759. *Griseofulvin. Part I.*

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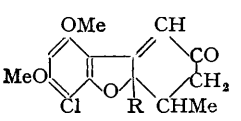
The structures proposed for griseofulvin, $C_{17}H_{17}O_6Cl$, by Oxford, Raistrick, and Simonart (*Biochem. J.*, 1939, **33**, 240) and by Grove and McGowan (*Nature*, 1947, **160**, 574) are inconsistent with the ultra-violet and infra-red absorption spectra. Grove and McGowan's suggestion (*Chem. and Ind.*, 1949, 647), based on spectroscopic data, that griseofulvin is the methyl ether of a 1 : 3-diketone has been proved conclusively by catalytic reduction of griseofulvic acid, $C_{16}H_{15}O_6Cl$, to two neutral non-lactonic alcohols, $C_{16}H_{19}O_6Cl$ and $C_{16}H_{19}O_5Cl$, and by other evidence. The formation of decarboxygriseofulvic acid by the action of alkali on griseofulvic acid is shown by ultra-violet and infra-red spectroscopic evidence to involve a rearrangement of the molecule.

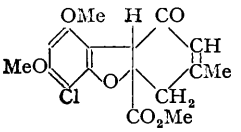
GRISEOFULVIN, $C_{17}H_{17}O_6Cl$, m. p. 220°, $[\alpha]_{5790}^{19}$ +354°, a colourless neutral compound containing three methoxyl groups, was isolated from the mycelium of *Penicillium griseofulvum* Dierckx by Oxford, Raistrick, and Simonart (*Biochem. J.*, 1939, **33**, 240). Subsequently, it was isolated from *P. janczewskii* Zal. [= *P. nigricans* (Bainier) Thom] and its unique biological activity on moulds noted by Brian, Curtis, and Hemming (*Trans. Brit. Mycol. Soc.*, 1946, **29**, 173; see also Brian, *Ann. Bot.*, 1949, **13**, 59) and McGowan (*Trans. Brit. Mycol. Soc.*, 1946, **29**, 188) who originally called it "curling factor" before the identity with griseofulvin was established (Grove and McGowan, *Nature*, 1947, **160**, 574; Brian, Curtis, and Hemming, *Trans. Brit. Mycol. Soc.*, 1949, **32**, 30).

Although the chemical and biological properties of "curling factor" and griseofulvin were identical, the analytical data and molecular-weight determinations quoted by McGowan (*loc. cit.*) in support of his original formula $C_{20}H_{20}O_9$ agreed more closely with $C_{20}H_{19}O_7Cl$ than with $C_{17}H_{17}O_6Cl$. However, a careful reinvestigation has established the empirical formula as $C_{17}H_{17}O_6Cl$, in agreement with the earlier work of Oxford *et al.* and this is supported by molecular-weight determinations by both the Rast and crystallographic methods (the latter by Dr. A. F. Wells). Nevertheless, the structure (I) tentatively proposed by Oxford *et al.* (*loc. cit.*) is not entirely satisfactory even on the basis of the facts which they reported, and we have accumulated considerable evidence which cannot be reconciled with it. We have repeated practically all the experimental work

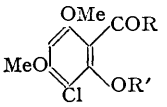
described by Oxford *et al.* and agree with the great majority of it. These facts alone, however, are not sufficient to support a structural formula.



(I; R = CO_2Me)
(I*a*; R = H)



(II)



(III)

Oxford *et al.* showed that griseofulvin contained an ethylenic bond and a chemically reactive carbonyl group and these observations have been confirmed by microhydrogenation and preparation of a crystalline 2 : 4-dinitrophenylhydrazone. In addition, griseofulvin

FIG. 1. FIG. 2.

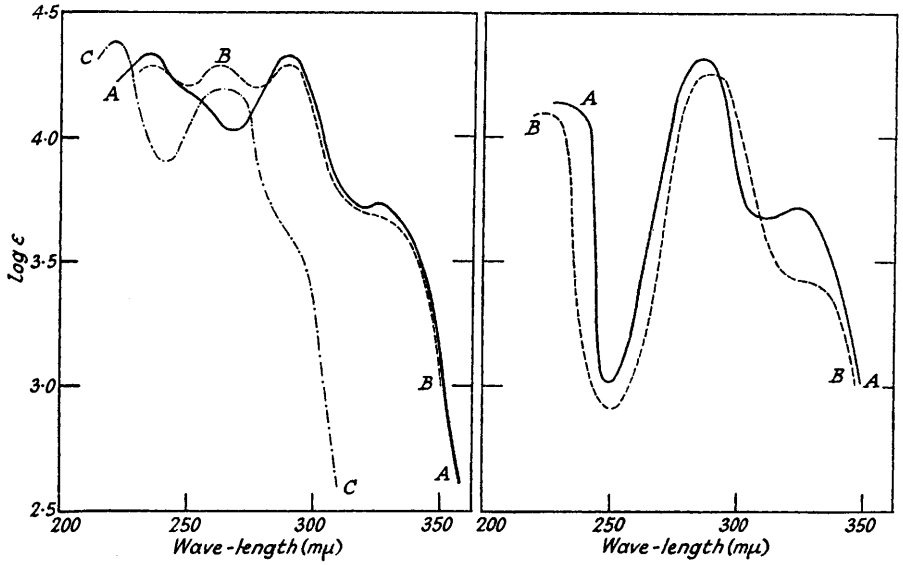


FIG. 1. *A, Griseofulvin* ($\lambda_{max.}$ 324, 291, ~252, 236 mμ; log ε 3·72, 4·34, 4·10, 4·33 *respectively*). *B,* iso-*Griseofulvin* ($\lambda_{max.}$ 326, 291, 263, 235 mμ; log ε 3·71, 4·29, 4·29, 4·28 *respectively*). *C, Decarboxy-griseofulvic acid* ($\lambda_{max.}$ ~295, 265, 220 mμ; log ε 3·47, 4·20, 4·39 *respectively*) (*all in methanol*).

