XXIX. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

LX. GRISEOFULVIN, $C_{17}H_{17}O_6Cl$, A METABOLIC PRODUCT OF *PENICILLIUM GRISEO-FULVUM* DIERCKX

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(*Received 22 December 1938*)

THE following metabolic products, present in the metabolism solution of *Penicillium griseo-fulvum* Dierckx when this mould is grown under different cultural conditions and on different culture solutions, have been isolated in this laboratory: 6-hydroxy-2-methylbenzoic acid [Anslow & Raistrick, 1931], 2:5-dihydroxybenzoic acid (gentisic acid), fumaric acid and mannitol [Raistrick & Simonart, 1933] and fulvic acid, $C_{14}H_{12}O_8$, a yellow crystalline substance of at present undetermined molecular constitution [Oxford *et al.* 1935]. The purpose of the present communication is to record observations on a hitherto undescribed chlorine-containing metabolic product of *P. griseo-fulvum* which has been isolated from the mycelium of this mould and for which the name *griseofulvin* is proposed.

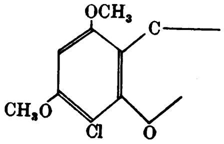
Griseofulvin, $C_{17}H_{17}O_6Cl$, M.P. 218–219°, is a colourless, crystalline, neutral compound giving no colour with $FeCl_3$ and containing no free hydroxyl or carboxyl groups. It is highly dextrorotatory, $[\alpha]_{5461} + 417°$.

One oxygen atom is present as $>CO$ since a crystalline mono-oxime, $C_{17}H_{18}O_6NCl$, was readily obtained; derivatives are also formed with phenylhydrazine and with 2:4-dinitrophenylhydrazine, but these could not be obtained crystalline. The function of three other oxygen atoms is obvious since griseofulvin contains three methoxyl groups. A study of the products obtained on acid and alkaline hydrolysis showed that griseofulvin must be the methyl ester of a carboxylic acid, and hence the function of a fifth oxygen atom is accounted for. The function of the sixth oxygen atom has not been definitely settled though evidence given later indicates that it is present as an oxygen bridge.

Griseofulvin, when hydrolysed with boiling *N* aqueous-alcoholic H_2SO_4, yields *griseofulvic acid*, $C_{16}H_{15}O_6Cl$, $[\alpha]_{5461} + 508°$, a monobasic acid containing two methoxyl groups and giving only a feeble colour with $FeCl_3$, but these facts in themselves are not sufficient to establish the presence of the grouping $—COO.CH_3$. However, hydrolysis of griseofulvin, or further hydrolysis of griseofulvic acid, with boiling aqueous *N*/2 NaOH yields nor*griseofulvic acid*, $C_{15}H_{13}O_6Cl$, $[\alpha]_{5461} + 609°$, a dibasic acid containing only one methoxyl group, together with *decarboxygriseofulvic acid*, $C_{15}H_{15}O_4Cl$, $[\alpha]_{5461} - 31°$, an insoluble *neutral* compound containing two methoxyl groups, giving no colour with $FeCl_3$, and derived from griseofulvic acid by the loss of 1 mol. of CO_2. Decarboxygriseofulvic acid is stable to acid hydrolysis and hence it seems certain that griseofulvin contains only one $—COO.CH_3$ group and that the second acidic group in *nor*griseofulvic acid is a phenolic and not a carboxyl group. This conclusion is supported by the colour reactions of *nor*griseofulvic acid, an intense brown colour with alcoholic $FeCl_3$, an intense orange brown colour with diazotized sulphanilic acid in Na_2CO_3 solution and a positive Millon reaction.

