) and filtrate A. The former after chromatography on alumina crystallised from methanol in needles (2·6 g.), m. p. and mixed m. p. 179—181°.

The gum recovered from filtrate A was separated into a light petroleum (b. p. 60—80°)-soluble oil, which was retained, and an insoluble fraction, a solution of which in ethyl acetate was extracted in turn with sodium hydrogen carbonate and sodium hydroxide (acidification of these extracts yielded intractable gums). The neutral gum, obtained by evaporation of the ethyl acetate layer, crystallised from methanol, giving dechlorogriseofulvin (0·8 g.), m. p. 176—179°; the gum from the mother-liquors in benzene (15 ml.) was chromatographed on alumina (10 × 1 cm.), and the band fluorescing blue in ultra-violet light was eluted with benzene. Fractional crystallisation of the recovered solid from methanol gave the bromo-compound (I; R = Br) (67 mg.), m. p. 204—205°, and dechlorogriseofulvin (50 mg.), m. p. 178—180°.

7-Bromo-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione crystallised from benzene or methanol in needles, m. p. 204—205° (Found : C, 51·4, 51·3; H, 4·5, 4·45; Br, 19·7; OMe, 23·3. $C_{17}H_{17}O_6Br$ requires C, 51·4; H, 4·3; Br, 20·1; 3OMe, 23·4%). Light absorption in ethanol : Max. ~325, 292, ~255, 235 (log ε, 3·75, 4·365, 4·22, 4·38). The infra-red spectrum, determined on a Nujol mull, showed absorption max. at 1703, 1653, 1613, and 1577 $cm.^{-1}$ in the double-bond stretching regions. The bromo-compound gave a pale yellow colour with concentrated nitric acid at room temperature.

*Oxidation of 7-Bromo-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione.*—(a) *Chromic oxide.* The bromo-compound (35 mg.) in warm acetic acid (0·5 ml.) was oxidised with chromic oxide (100 mg.) in acetic acid (0·5 ml.) and water (0·2 ml.), as described for griseofulvin (Grove *et al.*, *J.*, 1952, 3967) giving 3-methoxy-2 : 5-toluquinone, m. p. and mixed m. p. 147—149°.

(b) *Potassium permanganate.* To the bromo-compound (94 mg.) in acetone (25 ml.) at room temperature, powdered potassium permanganate (380 mg.) was added in portions (3 hr.) with occasional shaking. The brown precipitate was collected after 15 hr. and was extracted with dilute aqueous ammonia (3 × 2·5 ml.); acidification and recovery gave 3-bromo-2-hydroxy-4 : 6-dimethoxybenzoic acid (3 mg.) which crystallised from acetic acid in needles, m. p. 225—227° (decomp.), and was identical (mixed m. p. and infra-red spectrum) with the synthetic specimen described below.

*Bromination of Methyl 4-Hydroxy-2 : 6-dimethoxybenzoate.*—(*a*) The ester (140 mg.) (Pfeffer and Fischer, *Annalen*, 1912, **389**, 207) in chloroform (60 ml.) was treated with bromine (106 mg.) in chloroform (1 ml.) and set aside for 48 hr. After being washed with water, the dried chloroform solution was concentrated and the resulting solid was fractionally crystallised from benzene, yielding (i) the *3-bromo*-derivative, needles (100 mg.), m. p. 158—159° (Found : C, 41·6; H, 3·9; Br, 27·15. $C_{10}H_{11}O_5Br$ requires C, 41·3; H, 3·8; Br, 27·45%), and (ii) the *3 : 5-dibromo*-derivative which, after sublimation at 60°/$10^{-4}$ mm., crystallised from light petroleum (b. p.

40—60°) in needles (15 mg.), m. p. 82—83° depressed below 60° on admixture with 2-bromo-3 : 5-dimethoxyphenol, m. p. 79—81° (Found: C, 32·7; H, 2·85. $C_{10}H_{10}O_5Br_2$ requires C, 32·45; H, 2·7%). The infra-red spectrum of the 2 : 6-dibromo-compound showed carbonyl and hydroxyl absorption at 1725 and 3400 cm.$^{-1}$ respectively.

(*b*) Bromine (755 mg.) in acetic acid (5 ml.) was added to the ester (1·0 g.) in acetic acid (150 ml.) and, after 24 hr., the solvent was removed. Fractional crystallisation of the residual gum from benzene gave the 3-bromo-derivative (750 mg.), m. p. 158—159°, and 2 : 6-*dibromo*-3 : 5-*dimethoxyphenol* (150 mg.), m. p. 164—165° (Found: C, 30·6; H, 2·9; Br, 51·1. $C_8H_8O_3Br_2$ requires C, 30·8; H, 2·6; Br, 51·2%).