FIG. 2. *A, Griseofulvic acid reduction product A in methanol. B, Methylphloracetophenone.*

reacts normally with Girard's reagent P, forming a derivative from which it can be recovered unchanged. With semicarbazide in pyridine griseofulvin gave, not the normal semicarbazone, but an isomeric base which was readily hydrolysed by dilute mineral acids to griseofulvic acid. The properties of this derivative suggest that it is a pyrazoline although it did not respond to the Knorr test. Oxford *et al.* reported evidence suggesting that the double bond in griseofulvin was conjugated with the carbonyl group, and the reaction with semicarbazide provides additional evidence for the presence of an αβ-unsaturated ketone grouping. Griseofulvin reacts readily with bromine in chloroform, but hydrogen bromide is eliminated and the product is bromogriseofulvin.

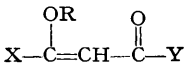
Grove and McGowan (*loc. cit.*) pointed out that structure (II), a slight modification of (I), accounted more satisfactorily for the properties of griseofulvin as then known. But consideration of the ultra-violet and infra-red absorption spectra of griseofulvin and some of its derivatives led these authors (*Chem. and Ind.*, 1949, 647) ultimately to reject both structures. The ultra-violet absorption spectrum of griseofulvin (Fig. 1) showed that the αβ-unsaturated ketone system postulated by Oxford *et al.* could not be conjugated with the aromatic nucleus in griseofulvin as in (I). Neither did the absorption of griseofulvin agree particularly well with that predicted for (II). Rather, it was typical of a compound in

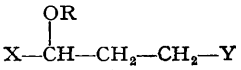
which phloroglucinol and carbonyl chromophores were conjugated, as in pinobanksin dimethyl ether (Lindstedt, *Acta Chem. Scand.*, 1950, **4**, 772) and some of the derivatives and analogues of rottlerin investigated by Morton and Sawires (*J.*, 1940, 1052), and it strongly suggested that griseofulvin contained the partial structure (III). The curve for methylphloracetophenone taken from the paper of Morton and Sawires is plotted in Fig. 2.

There is, moreover, chemical evidence for the inadequacy of formulæ (I) and (II). Thus, while griseofulvin on mild hydrolysis with aqueous alcoholic alkali gives griseofulvic acid, $C_{16}H_{15}O_6Cl$, one of the reduction products, tetrahydrodeoxygriseofulvin, is very resistant to hydrolysis and this stability cannot easily be dismissed in terms of steric hindrance (cf. Oxford *et al.*, *loc. cit.*; see also Part VI, *J.*, 1952, 3994). Further, the fact that on slightly more vigorous hydrolysis with aqueous alkali griseofulvic acid gives as one of the products decarboxygriseofulvic acid, $C_{15}H_{15}O_4Cl$, cannot be taken as proving the presence of the carboxylic acid group. In fact, the stability which we find griseofulvic acid possesses towards acid hydrolysis (24 hours' heating under reflux in 6N-sulphuric acid and 70% recovery of griseofulvic acid), suggests that the carbon dioxide lost in the alkaline hydrolysis is derived from a carboxyl group which makes its appearance as a result of a molecular rearrangement under alkaline conditions. Support for this is obtained from a comparison of the ultra-violet absorption curves of griseofulvin and decarboxygriseofulvic acid (Fig. 1) which show a profound alteration in the chromophoric system to have occurred (Grove and McGowan, *Chem. and Ind.*, *loc. cit.*), as well as from the infra-red spectra (see below) of these two compounds (Fig. 3).

In addition, the ultra-violet absorption of decarboxygriseofulvic acid is clearly inconsistent with the structure (I*a*) suggested for this compound by Oxford *et al.* A compound in which the αβ-unsaturated ketone grouping is conjugated with the aromatic nucleus should show a strong absorption band in the region of 330 mμ (Grove and McGowan, *loc. cit.*).

The difference in behaviour of griseofulvin and tetrahydrodeoxygriseofulvin towards hydrolysis caused Grove and McGowan to suggest that griseofulvin contained the grouping (IV; R = Me), being a methyl enol ether of a 1 : 3-diketone, griseofulvic acid (IV; R = H). Tetrahydrodeoxygriseofulvin (V; R = Me) in which the methoxyl group is attached to a saturated carbon atom would then resist hydrolysis. Oxford *et al.* observed that an isomer, m. p. 200—201°, $[\alpha]_{5790}^{19}$ +223°, of griseofulvin was formed, mixed with an approximately equal amount of griseofulvin, when griseofulvic acid or norgriseofulvic acid was methylated with diazomethane. It appeared likely on the above hypothesis that *iso*griseofulvin would prove to be the isomeric methyl ether (VI), although no evidence was offered at that time. We have now shown that *iso*griseofulvin can be made easily and in good yield by the action of methanolic hydrochloric acid on griseofulvin. *iso*Griseofulvin is easily hydrolysed by dilute aqueous-alcoholic alkali to griseofulvic acid, which has the same optical rotation and configuration as that prepared from griseofulvin; the isomerism is thus not connected with asymmetry round a particular carbon atom, but arises from the presence of a tautomeric system in griseofulvic acid. As is then to be expected, the ultra-violet absorption of *iso*griseofulvin (Fig. 1) is similar to that of griseofulvin: nevertheless, *iso*griseofulvin shows an additional and characteristic band at 263 mμ. *iso*Griseofulvin also differs from griseofulvin in that it does not readily form derivatives with ketonic reagents.