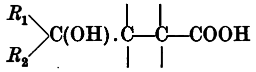
On catalytic reduction with palladium-charcoal-hydrogen, griseofulvin gives rise to two reduction products: *dihydrogriseofulvin*, $C_{17}H_{19}O_6Cl$, $[\alpha]_{5461}-33°$, and *tetrahydrodeoxygriseofulvin*, $C_{17}H_{21}O_5Cl$. Dihydrogriseofulvin still contains the $>CO$ group present in griseofulvin since it forms a 2:4-dinitrophenylhydrazone with Brady's reagent. Hence the two hydrogen atoms taken up in the formation of dihydrogriseofulvin must have been absorbed in the saturation of a $—C{=}C—$ linkage. This conclusion is supported by the fact that whereas griseofulvin gives no coloration with Täufel & Thaler's [1932] reagent—warming with salicylaldehyde and 45 % aqueous H_2SO_4—dihydrogriseofulvin gives a deep red colour with this reagent. This indicates clearly the presence in dihydrogriseofulvin of the grouping $—CH_2.CO.CH_2—$ so that griseofulvin itself must contain the grouping $—CH_2.CO.CH{=}C<$. Tetrahydrodeoxygriseofulvin contains two hydrogen atoms more and one oxygen atom less than dihydrogriseofulvin. This fact is most readily explained by assuming that the grouping $—CH_2.CO.CH_2—$ in dihydrogriseofulvin becomes $—CH_2.CH_2.CH_2—$ in tetrahydrodeoxygriseofulvin, an assumption that is supported by the fact that tetrahydrodeoxygriseofulvin does not react with Brady's reagent.

On oxidation of griseofulvin with $KMnO_4$ in acetone at room temperature two degradation products were isolated. The first of these, $C_9H_9O_5Cl$, is a phenolic monobasic acid containing two methoxyl groups. It must be a chlorohydroxydimethoxybenzoic acid since, with diazomethane, it gave methyl 3-chloro-2:4:6-trimethoxybenzoate identical with that prepared by Calam & Oxford [1939] from phloroglucinol carboxylic acid. Since the griseofulvin degradation product gives a deep purple colour with $FeCl_3$ it is clearly a derivative of salicylic acid. Hence it must be either 5-chloro-2-hydroxy-4:6-dimethoxybenzoic acid or *3-chloro-2-hydroxy-4:6-dimethoxybenzoic acid*, (VII), and since it gives a negative Millon reaction, indicating the absence of an unsubstituted carbon *ortho* to the phenolic group, it must have the latter orientation. The same degradation product is also obtained under similar conditions of oxidation from griseofulvic acid, $C_{16}H_{15}O_6Cl$, and from decarboxygriseofulvic acid, $C_{15}H_{15}O_4Cl$. These facts lead to the following conclusions: (*a*) griseofulvin itself contains a benzene ring to which are attached two of the three $—O.CH_3$ groups and also the chlorine atom; (*b*) the $—COO.CH_3$ group present in griseofulvin is almost certainly not attached to this benzene ring; (*c*) the —COOH and —OH groups in the degradation product arise respectively from the oxidation of a —C— and a —O— linkage. There is present, therefore, in griseofulvin the nucleus



So far as we are aware griseofulvin affords the first recorded instance of the occurrence of the phloroglucinol nucleus in a mould metabolic product.

That griseofulvin, in addition to the above nucleus, also contains a second ring or a long side chain attached to the benzene ring through the —C— and —O— groups, and containing one or more centres of asymmetry, is indicated by the second degradation product referred to above. This substance, $C_{14}H_{15}O_7Cl$, $[\alpha]_{5790} - 24°$, is a monobasic acid giving no colour with $FeCl_3$. It contains two methoxyl groups, a —CO— group which cannot be present as $—OC.CH_3$ since the substance does not give iodoform with alkaline iodine and a hydroxyl group which must be tertiary since no reaction is given with the Fearon-Mitchell [1932] reagent for primary and secondary alcohols. On treatment with acetic anhydride and pyridine it did not give an acetate, but the elements of water were eliminated to give a neutral substance, $C_{14}H_{13}O_6Cl$. The original substance, $C_{14}H_{15}O_7Cl$, is therefore probably a γ-hydroxy-acid of the form



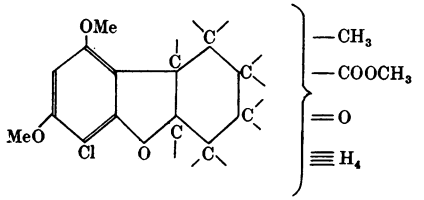
$$\begin{matrix}R_1\\R_2\end{matrix}\!\!>C(OH).\underset{|}{\overset{|}{C}}-\underset{|}{\overset{|}{C}}-COOH$$

where neither R_1 nor R_2 is hydrogen.