2-*Bromo*-3 : 5-*dimethoxyphenol*.—Methyl 3-bromo-4-hydroxy-2 : 6-dimethoxybenzoate (100 mg.) in concentrated sulphuric acid (0·3 ml.) was set aside for 1 hr. at 18° and was then poured over crushed ice. The precipitated solid A was collected and the filtrate, after several days, deposited 2-bromo-3 : 5-dimethoxyphenol which was sublimed at 60°/$10^{-3}$ mm. and crystallised from light petroleum in needles, m. p. 78—80°, identical (mixed m. p. and infra-red spectrum) with the monobromo-compound obtained as below by bromination of 3 : 5-dimethoxyphenol.

The solid A consisted of starting material (m. p. and mixed m. p. 154—156°) and a fraction, m. p. 45—90°, soluble in sodium hydrogen carbonate solution.

*Bromination of* 3 : 5-*Dimethoxyphenol*.—Bromine (7·0 g.) in chloroform (15 ml.) was added slowly and with shaking to the phenol (6·7 g.) in chloroform (15 ml.) at room temperature. After 48 hr., the solvent was removed and the residual gum was distilled at 103°/0·3 mm., giving the 2-*bromo*-derivative (6·0 g.), needles, m. p. 79—81°, from benzene–light petroleum (b. p. 40—60°) (Found: C, 41·1; H, 4·2; Br, 34·4. $C_8H_9O_3Br$ requires C, 41·2; H, 3·9; Br, 34·3%); the *acetate* crystallised from methanol in plates, m. p. 92—93° (Found: C, 43·7; H, 4·3; Br, 29·2. $C_{10}H_{11}O_4Br$ requires C, 43·65; H, 4·0; Br, 29·6%).

Sublimation of the residue from the distillation of the 2-bromo-compound yielded the 2 : 6-dibromo-derivative which crystallised from benzene in needles (1·1 g.), m. p. 165—167°, identical (m. p. and mixed m. p.) with the dibromo-compound obtained in the bromination of methyl 4-hydroxy-2 : 6-dimethoxybenzoate (Found: C, 30·7; H, 2·8; Br, 51·4%); the *acetate* crystallised from methanol in prisms, m. p. 152—153° (Found: C, 34·2; H, 3·0; Br, 45·1. $C_{10}H_{10}O_4Br_2$ requires C, 33·9; H, 2·9; Br, 45·2%).

3-*Bromo*-ω-*chloro*-2-*hydroxy*-4 : 6-*dimethoxyacetophenone*.—2-Bromo-3 : 5-dimethoxyphenol (1·0 g.), chloroacetyl chloride (0·9 g.), nitrobenzene (1·5 g.), and aluminium chloride (1·4 g.) were allowed to stand at 18° for 4 days. After the addition of ice-water (20 ml.) and concentrated hydrochloric acid (4 ml.), the nitrobenzene was removed by steam-distillation; the *acetophenone* which separated as a gummy solid on cooling crystallised from dioxan in pale yellow needles (25% yield), m. p. 211—213° [Found: C, 38·8; H, 3·4; Hal. (calc. as Cl), 26·4. $C_{10}H_{10}O_4BrCl$ requires C, 38·8; H, 3·3; Hal., 26·45%].

7-*Bromo*-4 : 6-*dimethoxycoumaran*-3-*one*.—The above ω-chloroacetophenone (350 mg.) was heated under reflux for 2·5 hr. with methanol (40 ml.) and crystalline sodium acetate (500 mg.). The *coumaranone*, which separated on cooling, crystallised from dioxan in needles (265 mg.), m. p. 219—221° (decomp.) (Found: C, 43·9; H, 3·7; Br, 29·4. $C_{10}H_9O_4Br$ requires C, 44·0; H, 3·3; Br, 29·4%). The 2 : 4-*dinitrophenylhydrazone* crystallised from nitrobenzene–methanol in crimson needles, m. p. 236° (decomp.) (Found: C, 42·9; H, 2·8; N, 12·4. $C_{16}H_{13}O_7N_4Br$ requires C, 42·4; H, 2·9; N, 12·4%).

3-*Bromo*-2-*hydroxy*-4 : 6-*dimethoxybenzoic Acid*.—The foregoing coumaranone (220 mg.) in acetone (40 ml.) was treated with potassium permanganate (500 mg.) in acetone (20 ml.), and the mixture was set aside for 2 days. Extraction of the brown precipitate with dilute ammonia solution (25 ml.) and acidification gave 3-*bromo*-2-*hydroxy*-4 : 6-*dimethoxybenzoic acid* which crystallised from acetic acid in needles (65 mg.), m. p. 224—226° (decomp.) (Found: C, 39·2; H, 3·5; Br, 29·0. $C_9H_9O_5Br$ requires C, 39·0; H, 3·5; Br, 28·8%). Starting material (55 mg.) was recovered from the acetone mother-liquors.

I am indebted to Dr. L. A. Duncanson for determining the infra-red absorption spectra; to Mr. H. G. Hemming for the biological assay; to Mr. P. J. Suter for assistance in the preparation of 2-bromo-3 : 5-dimethoxyphenol; and to many colleagues for their suggestions and criticisms.

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[*Received, April 6th*, 1954.]