(IV)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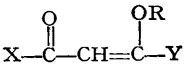

(VI)

Recent work on the correlation of absorption frequency and structure for the C=O and –OH stretching vibrations has been summarised by Grove and Willis (*J.*, 1951, 877). Normal carboxylic esters generally absorb between 1715 (conjugated) and 1745 cm.$^{-1}$ (unconjugated). These frequencies may be raised by the presence of powerful electron-attracting substituents (*e.g.*, NO_2) or lowered by intramolecular hydrogen bonding (chelation); however, neither of these complications is present in the griseofulvin molecule. Griseofulvin (Fig. 3*b*) has no absorption bands in the carboxylic ester range, and the

highest >C=O absorption occurs at 1700 cm.$^{-1}$. Carboxylic acids in the solid (dimeric) state show a broad absorption in the region 2500—2700 cm.$^{-1}$ attributed to –OH stretching vibrations and a band in the range 1680 (conjugated) to 1720 cm.$^{-1}$ (unconjugated) due to the >C=O group. The highest absorption frequency in the double-bond stretching region of griseofulvic acid (Fig. 3*a*) occurs at 1655 cm.$^{-1}$; the compound does not absorb in the region 2500—2700 cm.$^{-1}$ but has a strong alcoholic (or enolic) –OH absorption at 3225 cm.$^{-1}$.

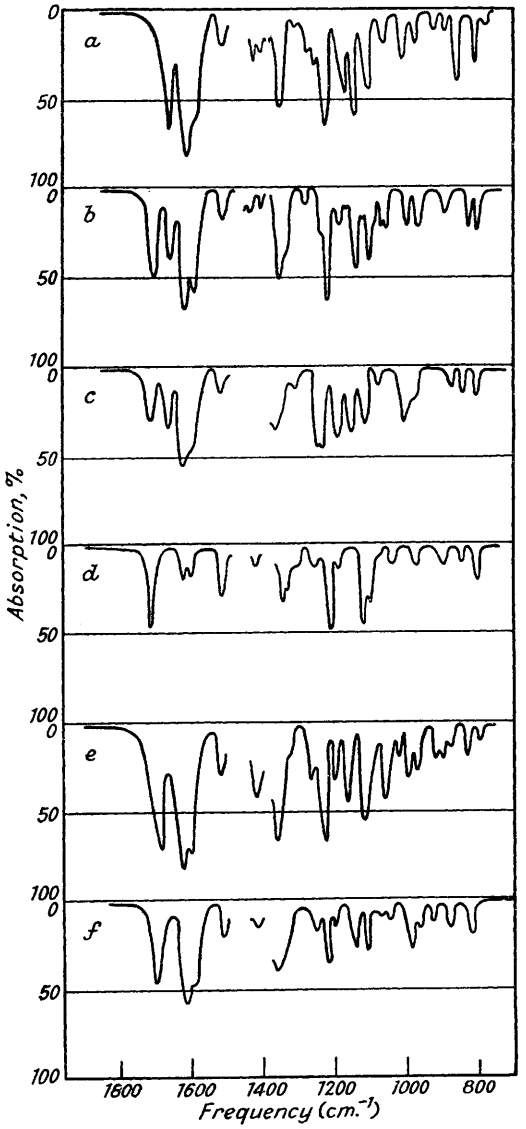
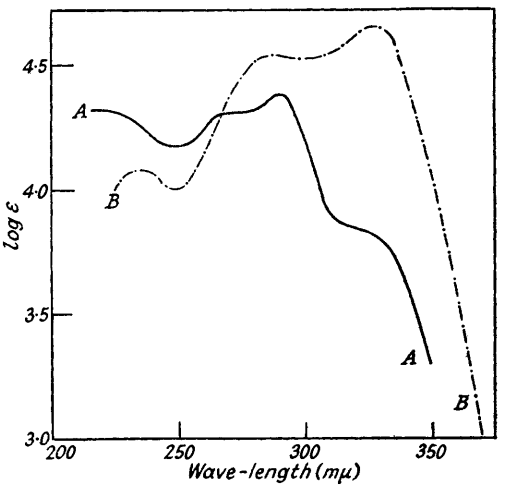


FIG. 3. *a, Griseofulvic acid; b, griseofulvin; c,* iso*griseofulvin; d, decarboxygriseofulvic acid; e, griseofulvic acid reduction product A; f, griseofulvic acid reduction product C.*

FIG. 4.



Norgriseofulvic acid, (*A*) *in methanol* ($\lambda_{max.}$ 332, 290, 269, 223 mμ; log ε 3·77, 4·38, 4·32, 4·32 *respectively*) *and* (*B*) *in* 0·1N-*sodium hydroxide* ($\lambda_{max.}$ 327, 277, 234 mμ; log ε 4·65, 4·53, 4·08 *respectively*).

These results, briefly reported by Grove and McGowan (*Chem. and Ind.*, 1949, 647), indicate the absence of carboxylic ester and carboxylic acid groups in griseofulvin and griseofulvic acid respectively, and can be interpreted as supporting the hypothesis outlined above. Furthermore, the presence of two carbonyl groups absorbing at 1700 and 1650 cm.$^{-1}$, respectively, in griseofulvin is clearly shown, and one of these persists in the spectra of those compounds in which the reactive carbonyl group has been removed, for example, by hydrogenation (see below; see also Part V, *J.*, 1952, 3987).

The infra-red spectra of griseofulvin and *iso*griseofulvin (Figs. 3*b* and *c*) are very similar,

particularly in the double-bond stretching region, *iso*griseofulvin showing two carbonyl bands at 1704 and 1658 cm.$^{-1}$ respectively. Griseofulvin, *iso*griseofulvin, and griseofulvic acid all show an intense broad band between 1580 and 1620 cm.$^{-1}$, arising presumably from unsaturation in the skeletal ring system. These intense bands are absent in the spectrum of decarboxygriseofulvic acid (Fig. 3*d*), which shows >C=O absorption at 1710 cm.$^{-1}$ attributed to an unconjugated six-membered ring keto-group and no OH absorption.