Griseofulvin, on fusion with KOH, gives orcinol (3:5-dihydroxytoluene, IX). It is difficult, if not impossible, to explain how the substituted benzene ring which yields 3-chloro-2-hydroxy-4:6-dimethoxybenzoic acid on oxidation of griseofulvin with $KMnO_4$ at room temperature could also yield orcinol on KOH fusion. Hence the conclusion seems irresistible that 3-chloro-2-hydroxy-4:6-dimethoxybenzoic acid and orcinol arise from separate halves of the griseofulvin molecule and, as was indicated earlier, that the $—COOCH_3$ group is attached to the orcinol-yielding half.

Finally, both griseofulvic acid, $C_{16}H_{15}O_6Cl$, and *nor*griseofulvic acid, $C_{15}H_{13}O_6Cl$, with diazomethane, give not only griseofulvin, identical in its properties with the natural product, but also an *iso*griseofulvin, having the same empirical formula as griseofulvin but with a different M.P. and having $[\alpha]_{5461} + 265°$ instead of $+417°$.

The experimental facts at present available do not lead with certainty to a structural formula for griseofulvin. If, however, it is accepted that 3-chloro-2-hydroxy-4:6-dimethoxybenzoic acid and orcinol arise from separate halves of the griseofulvin molecule, and if it is further assumed that orcinol arises from a preformed six-carbon ring with a methyl side-chain attached, then the skeleton



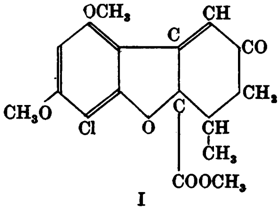
seems inevitable. The non-chlorinated ring must contain the grouping

$$>C=CH.CO.CH_2—$$

(see under dihydrogriseofulvin) and also the centre or centres of optical activity. A number of possible structures, which would satisfy the experimental facts available, could be advanced, but of these (I) appears to offer the best working hypothesis and is suggested as a tentative formula.

Accepting (I) for griseofulvin, $C_{17}H_{17}O_6Cl$, then griseofulvic acid, $C_{16}H_{15}O_6Cl$, would be (II), *nor*griseofulvic acid, $C_{15}H_{13}O_6Cl$ (III), decarboxygriseofulvic acid, $C_{15}H_{15}O_4Cl$ (IV), dihydrogriseofulvin, $C_{17}H_{19}O_6Cl$ (V), tetrahydrodeoxygriseofulvin, $C_{17}H_{21}O_5Cl$ (VI), and the $KMnO_4$ oxidation product of griseofulvin,

$C_{14}H_{15}O_7Cl$ (VIII). The known properties of these substances can be readily explained on the basis of the structures proposed for them.



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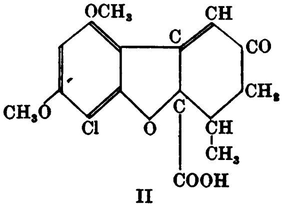

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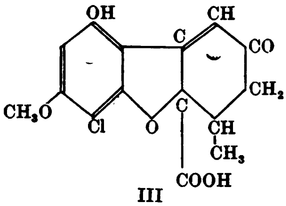

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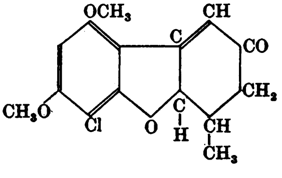

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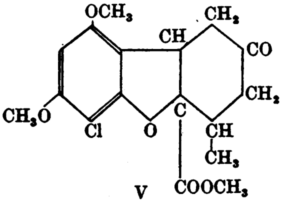

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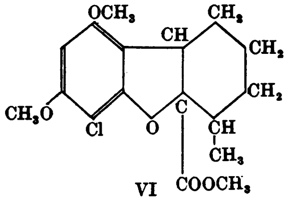

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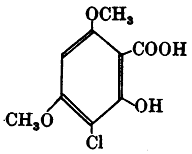

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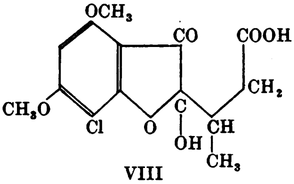

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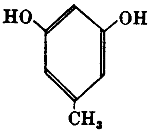

IX

Experimental

History of the culture used

The culture of *Penicillium griseo-fulvum* Dierckx used throughout this work was received in December 1929 from Prof. Ph. Biourge of the University of Louvain, and was numbered B. 34. It bears the L.S.H.T.M. Catalogue No. P. 38.

