762. *Griseofulvin. Part IV.* * *Structure.*
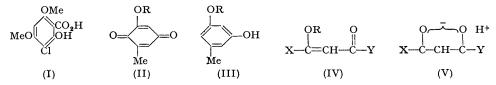
By John Frederick Grove, J. MacMillan, T. P. C. Mulholland, and
M. A. Thorold Rogers.
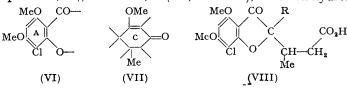
The structure 7-chloro-4 : 6-dimethoxycoumaran-3-one-2-*spiro*-1′-(2′-methoxy-6′-methyl*cyclo*hex-2′-en-4′-one) (IX; R = Me) accounts for the known physical and chemical properties of griseofulvin and for the degradation products described in Parts I—III (preceding papers).

FROM the oxidative degradations described in Part II (*J.*, 1952, 3958), it was concluded that griseofulvin possesses a benzenoid ring (A) and a hydroaromatic six-membered ring (C) thereby confirming the views of Oxford, Raistrick, and Simonart (*Biochem. J.*, 1939, **33**, 240). The nature and orientation of ring A follows from the formation of 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (I) from griseofulvin by oxidation with zinc permanganate in acetone—conditions which are considered to preclude rearrangement. The aromatic character of ring A is borne out by the lack of reactivity of the chlorine substituent towards aqueous hydrolytic reagents, and towards sodium alkoxides. Thus, griseofulvic acid was recovered unchanged by refluxing 2N-sodium methoxide and, while treatment with N/25-sodium ethoxide replaces a labile methoxyl group by ethoxyl, forming an acidic product, $C_{17}H_{17}O_6Cl$, the chlorine atom survives. Similarly, the two labile methoxyl groups in griseofulvin which are readily hydrolysed with aqueous alkali in the formation of norgriseofulvic acid (Oxford *et al., loc. cit.*) are exchanged for ethoxyl on treatment with N/25-sodium ethoxide; the chlorine atom is however retained in the product $C_{19}H_{21}O_6Cl$.
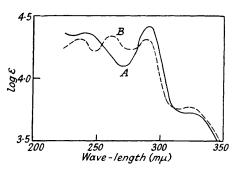

(I)   (II)   (III)   (IV)   (V)

The presence of a second six-membered ring (C) in griseofulvin is indicated by oxidation with chromic oxide to 3-methoxy-2 : 5-toluquinone (II; R = Me) (Part II) and by formation of orcinol (III; R = H) by potassium hydroxide fusion (Oxford *et al., loc. cit.*). Since the three methoxyl groups in griseofulvin appear in the oxidation products (I) and (II; R = Me), it is evident that griseofulvin is not the methyl ester of a carboxylic acid (contrast Oxford *et al., loc. cit.*; see also Part I). Furthermore, it has been established that the

* Part III, preceding paper.

methoxyl group in the quinone (II; R = Me) is derived from a methyl enol ether of the type (IV) (Part I; *J.*, 1952, 3949) as follows. Ethylation of griseofulvic acid with diazo-ethane or ethanolic hydrogen chloride affords two isomeric ethyl ethers $C_{18}H_{19}O_6Cl$, one of which, of m. p. 205—206°, is oxidised by chromic oxide to 3-ethoxy-2 : 5-toluquinone (II; R = Et), identical with a synthetic specimen. Both ethyl ethers give 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid and 7-chloro-2-hydroxy-4 : 6-dimethoxycoumaran-3-one-2-β-butyric acid on permanganate oxidation. These facts can only be explained by postulating the presence, in griseofulvin, of (IV; R = Me), which on hydrolysis affords the

(VI)   (VII)   (VIII)

tautomeric system (V) in griseofulvic acid; ethylation of (V) then affords two isomeric ethyl ethers. The second ethyl ether is dimorphous, forms of m. p. 192—193° and 167—168° having been isolated, and like *iso*griseofulvin, does not yield a quinone with chromic oxide. The two ethyl ethers are assigned the normal and the *iso*structure respectively, relative to griseofulvin and *iso*griseofulvin, from a consideration of their ultra-violet absorption spectra [Figure : the band at 262 mμ in the ether, m. p. 192—193°, corresponds

A, Ethyl ether, m. p. 205—206° ($\lambda_{max.}$ 328, 289, 239 mμ; log ε 3·73, 4·41, 4·36 *respectively*).

B, Ethyl ether, m. p. 192—193° ($\lambda_{max.}$ 325, 290, 262, 235 mμ; log ε 3·77, 4·31, 4·34, 4·31 *respectively*).
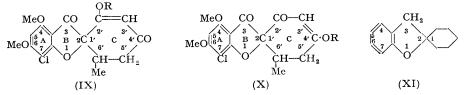
(*Both in methanol.*)

to a similar band in *iso*griseofulvin (Part I)] and because of their behaviour towards chromic oxide oxidation and the lack of carbonyl reactivity in the lower-melting ether which is paralleled in *iso*griseofulvin (Part I).