Set against the spectroscopic evidence may be mentioned three facts which, though inconclusive, might suggest the presence of a carboxylic acid group in griseofulvic acid. Pyrolysis of griseofulvic acid gives some carbon dioxide, but the amorphous residue is not decarboxygriseofulvic acid and it may be argued that a profound structural alteration is involved. Griseofulvic acid may be "esterified" with methanolic hydrochloric acid under Fischer–Speier conditions; methylation under these conditions is, however, merely an indication of acidity (griseofulvic acid has K 3·2 × 10^{-5} at 18°) and, though not a normal method of preparing enol ethers, has analogies (cf. the *O*-alkylation of phloroglucinol). Thirdly, a keto–enol system of the type postulated might be expected to give more than a pale orange colour with ferric chloride; however, Morgan and Drew (*J.*, 1924, **125**, 752) reported, and it has been confirmed, that dimedone, in common with some other cyclic

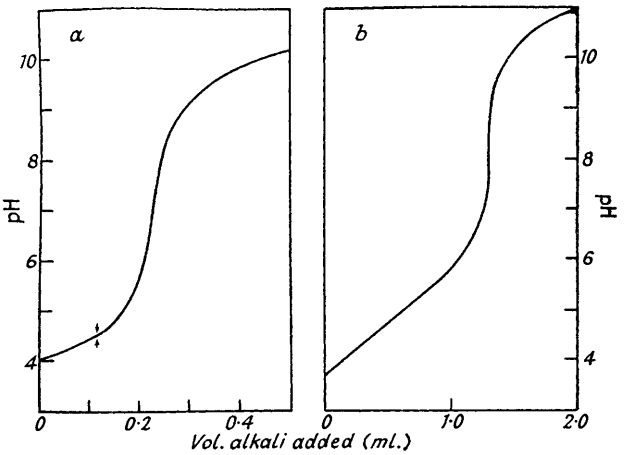
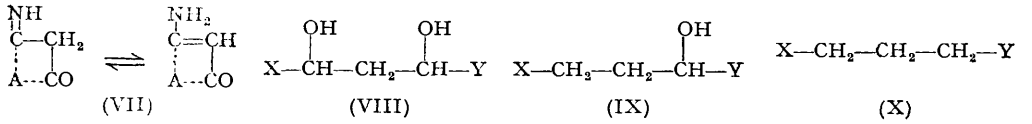


FIG. 5. *a, Griseofulvic acid, c* = 1·1 × 10^{-4}M *in water; b, norgriseofulvic acid, c* = 2·25 × 10^{-3}M *in aqueous alcohol.*

1 : 3-diketones, gives no colour with ferric chloride in an alcoholic solution of concentration comparable to that attainable with griseofulvic acid.

However, further observations provide conclusive evidence against the presence of a carboxylic acid residue. First, griseofulvin and griseofulvic acid give negative reactions in the hydroxamic acid test for carboxylic ester and acid groupings. Secondly, griseofulvic acid is a mild reducing agent and is oxidised by warm ammoniacal silver and yellow mercuric oxide; griseofulvin has no reducing activity. Thirdly, griseofulvin reacts with ammonia in methanol to give a compound, griseofulvamine, $C_{16}H_{16}O_5NCl,H_2O$ which is certainly not an amide. The properties of this substance, which contains only two methoxyl groups, are typical of compounds formed by cyclic β-diketones, *e.g.*, dimedone (Haas, *J.*, 1906, **89**, 187) with ammonia and are in accord with the structures of the type (VII). Finally, reduction of griseofulvic acid provides the strongest evidence against the presence of a carboxylic acid group. When Adams's platinum oxide catalyst in acetic acid was used, two neutral non-lactonic alcohols, $C_{16}H_{19}O_6Cl$ (A) and $C_{16}H_{19}O_5Cl$ (B), were isolated together with small amounts of a neutral non-lactonic compound, $C_{16}H_{19}O_4Cl$ (C). If the acidic component in griseofulvic acid is (IV; R = H), these compounds can be written with the partial structures (VIII) (IX or V; R = H), and (X), respectively. The reduction product B was oxidised by chromic acid to the corresponding ketone, $C_{16}H_{17}O_5Cl$, indicating that the hydroxyl group is secondary. The ultra-violet absorption curve of the reduction product A (Fig. 2) shows that the main chromophoric system (III) of griseofulvin is unaffected by the reduction and since the chemically unreactive carbonyl

group of griseofulvin can be identified in A by its infra-red spectrum (Fig. 3*e*; band at 1685 cm.$^{-1}$) it follows that this carbonyl group must be the one in the partial structure (III). This unreactive carbonyl group can also be identified in the spectra of the reduction products B (band at 1682 cm.$^{-1}$) and C (Fig. 3*f*; band at 1695 cm.$^{-1}$); A and B show typical alcoholic –OH absorption (at 3401 and 3425 cm.$^{-1}$ respectively), which is absent in the spectrum of reduction product C.



(VII) (VIII) (IX) (X)

It is considered that all the facts reported above are satisfactorily explained by the hypothesis that griseofulvin contains two carbonyl groups, one of which is unreactive chemically. The reactive carbonyl group is contained in the partial structure (IV; R = Me), griseofulvic acid being the corresponding 1 : 3-diketone (IV; R = H, in the enolic form). The formation of decarboxygriseofulvic acid involves a profound rearrangement.

The behaviour of griseofulvin with alkali is complex. In some experiments, the hydrolysis proceeded as described by Oxford *et al.* and the neutral " decarboxygriseofulvic acid," m. p. 138°, and the dibasic norgriseofulvic acid were isolated. In other experiments, decarboxygriseofulvic acid was accompanied by a neutral isomer, m. p. 204—206°; the yield of crude decarboxygriseofulvic acid was increased when the hydrolysis was conducted in an atmosphere of nitrogen and greatly reduced by a stream of air.