Cultural conditions

Batches of 100 flasks of the following solution (glucose, 80 g.; $NaNO_3$, 2·5 g.; KH_2PO_4, 1·0 g.; KCl, 0·5 g.; $MgSO_4$, $7H_2O$, 0·5 g.; $FeSO_4$, $7H_2O$, 0·02 g.; distilled water, 1000 ml.), 350 ml. in each litre conical flask, were sterilized, sown with a spore suspension of *P. griseo-fulvum*, and incubated at 30° for 65–85 days when the glucose had been reduced to 0·6–0·8 %. At the end of the incubation period the mycelium was separated by filtration, washed with cold water, and dried in a vacuum oven at 50°. Weight of dry mycelium from 740 flasks = 2970 g.

Extraction of griseofulvin

The mycelium was finely ground in a coffee mill, mixed with pumice stone which had been previously washed with ether, and extracted in a Soxhlet apparatus for 3 days with light petroleum (b.p. 40–50°). The extract consisted of an oil containing small amounts of griseofulvin. The mycelium was then air-dried and re-extracted with ether for 4 days and gave 286 g. of crude solid material = 9·6 % of the dried mycelium. This crude solid material consists of a mixture of griseofulvin and a hitherto undescribed mould metabolic product containing nitrogen, which will form the subject of a future communication.

The constituents were separated as follows: 10–15 g. lots were extracted with boiling benzene (350 ml.). The benzene solution, on cooling, deposited the nitrogenous compound in a fairly pure condition. The benzene mother liquors, by evaporation in stages, yielded successive crops of griseofulvin which was purified by crystallization from ethanol. Total yield of pure griseofulvin, 48·9 g.

When *P. griseo-fulvum* was grown on the medium described above but with an equivalent amount of KBr in place of KCl, no metabolic product containing bromine could be isolated although good growth of the mould and metabolism of the glucose occurred. No griseofulvin could be detected in the mycelium of *P. griseo-fulvum* grown on the medium used by Oxford *et al.* [1935] for the isolation of fulvic acid. This medium contains glucose and ammonium tartrate as sources of C and N and KCl as source of Cl.

Analysis and general properties of griseofulvin

Griseofulvin forms massive, colourless, rhombic crystals by slow deposition from ethanol, M.P. 218–219° without decomposition. (Found: C, 58·05, 57·86; H, 4·93, 4·90; Cl, 9·98, 10·09; CH_3O, 26·29, 26·91 %; N, nil; mol. wt. (cryoscopic in dioxane), 309; (cryoscopic in camphor), 348, 361. $C_{14}H_8O_3Cl(CH_3O)_3$, i.e. $C_{17}H_{17}O_6Cl$ requires C, 57·86; H, 4·85; Cl, 10·06; $3CH_3O$, 26·40 %; mol. wt. 352·5.) The substance is quite neutral; 0·0960 g. dissolved in aqueous ethanol required only 0·05 ml. *N*/10 NaOH for neutralization to phenolphthalein. Optical rotation, $[\alpha]_{5790}^{19^\circ}+354^\circ$; $[\alpha]_{5461}^{19^\circ}+417^\circ$. ($c=1{\cdot}0127$ in acetone.) It is sparingly soluble in the cold, in chloroform, ethyl acetate, benzene, toluene, ethanol, acetone and dioxane, and is quite insoluble in water. The small solubility in cold dioxane renders an exact determination of the mol. wt. impossible. It gives no colour with ferric chloride in alcoholic solution, but gives a yellow colour without fluorescence in cold conc. H_2SO_4 and a similar colour in cold conc. HNO_3. It does not decolor bromine in chloroform solution and gives no reactions with concentrated HBr in glacial acetic acid (not a γ-pyrone) or with alkaline sodium nitroprusside and pyridine (not a $\beta\gamma$-unsaturated lactone [Jacobs & Hoffman, 1926]). It cannot be acetylated. It reacts very readily with phenylhydrazine and with 2:4-dinitrophenylhydrazine but crystalline derivatives could not be isolated in a pure state. It does not react with diazomethane or semicarbazide.

Identification of the alkoxyl groups in griseofulvin. The alkyl iodide from a Zeisel decomposition of griseofulvin (0·1655 g.) was passed into dimethylaniline. The white crystalline precipitate formed (0·3590 g., theoretical for $3CH_3O$ groups in griseofulvin 0·3705 g.) behaved on heating in an exactly similar way to authentic dimethylaniline methiodide, i.e. it melted at 206° in a sealed tube and was completely volatilized at 220°. (Found: I, 48·41 %. Calc. for $C_6H_5(CH_3)_3NI$; I, 48·27 %.) All three alkoxyl groups in griseofulvin are thus methoxyl groups.