Norgriseofulvic acid was formulated by Oxford *et al.* (*loc. cit.*) as a phenolic acid. We know of no strictly analogous case where an aryl methyl ether group has been hydrolysed with such ease by dilute alkali without showing a high lability to acid. Nevertheless, we agree that the second acidic group in norgriseofulvic acid is indeed phenolic, and consider that its enhanced acidity is due to the environment of the remainder of the molecule. Support for the presence of the phenolic group comes from the ultra-violet absorption maximum which shifts to longer wave-lengths in alkaline solution (Fig. 4), as occurs with hydroxyacetophenones (Morton and Stubbs, *J.*, 1940, 1347). The potentiometric titration curve (Fig. 5*b*) shows only one point of inflexion.

We have confirmed the formation of orcinol in high yield (together with a little acidic material, m. p. 180°) on alkali fusion of griseofulvin. On the other hand, none was obtained from decarboxygriseofulvic acid. This is further evidence that rearrangement of the molecule is involved in the formation of the latter.

Experimental

M. p.s are corrected. Mixed m. p. determinations with griseofulvic acid are unreliable and identification was established, except where stated, by comparison of the *X*-ray powder patterns.

Infra-red spectra of solids were obtained in Nujol " mulls " in the apparatus described by Grove and Willis (*loc. cit.*). Calibration accuracy: ±3 at 1700 cm.$^{-1}$, ±10 at 3000 cm.$^{-1}$. Concentration and thickness of " mulls " were not determined and it is not possible to compare intensities in the spectrum of one compound with another.

Microanalyses are by Drs. Strauss and Weiler, Oxford, by Mr. W. Brown, Imperial Chemical Industries Limited, Butterwick Research Laboratories, and by the Analytical Department, Imperial Chemical Industries Limited, Dyestuffs Division.

Crude Griseofulvin.—Initially, crude griseofulvin was supplied by Mr. P. J. Curtis and was obtained by chloroform extraction of the culture filtrates of *P. janczewskii* and *P. griseofulvum*. It contained a new mould metabolite having properties very similar to those of griseofulvin and was identified as the dechloro-analogue, dechlorogriseofulvin (MacMillan, *Chem. and Ind.*, 1951, 719).

Griseofulvin and dechlorogriseofulvin could be separated by chromatography on activated alumina or by fractional crystallisation but both methods were time-consuming and tedious. Accordingly, the large-scale production of griseofulvin from the mycelium of *P. griseofulvum* was undertaken by Dr. C. T. Calam, Imperial Chemical Industries Limited, Dyestuffs Division, to whom we are indebted. Crude griseofulvin from this source did not contain dechloro-

griseofulvin but contained another impurity, identified as mycelianamide (Oxford and Raistrick, *Biochem. J.*, 1948, **42**, 323) by direct comparison with an authentic sample kindly provided by Prof. H. Raistrick.

Griseofulvin.—Griseofulvin, obtained from the mycelium of *P. griseofulvum* Dierckx by the method of Oxford *et al.* (*loc. cit.*), and crystallised from ethanol, was purified further by chromatography on activated alumina. In a typical experiment, griseofulvin (43·5 g.) in benzene (4 l.) was filtered from *ca.* 1·5 g. of insoluble material and chromatographed on a column (21·5 × 2·5 cm.) of alumina of pH 4, activated at 130—140°. A dark impurity was strongly adsorbed at the top of the column. Griseofulvin was only weakly adsorbed and soon started to pass through with the eluant (A). The chromatogram was developed with benzene (300 ml.) containing 1% of ethanol and elution continued until a thin brown band reached the bottom of the column (eluant B). Recovery of the combined eluants A and B gave pure griseofulvin (34·8 g.), m. p. 218—219°, together with a less pure fraction (3·8 g.), m. p. 217—218°. Analytical specimens, further purified by crystallisation from ethanol or benzene, formed needles or prisms, m. p. 220—221°, $[\alpha]_D^{21}$ +337° (*c*, 1·00 in acetone) [Found : C, 58·0, 58·0; H, 5·1, 5·0; Cl, 9·9, 10·1; OMe, 25·8; *C*-Me (Kühn–Roth), 4·1%; *M* (Rast), 355. Calc. for $C_{17}H_{17}O_6Cl$: C, 57·9; H, 4·85; Cl, 10·1; 3OMe, 26·4; *C*-Me, 4·1%; *M*, 352·5].

The unit cell was tetragonal and had *a* 8·93, *c* 19·83 Å. The density, determined by flotation in sodium iodide solution, was 1·462. Hence the molecular weight per unit cell is 1394; *i.e.*, there are four molecules (Found : *M*, 348·5). We are indebted to Dr. A. F. Wells for this information.

On hydrogenation of griseofulvin (4·528 mg.) in the apparatus described by Clauson-Kaas and Limborg (*Acta Chem. Scand.*, 1947, **1**, 884) with a palladium–charcoal catalyst in acetic acid solution, hydrogen equivalent to 1·33 double bonds (0·42 ml. at 23°/749 mm.) was taken up in 10 minutes; this was followed by uptake of a further 0·17 ml. in 50 minutes. This slow uptake has been shown by experiments with model compounds to be characteristic of the reduction of a keto-group (cf. Part V, *loc. cit.*).

Derivatives. Griseofulvin (0·4 g.) and bromine (0·6 g.) were dissolved in chloroform. After 30 minutes the solvent and excess of bromine were removed. The residual *bromo*-derivative, crystallised from ethanol, had m. p. 255° (decomp.) [Found : C, 46·2; H, 3·5; Hal. (1Cl + 1Br), 27·5. $C_{17}H_{16}O_6ClBr$ requires C, 47·2; H, 3·7; Hal., 26·8%].