Griseofulvin mono-oxime. Griseofulvin (0·7 g.) in ethanol (20 ml.) was refluxed for 7 hr. with hydroxylamine hydrochloride (1·0 g.) and *N* NaOH (14·3 ml.). On dilution with water the crude oxime separated and was purified by repeated crystallization from benzene-light petroleum. Clusters of shining, colourless leaflets which retained solvent very tenaciously. The air-dried crystals sintered at 120° and melted with gas evolution at 120–40° (probably loss of solvent); the melt reset on further heating and remelted sharply at 226–7° without decomp. (Found, on material dried to constant weight at 110°: C, 55·33; H, 5·23; N, 3·46; Cl, 9·06 CH_3O, 25·25 %. $C_{17}H_{18}O_6NCl$ requires

C, 55·49; H, 4·92; N, 3·81; Cl, 9·64; 3(CH_3O), 25·31 %.) In spite of the large excess of hydroxylamine used in this preparation there was no evidence of the formation of a di-oxime.

Catalytic reduction of griseofulvin. Dihydrogriseofulvin and tetrahydrodeoxygriseofulvin

To a solution of griseofulvin (0·9 g.) in ethyl acetate (140 ml.) were added norite (2·5 g.) and a suspension of powdered $PdCl_2$ (0·7 g.) in water (50 ml.) previously heated to boiling and then cooled. The mixture was vigorously shaken with hydrogen and after the initial very rapid uptake of hydrogen required for the reduction of the $PdCl_2$ and saturation of the catalyst a slow absorption set in. The reduction was stopped after 7 hr. when 61 ml. H_2 had been absorbed (theoretical for 1 mol. $H_2 = 57$ ml. at N.T.P.). The mixture was filtered, the ethyl acetate layer separated, dried and concentrated to low bulk. Addition of much light petroleum now gave a colourless precipitate (0·7 g., M.P. 184–186°) from which were obtained, by fractional crystallization from light petroleum (B.P. 80–100°) and aqueous ethanol, two pure substances, dihydrogriseofulvin and tetrahydrodeoxygriseofulvin.

Dihydrogriseofulvin. Colourless flat rods, M.P. 194–196° from light petroleum (B.P. 80–100°). (Found: C, 57·60, 57·57; H, 5·31, 5·25; Cl, 10·2 %. $C_{17}H_{19}O_6Cl$ requires C, 57·53; H, 5·40; Cl, 10·0 %.) Optical rotation, $[\alpha]_{5461}^{18^\circ} - 33^\circ$; $[\alpha]_{5790}^{18^\circ} - 27^\circ$. ($c = 0{\cdot}452$ in acetone.) Dihydrogriseofulvin is more readily soluble in most organic solvents than griseofulvin. It gives only a yellow colour with $FeCl_3$ in alcoholic solution. Unlike griseofulvin, which gives no colour, it gives a deep rose red colour on warming with salicylaldehyde and 45 % aqueous H_2SO_4. Addition of Brady's reagent [1931] to an alcoholic solution gives a yellow crystalline precipitate, M.P. 264–266°, which is probably a mono-2:4-dinitrophenylhydrazone since it gives a reddish brown colour with alcohol and a trace of aqueous KOH.

Tetrahydrodeoxygriseofulvin. Long colourless slender needles, M.P. 180°, from aqueous ethanol or methanol. (Found: C, 59·99, 60·00; H, 6·28, 6·43; Cl, 10·5, 10·6; CH_3O, 26·9 %. $C_{17}H_{21}O_5Cl$ requires C, 59·88; H, 6·22; Cl, 10·4; $3CH_3O$, 27·3 %.) The substance is quite insoluble in dilute KOH. It gives an intense greenish yellow colour with cold concentrated H_2SO_4 becoming wine-red on warming. A solution in aqueous alcohol gives no precipitate with Brady's reagent even on heating. It is not hydrolysed by boiling for 4 hr. with aqueous-alcoholic N H_2SO_4 or by boiling for 7 hr. with $N/2$ NaOH, facts which are difficult to explain unless a steric hindrance effect is assumed.