Griseofulvin in ethanol was treated with an excess of Brady's reagent (*J.*, 1951, 756). After 24 hours, the solution was warmed to 70° and cautiously diluted with water. On cooling, the *monodinitrophenylhydrazone* separated in red needles, m. p. 155—160° (decomp.) (Found : C, 51·6; H, 4·1; N, 10·7. $C_{23}H_{21}O_9N_4Cl$ requires C, 51·8; H, 4·0; N, 10·5%). It was insoluble in warm dilute hydrochloric acid.

Prepared by the method of Oxford *et al.* (*loc. cit.*) and air-dried, the mono-oxime melted at 120° with gas evolution, reset on further heating, and remelted sharply at 224°. A specimen, dried *in vacuo* at 100° for analysis, melted at 225° without previous melting or sintering (Found : C, 55·2; H, 5·0; N, 4·0; Cl, 9·1. Calc. for $C_{17}H_{18}O_6NCl$: C, 55·5; H, 4·9; N, 3·8; Cl, 9·5%).

Reaction with thiosemicarbazide. (*a*) Griseofulvin was recovered unchanged after being heated under reflux for 3 hours with thiosemicarbazide in ethanol.

(*b*) Griseofulvin (0·2 g.) was heated under reflux for 1 hour with Girard reagent P (0·7 g.) in acetic acid (2N in ethanol; 10 ml.), the solution was poured into ice and 2N-sodium hydroxide (10 ml.), and the whole rapidly adjusted to pH 6 (acidification of a portion, at this stage, with 6N-hydrochloric acid precipitated griseofulvin). To the filtered solution (85 ml.) were added thiosemicarbazide (0·8 g.) in 2N-hydrochloric acid (turbidity) and then a further 6 ml. of 2N-acid. The flocculent precipitate, collected after 15 minutes, had m. p. 196—198° (decomp.) with softening at 182°. Extraction with hot benzene (much semi-solid insoluble material) and cooling gave long colourless needles (70 mg.) of a *substance* which, recrystallised twice from benzene, had m. p. 190—200° (decomp.) after softening above 165° (Found : C, 55·45; H, 5·15; N, 8·4. $C_{18}H_{22}O_6N_3SCl,C_6H_6$ requires C, 55·2; H, 5·4; N, 8·1%).

Reaction with semicarbazide. Griseofulvin (500 mg.) in pyridine (3 ml.) was treated with semicarbazide hydrochloride (176 mg.) in water (1 ml.). Dilution of the yellow solution after 24 hours afforded a brown solid separable into two fractions. (*a*) A dioxan-soluble fraction crystallised in needles, decomp. 240—242°, from aqueous dioxan [Found : C, 52·1; H, 4·9; N, 9·6; OMe, 21·6. $C_{18}H_{20}O_6N_3Cl$ requires C, 52·75; H, 4·9; N, 10·3; 3OMe, 22·7%]. This (?) *pyrazoline* was soluble in dilute mineral acids, and gave a wine-red colour with ferric chloride in methanol, discharged on addition of water. The Knorr pyrazoline test was negative. Hydrolysis by dilute hydrochloric acid at 40° for 5 minutes gave griseofulvic acid. (*b*) A dioxan-

insoluble fraction, decomp. 200—240°, on crystallisation from dilute acetic acid, afforded griseofulvin, m. p. 216—218° (mixed m. p. and analysis).

Griseofulvamine.—A suspension of griseofulvin (0·48 g.) in methanol (50 ml.) was saturated with dry ammonia for 1½ hours (complete solution). After 48 hours, excess of ammonia and solvent were removed *in vacuo* and the resultant *amine* (0·11 g.) crystallised from aqueous methanol in colourless needles, m. p. 264° (decomp.) (Found: C, 54·1, 54·1; H, 4·8, 4·6; Cl, 9·6; OMe, 19·0; Loss of wt. at 150°, 3·9. $C_{16}H_{16}O_5NCl,H_2O$ requires C, 54·0; H, 5·0; N, 3·95; Cl, 10·0; 2OMe, 17·4; H_2O, 5·05%). Ultra-violet absorption in methanol: $\lambda_{max.}$ 288, ~235 mμ; log ε 4·65, 4·09. The amine was soluble in dilute mineral acids; its acid solution, on warming, deposited griseofulvic acid. The amine was insoluble in cold dilute alkali, but dissolved when heated, with evolution of ammonia. It did not react with Brady's reagent. With ferric chloride in methanol, it gave a wine-red colour, discharged on addition of water. It slowly reduced ammoniacal silver nitrate at 100°.

The neutral N-*acetyl* derivative, prepared by reaction with acetic anhydride in pyridine, crystallised from ethanol in colourless silky needles, m. p. 247—248° depressed on admixture with griseofulvamine (Found: C, 56·7; H, 4·6; N, 3·8. $C_{18}H_{18}O_6NCl$ requires C, 56·8; H, 4·7; N, 3·7%). It gave a red precipitate with Brady's reagent, but no colour with ferric chloride.

Hydrolysis of Griseofulvin.—(a) *With ethanolic* N-*hydrochloric acid.* The method described by Oxford *et al.* (*loc. cit.*) gave griseofulvic acid as colourless prisms, decomp. 255—258° (from acetic acid), $[\alpha]_D^{17}$ +406° (*c*, 0·176 as sodium salt in water), $[\alpha]_D^{22}$ +399° (*c*, 1·004 as sodium salt in aqueous methanol) [Found: C, 56·5; H, 4·5; Cl, 10·1; OMe, 17·9%; *M* (Rast), 340; equiv. (potentiometric), 358. Calc. for $C_{16}H_{15}O_6Cl$: C, 56·7; H, 4·5; Cl, 10·5; 2OMe, 18·3%; *M*, 338·5]. Ultra-violet absorption max.: 326, 291, 267, 236 mμ (log ε 3·72, 4·40, 4·30, 4·17) in methanol; ~332, 289, 235 mμ (log ε 3·78, 4·70, 4·13) in 0·1N-sodium hydroxide. The p*K*, from the potentiometric titration curve (Fig. 5*a*), was 4·50, whence $K = 3·2 \times 10^{-5}$ at 18°. Griseofulvic acid gave only a pale orange colour with ferric chloride in methanol, but coupled readily with diazotised *o*-nitraniline. The Millon and the Gibbs reaction and the hydroxamic acid test (Feigl, "Spot Tests," Elsevier, New York, 3rd edn., 1947, p. 355) were negative. Hot ammonical silver and yellow mercuric oxide, but not Fehling's solution, were slowly reduced by griseofulvic acid.