Hydrolysis of griseofulvin

(A) *With dilute aqueous-alcoholic H_2SO_4. Griseofulvic acid.* A mixture of griseofulvin (1·0 g.), ethanol (200 ml.) and $2N$ H_2SO_4 (250 ml.) was refluxed for 6 hr. The ethanol was removed *in vacuo*, the colourless precipitate collected, washed with water and then with dilute Na_2CO_3 in which it was almost completely soluble. The alkaline solution was acidified and the resulting precipitate (0·8 g.) was crystallized from boiling aqueous methanol in which it is only sparingly soluble. Colourless, flat, hexagonal prisms, M.P. 256–260°. (Found: C, 56·85, 56·77; H, 4·54, 4·61; Cl, 10·38, 10·14; CH_3O, 18·2 %. Equiv. by titration, 362. $C_{16}H_{15}O_6Cl$ requires C, 56·70; H, 4·46; Cl, 10·47; $2CH_3O$, 18·3 %. Equiv., as a monobasic acid, 339.) Optical rotation, $[\alpha]_{5461}^{19^\circ} + 508^\circ$; $[\alpha]_{5790}^{19^\circ} + 420^\circ$. ($c = 0{\cdot}2256$ as the sodium salt in aqueous methanol.)

Griseofulvic acid gives a yellowish brown colour with $FeCl_3$, a yellow colour with cold conc. H_2SO_4, and only a faint yellow colour with diazotized sulphanilic acid in Na_2CO_3 solution. The Millon reaction is also negative. It is readily soluble in aqueous $NaHCO_3$ and the resulting solution slowly decolorizes very dilute $KMnO_4$ solution. It gives a dark red amorphous precipitate with Brady's reagent.

(B) *With dilute aqueous-alcoholic NaOH.* A mixture of griseofulvin (2·25 g.), ethanol (300 ml.) and $N/4$ NaOH (200 ml.) was refluxed for 3 hr. The solution was cooled, acidified, the ethanol removed *in vacuo* and the colourless precipitate collected, washed and dried (2·3 g.). It was crystallized from aqueous methanol in colourless hexagonal prisms, M.P. 255–262°, alone or in admixture with griseofulvic acid formed on acid hydrolysis of griseofulvin (see (A) above). Equiv. by titration, 374. $C_{16}H_{15}O_6Cl$, as a monobasic acid, requires 339.

(C) *With dilute aqueous NaOH.* A mixture of griseofulvin (3·82 g.), N NaOH (250 ml.) and water (350 ml.) was refluxed for 5 hr. At first part of the solid appeared to dissolve, but later much colourless material separated. Next day this was collected, washed and dried (fraction I, wt. 1·04 g.). The alkaline filtrate was acidified, filtered from a little dark coloured amorphous material and set aside. An almost colourless, crystalline substance (fraction II, wt. 1·6 g.) separated.

Purification and properties of fraction I. Decarboxygriseofulvic acid. Colourless needles from aqueous ethanol, M.P. 138–140°. Contains chlorine. (Found: C, 61·25, 61·15; H, 5·09, 5·10; CH_3O, 21·01, 21·37; mol. wt. (cryoscopic in dioxane), 279. Equiv. nil. $C_{15}H_{15}O_4Cl$ requires C, 61·10; H, 5·13; $2CH_3O$, 21·05 %; mol. wt. 295.) Optical rotation, $[\alpha]^{18°}_{5461} - 31°$. ($c = 0·163$ in acetone.)

Decarboxygriseofulvic acid is much more soluble in the usual organic solvents than is griseofulvin. It gives no colour with $FeCl_3$ in aqueous alcoholic solution. With cold conc. H_2SO_4 it gives an immediate intense yellow colour, quickly changing to brown and finally to a deep purple (permanganate) colour. It gives a brownish red colour with saturated alcoholic picric acid but no picrate separates. It is unchanged by boiling with dilute aqueous-alcoholic H_2SO_4.

Purification and properties of fraction II. nor*Griseofulvic acid.* Long, colourless, pointed needles from aqueous methanol, M.P. 260° decomp. (Found: C, 55·67; H, 4·18; Cl, 10·46, 10·61; CH_3O, 10·6 %. Equiv. by titration, 165. $C_{15}H_{13}O_6Cl$ requires C, 55·46; H, 4·04; Cl, 10·92; $1CH_3O$, 9·6 %. Equiv. as a dibasic acid, 162.) Optical rotation, $[\alpha]^{18°}_{5461} + 609°$; $[\alpha]^{18°}_{5790} + 505°$. ($c = 0·2262$ as sodium salt in water.) *nor*Griseofulvic acid is readily soluble in hot methanol but is almost insoluble in boiling chloroform. It gives a positive Millon reaction, an intense brown colour with $FeCl_3$ in aqueous methanol, an intense orange brown colour with diazotized sulphanilic acid in Na_2CO_3 and a yellow colour with cold conc. H_2SO_4. It was recovered unchanged after boiling with N aqueous-alcoholic H_2SO_4.

(D) *Hydrolysis of griseofulvic acid with* N/2 *aqueous NaOH.* Griseofulvic acid ($C_{16}H_{15}O_6Cl$, ((A) above) 0·53 g.) was boiled with $N/2$ aqueous NaOH (70 ml.) for 4 hr. The products of hydrolysis were decarboxygriseofulvic acid, $C_{15}H_{15}O_4Cl$ ((C) above, fraction I, 0·13 g., M.P. 138–140° alone or in admixture) and *nor*griseofulvic acid, $C_{15}H_{13}O_6Cl$ ((C) above, fraction II, 0·15 g., M.P. 260° alone or in admixture).

Methylation of griseofulvic acid and nor*griseofulvic acid with diazomethane*

*nor*Griseofulvic acid, $C_{15}H_{13}O_6Cl$ (0·38 g.), suspended in ether was methylated by the addition of diazomethane prepared from nitrosomethylurethane (7 ml.). After several hours the solution was separated from a little insoluble precipitate, the solvent removed and the crystalline residue (0·43 g.) fractionally crystallized from ethanol. The more soluble component proved to be griseofulvin. Prisms, m.p. 217–218°, alone or in admixture with authentic griseofulvin. The less soluble component proved to be an iso*griseofulvin,* long colourless needles from ethanol (0·15 g.), m.p. 198–200°, and giving a large depression on admixture with griseofulvin. (Found: C, 57·82, 57·88; H, 4·98, 4·99; N, nil; Cl, 10·20; CH_3O, 26·51 %. $C_{17}H_{17}O_6Cl$ requires C, 57·86; H, 4·85, Cl, 10·06; $3CH_3O$, 26·40 %.) Optical rotation, $[\alpha]^{19°}_{5461} + 265°$; $[\alpha]^{19°}_{5790} + 223°$. ($c = 0·1424$ in acetone.) It is very similar in all its properties to griseofulvin except that it is less soluble in organic solvents.

Methylation of griseofulvic acid, $C_{16}H_{15}O_6Cl$, with diazomethane also gave a mixture of griseofulvin and *iso*griseofulvin, though in this case griseofulvin was formed in the larger amount.

Oxidation of griseofulvin by $KMnO_4$ in acetone

Griseofulvin (4 g.) dissolved in pure acetone (1 litre) was treated with finely powdered $KMnO_4$ (16 g.). The mixture was kept cool and shaken occasionally during 5 hr. The MnO_2 and insoluble potassium salts formed were separated, washed with acetone, dried and ground with dilute ammonia. The filtered ammoniacal solution was acidified to Congo red with dilute H_2SO_4. A gum was precipitated which later solidified (0·8 g.). (For treatment of filtrate from gum, see below.) The gum was repeatedly crystallized from ethyl acetate to yield 0·2 g. of colourless needles, m.p. 224° decomp., of *3-chloro-2-hydroxy-4:6-dimethoxybenzoic acid.* (Found: C, 46·52, 46·49; H, 4·01, 3·96; Cl, 15·36, 15·64; CH_3O, 26·18, 26·42 %. Equiv. 231. $C_9H_9O_5Cl$ requires C, 46·45; H, 3·90; Cl, 15·25; $2CH_3O$, 26·67 %. Equiv. as a monobasic acid, 232·5.) This acid is insoluble in water and sparingly soluble in all organic solvents. It gives a beautiful purple colour with $FeCl_3$ in alcoholic solution but gives a negative Millon reaction.