(b) *With* 6N-*sulphuric acid.* Griseofulvic acid was obtained in 70% yield when griseofulvin was heated under reflux in a stream of nitrogen for 24 hours with 6N-sulphuric acid in an equal volume of ethanol.

(c) *With aqueous* 0·5N-*sodium hydroxide.* (i) In two experiments the hydrolysis proceeded as described by Oxford *et al.*, yielding decarboxygriseofulvic acid and norgriseofulvic acid. The former crystallised from ethanol in needles, m. p. 138°, $[\alpha]_D^{21}$ −29° (*c*, 1·00 in acetone) [Found: C, 61·2, 61·1; H, 5·1, 5·1; Cl, 11·5, 12·2; OMe, 19·9%; *M* (Rast), 283. Calc. for $C_{15}H_{15}O_4Cl$: C, 61·1; H, 5·1; Cl, 12·0; 2OMe, 21·0%; *M*, 294·6], and its 2:4-*dinitrophenylhydrazone* was obtained as red needles (from nitrobenzene), m. p. 242—244° (decomp.) (Found: C, 53·5; H, 3·9; N, 11·7. $C_{21}H_{19}O_7N_4Cl$ requires C, 53·3; H, 4·0; N, 11·8%). Norgriseofulvic acid formed needles, $[\alpha]_D^{18}$ +298° (*c*, 1·00 in acetone), m. p. 262° (decomp.), from methanol [Found: C, 55·6; H, 4·3; Cl, 11·1; OMe, 9·9, 9·45%; *M* (Rast), 310; equiv. (potentiometric), 158. Calc. for $C_{15}H_{13}O_6Cl$: C, 55·5; H, 4·0; Cl, 10·9; 1OMe, 9·6%; *M*, 324·6; equiv., 162 (dibasic acid)].

(ii) In all other experiments the reaction proceeded as follows: Powdered griseofulvin (5·0 g.) was heated under reflux with 0·5N-sodium hydroxide (750 ml.) for 5 hours. After cooling, the alkaline solution was filtered from the neutral solid (1·1 g.) and acidified, giving norgriseofulvic acid (2·8 g.). The neutral product, recrystallised twice from methanol (charcoal), gave needles, m. p. 148—159°, $[\alpha]_D^{20}$ −13° (*c*, 1·0 in acetone). A mixture with decarboxygriseofulvic acid of m. p. 138° melted at 138—158°. The product, m. p. 148—159°, sublimed without change in m. p. and gave the same dinitrophenylhydrazone as decarboxygriseofulvic acid of m. p. 138°. It was also obtained when the hydrolysis was carried out in an atmosphere of nitrogen although the reaction was much cleaner than in the presence of air. The neutral product, m. p. 148—159° (1·168 g.), in benzene (50 ml.) was adsorbed on alumina (12·5 × 1 cm.) activated at 150°/14 mm. for 3 hours. Decomposition appeared to be rapid on the column in ultra-violet light and to some extent in daylight. Adsorption, development, and elution were therefore carried out as quickly as possible in the dark. The following bands (from bottom to top) were obtained on development with benzene. First, a broad colourless band (grey-green fluorescence in ultra-violet light), which by elution with benzene and crystallisation from methanol gave decarboxy-

griseofulvic acid (880 mg.), m. p. 137°. The methanolic mother-liquors, on recovery, gave needles (125 mg.), m. p. 142—160°, unchanged by repeated crystallisation from methanol. Secondly, a narrow yellow band, from which elution with benzene and recovery afforded pale yellow prisms (80 mg.); after recrystallisation from xylene, this *substance* had m. p. 204—206° (with softening above 190°) (Found: C, 61·1; H, 5·2; Cl, 11·5; OMe, 20·1. $C_{15}H_{15}O_4Cl$ requires C, 61·1; H, 5·1; Cl, 12·0; 2OMe, 21·0%). The 2 : 4-dinitrophenylhydrazone crystallised from nitrobenzene in red needles, decomp. 216—218°. Finally, an orange band which, eluted with benzene + 1% of ethanol, gave a red gum.

When decarboxygriseofulvic acid m. p. 134—137° (135 mg.), obtained as in (i), was adsorbed on alumina from a solution in benzene (20 ml.) as described above, the column consisted of one band only, namely the first. Elution and recovery yielded only decarboxygriseofulvic acid (120 mg.), colourless needles (from methanol), m. p. 136—137°.

iso*Griseofulvin*.—(*a*) Methylation of norgriseofulvic acid (90 mg.) with excess of ethereal diazomethane (Oxford *et al.*, *loc. cit.*) gave griseofulvin (40 mg.) and *iso*griseofulvin (30 mg.), long colourless needles (from ethanol), m. p. 200—201° (depressed below 180° on admixture with griseofulvin), $[\alpha]_D^{21}$ +215° (*c*, 1·3696 in acetone) (Found: C, 58·0; H, 5·0; Cl, 10·2; OMe, 25·6. Calc. for $C_{17}H_{17}O_6Cl$: C, 57·9; H, 4·85; Cl, 10·1; 3OMe, 26·4%).