0·3 g. of the acid, on methylation with ethereal diazomethane, yielded 0·15 g. of the methyl ester of 3-chloro-2:4:6-trimethoxybenzoic acid [Calam & Oxford, 1939]. Colourless hexagonal platelets from light petroleum, m.p. 127–128°, alone or in admixture with an authentic specimen. (Found: C, 50·55, 50·40; H, 5·01, 5·04; CH_3O, 47·20, 46·79 %. Mol. wt., cryoscopic in dioxane, 248. $C_{11}H_{13}O_5Cl$ requires C, 50·67; H, 5·03; $4CH_3O$, 47·63 %; mol. wt. 260·5.) The substance is quite insoluble in KOH solution and gives no colour with $FeCl_3$.

3-Chloro-2-hydroxy-4:6-dimethoxybenzoic acid is also formed by the oxidation with $KMnO_4$ in acetone of griseofulvic acid, $C_{16}H_{15}O_6Cl$, and decarboxygriseofulvic acid, $C_{15}H_{15}O_4Cl$.

The filtrate from the gum referred to above was extracted with ether. On removal of the solvent a small amount of colourless material remained which, on crystallization from benzene-light petroleum-chloroform, yielded clusters of minute rods, m.p. 200° decomp. (Found: C, 50·70, 50·79; H, 4·48, 4·45; Cl, 10·7, 11·1; CH_3O, 18·4, 18·3 %. Equiv., 320. $C_{14}H_{15}O_7Cl$ requires C, 50·82; H, 4·57; Cl, 10·73; $2CH_3O$, 18·77 %. Equiv. as a monobasic acid, 330·5.) Optical rotation, $[\alpha]^{18°}_{5790} - 24°$. ($c = 0·8506$ as the sodium salt in 20 % aqueous methanol.) The acid is appreciably soluble in water, readily so in ethanol, and gives no colour with $FeCl_3$. It appears to contain a —CO— group since, although its aqueous solution does not give an immediate reaction with Brady's reagent, a good yellow precipitate forms after 2 days. It also appears to contain an OH group since, on treatment with acetic anhydride in pyridine at 37° for several days, although no acetyl derivative is formed, the elements of water are eliminated to give a *neutral substance,* $C_{14}H_{13}O_6Cl$, as colourless needles from aqueous ethanol

M.P. 220° (no decomp.). (Found: C, 53·87; H, 4·18; Cl, 11·14%. $C_{14}H_{13}O_6Cl$ requires C, 53·74; H, 4·19; Cl, 11·34%.) The acid $C_{14}H_{15}O_7Cl$ gives a negative iodoform reaction with alkaline iodine, a negative Fearon-Mitchell reaction [1932] for primary and secondary alcohols and it does not appear to contain a lactone grouping since treatment with excess NaOH for some time at 37° does not unmask additional acidity. It reduces Fehling's solution on boiling. It is also formed by the $KMnO_4$ oxidation of griseofulvic acid but not of decarboxygriseofulvic acid.

Fusion of griseofulvin with KOH

Griseofulvin (1 g.) was fused for 1 hr. at 225–50° with solid KOH (3·5 g.) and water (1·5 ml.) in a nickel crucible. The melt was cooled and dissolved in water, and the solution saturated with CO_2 and extracted with ether. Evaporation of the dried extract gave a colourless crystalline residue (0·26 g.), which, when fractionally crystallized from light petroleum gave three fractions melting at 55, 95 and 107°. All three fractions gave the characteristic reactions of orcinol, i.e. a neutral aqueous solution giving a bluish purple colour with $FeCl_3$, a red colour with sodium hypochlorite and with ammonia and a precipitate with bromine water. A pink colour with a strong green fluorescence was observed when any of the fractions was dissolved in caustic potash and shaken with chloroform. The first fraction was hydrated orcinol (M.P. 58°) and the other two essentially the anhydrous phenol (M.P. 107°). All three fractions, on sublimation in a high vacuum at 55–60° gave a sublimate melting at 96°, not depressed on admixture with authentic anhydrous orcinol. (Found: C, 67·39, 67·38; H, 6·69, 6·40%. Mol. wt. (in camphor), 129, 130. $C_7H_8O_2$ requires C, 67·70; H, 6·49; mol. wt. 124.)

SUMMARY

Griseofulvin, $C_{17}H_{17}O_6Cl$, a hitherto undescribed mould metabolic product, has been isolated from the mycelium of *Penicillium griseo-fulvum* Dierckx grown on a modified Czapek-Dox solution. The general properties of griseofulvin are described together with a number of derivatives and degradation products. A provisional structural formula for griseofulvin is suggested which illustrates the experimental findings.

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