(*b*) A suspension of griseofulvin (4 g.) in " AnalaR " methanol (270 ml.) was saturated with dry hydrogen chloride and then heated under reflux for 2 hours. Removal of most of the solvent *in vacuo* and pouring into ice-water afforded a precipitate which was collected and extracted with sodium carbonate solution. Acidification of the extract gave griseofulvic acid (1·3 g.). The dried neutral fraction (2·2 g.), crystallised from ethanol, gave *iso*griseofulvin (0·61 g.), m. p. 195—197°. The material recovered from the mother-liquor was chromatographed in benzene (40 ml.) on acid-washed alumina (pH 4, activated at 250° *in vacuo*). Gummy benzene-insoluble material was rejected. The column was eluted with ether containing 1% of methanol (fractions were collected in ultra-violet light). It yielded a narrow greyish band (evaporation gave a trace of oil), a purple band [evaporation gave *iso*griseofulvin (0·68 g.), m. p. 193—195°], and a violet-purple band [evaporation gave griseofulvin (0·33 g.), m. p. 210°].

Griseofulvic acid treated in the same way also gave *iso*griseofulvin and griseofulvin.

Contrary to the report by Oxford *et al.*, *iso*griseofulvin is much more soluble in most organic solvents than is griseofulvin. *iso*Griseofulvin did not yield carbonyl derivatives with Brady's reagent, *p*-nitrophenylhydrazine, hydroxylamine, or semicarbazide.

Hydrolysis of iso*Griseofulvin*.—*iso*Griseofulvin (0·5 g.) and N/8-sodium hydroxide (160 ml.) were heated to effect solution (30 minutes). Acidification afforded griseofulvic acid (0·41 g.), m. p. 256° (decomp.), $[\alpha]_D^{22}$ +388° (*c*, 0·4064 as sodium salt in aqueous methanol).

Hydrogenation of Griseofulvic Acid.—Griseofulvic acid (3·0 g.) in glacial acetic acid (600 ml.) was hydrogenated by shaking it with Adams's platinum oxide (250 mg.) at room temperature and pressure; uptake (3 mols.) was complete in 5 hours. The gum, obtained by evaporation of the filtered solution, was triturated with benzene (50 ml.), furnishing the *alcohol A* (27%), colourless needles (from aqueous methanol), m. p. 222—224°. It crystallised from chloroform and methanol with solvent of crystallisation, and had m. p. 120—140°, resetting and then melting 222—224°, $[\alpha]_D^{15}$ −37·5° (*c*, 1·00 in acetone) (Found: C, 56·2; H, 5·85; OMe, 17·0. $C_{16}H_{19}O_6Cl$ requires C, 56·1; H, 5·55; 2OMe, 18·0%).

After extraction with sodium carbonate and drying, the benzene mother-liquors were passed through a column of acid-washed (pH 4) alumina (16·5 × 1·5 cm.), activated at 240°/12 mm. for 3 hours. Development with benzene gave the following colourless bands, identified in ultra-violet light: (a) Upper broad band, fluorescing light blue; elution with benzene and recovery afforded *product C* (10%), colourless prisms (from aqueous methanol), m. p. 147—148° (Found: C, 61·8; H, 6·1; Cl, 11·8; OMe, 19·6. $C_{16}H_{19}O_4Cl$ requires C, 61·8; H, 6·1; Cl, 11·5; 2OMe, 20·0%). (b) Lower broad band, fluorescing dark blue; elution with benzene and recovery furnished the *alcohol B* (45%), colourless prisms (from aqueous methanol), m. p. 198—200° (Found: C, 58·6; H, 5·9; Cl, 11·0; OMe, 19·1. $C_{16}H_{19}O_5Cl$ requires C, 58·8; H, 5·8; Cl, 10·9; 2OMe, 19·0%). Further elution with benzene containing 1% ethanol afforded a lower broad band, fluorescing mauve, which, on elution with benzene–ethanol (99 : 1), gave a gum (200 mg.), and an upper broad band, with blue fluorescence, whence elution with the same solvent yielded A (3%), m. p. and mixed m. p. 222—224°.

Oxidation of Griseofulvic Acid Reduction Product B.—Product B (100 mg.) in acetic acid (2 ml.) was treated during 30 minutes with chromic oxide (100 mg.) in acetic acid (0·5 ml.) and water (0·2 ml.). After each addition, the mixture was heated on a water-bath until the oxidant had been consumed, and finally at 100° for 15 minutes, and then poured into water (10 ml.).

The precipitated *ketone* crystallised from ethyl acetate in platelets, m. p. 200—201°, depressed below 180° on admixture with starting material (Found: C, 59·1; H, 5·4; Cl, 10·5; OMe, 18·3. $C_{16}H_{17}O_5Cl$ requires C, 59·2; H, 5·2; Cl, 10·0; 2OMe, 19·0%). It was neutral to Universal indicator but slowly gave a yellow solution in dilute aqueous sodium hydroxide. It gave no colour with ferric chloride and did not react with Brady's reagent (see Part VI).

Potassium Hydroxide Fusion of Decarboxygriseofulvic Acid.—Decarboxygriseofulvic acid, m. p. 138° (80 mg.), was added to a melt of potassium hydroxide (0·5 g.) and water (0·1 ml.) at 250° and the bath-temperature raised to 280° (gas evolution and production of some tar). Acidification of an aqueous solution of the cooled melt afforded a brown precipitate which was taken up in ether and recovered. The resultant tar was sublimed at 80°/10^{-4} mm. Only a little colourless material sublimed, having m. p. 140° (decomp.) after softening at 80—90°. It did not contain halogen, was soluble in sodium hydrogen carbonate solution, and gave a brownish colour with ferric chloride. Orcinol readily sublimes under the above conditions. The tarry residue was intractable.

Pyrolysis of Griseofulvic Acid.—Griseofulvic acid, heated in a stream of nitrogen, readily evolved carbon dioxide (baryta) at the m. p. (250—260°). Extraction of the residue with chloroform and chromatography on alumina gave only amorphous products.

Similar results were obtained when griseofulvic acid was heated to its m. p. in " Dowtherm " or in synthetic quinoline.

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