All the carbon atoms in griseofulvin are accounted for in the oxidation products, 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (I) and 3-methoxy-2 : 5-toluquinone (II ; R = Me). That none of them is common to both rings A and c has been demonstrated by cleavage of griseofulvin, in significant yield, into the acid (I) and orcinol monomethyl ether (III ; R = Me) by 2N-sodium methoxide (0·5N-sodium methoxide gave anomalous results described in the Experimental section). A similar fission, dealt with in detail in Part VI (*J.*, 1952, 3994), has been encountered in the alkaline hydrolysis of dihydrogriseofulvin which yields the salicylic acid (I) (derived from ring A) and *m*-cresol (from ring c). Moreover, formation of (I) under hydrolytic conditions provides convincing chemical proof that there is a carbonyl group directly attached to the benzenoid ring (A) as suggested by the spectroscopic evidence (Part I).

The hydroaromatic ring (c) must contain the *C*-methyl group and the olefinic double bond known to be present in griseofulvin (Part I). Moreover, if it is assumed that re-arrangement does not occur in the formation of orcinol (III ; R = H) and the monomethyl ether (III ; R = Me), two potential hydroxyl groups must be located in the 3 : 5-positions with respect to the *C*-methyl group. Finally, ring c must contain at least the methyl ether group of the system (IV ; R = Me). The most likely skeleton of ring c therefore appears to be (VII).

The manner in which the two partial structures (VI) and (VII) are linked in griseofulvin seems to us to be unequivocably determined by the formation of the acids $C_{14}H_{15}O_6Cl$

and $C_{14}H_{15}O_7Cl$, which were shown in Parts II and III to be (VIII; R = H and OH respectively). These acids, derived from griseofulvic acid by alkaline peroxide and by permanganate respectively, are substituted coumaran-3-ones in which the carbonyl and oxygen ether bridges from partial structure (VI) are linked to the same carbon atom, adjacent to a C-methyl group. Union of partial structures (VI) and (VII) in a like manner leads to the two spiran structures (IX) and (X) which are isomeric methyl ethers of the

(IX) (X) (XI)

tautomeric enol (XII) and are therefore considered to represent griseofulvin and *iso*griseofulvin although not necessarily respectively. It is considered that (IX; R = Me) is more likely to give rise to 3-methoxy-2 : 5-toluquinone (II; R = Me) on chromic oxide oxidation and therefore represents griseofulvin. *iso*Griseofulvin, on the other hand, does not yield a quinone with chromic oxide (Part II) and is therefore considered to be (X; R = Me) : the *o*-quinone which structure (X) might be expected to yield has not been isolated; it probably does not survive the oxidation. A conclusive decision that (IX; R = Me) and (X; R = Me) represent griseofulvin and *iso*griseofulvin respectively follows independently from hydrogenation studies (see Part VI).

The conclusion that griseofulvin is 7-chloro-4 : 6-dimethoxycoumaran-3-one-2-*spiro*-1′-(2′-methoxy-6′-methyl*cyclo*hex-2′-en-4′-one) (IX; R = Me) has already been briefly reported (Grove, Ismay, MacMillan, Mulholland, and Rogers, *Chem. and Ind.*, 1951, 219); *iso*griseofulvin is 7-chloro-4 : 6-dimethoxycoumaran-3-one-2-*spiro*-1′-(4′-methoxy-6′-methyl *cyclo*hex-3′-en-2′-one) (X; R = Me). There are difficulties inherent in the old nomenclature (Oxford *et al.*, *loc. cit.*) based on griseofulvic acid (which is not a carboxylic acid) which apply particularly to its ethers and reduction products; a nomenclature is required which will denote these derivatives unambiguously and will also be applicable to analogues (to be described later) throughout the griseofulvin chemistry. After consultation with the Editor, we propose the name grisan, for the ring system (XI). The new names corresponding to the old ones are given in the Table, but the trivial name griseofulvin will be retained. The neutral compound $C_{15}H_{15}O_4Cl$, a product of the alkaline hydrolysis of griseofulvin, is shown below to be a substituted tetrahydrodibenzfuran and not a derivative of grisan, and the trivial name decarboxygriseofulvic acid introduced for this compound by Oxford *et al.* will therefore be abandoned in future publications.
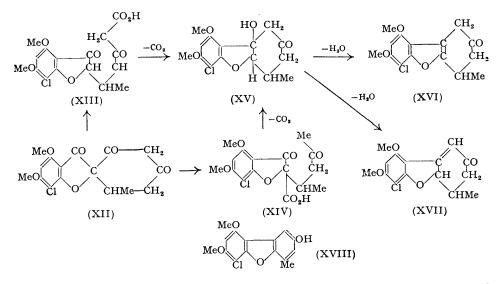
Old name	New name
Griseofulvin (IX; R = Me) ...	7-Chloro-4 : 6 : 2′-trimethoxy-6′-methylgris-2′-en-3 : 4′-dione.
Griseofulvic acid (XII)	7-Chloro-4 : 6-dimethoxy-2′-methylgrisan-3 : 4′ : 6′-trione.
*iso*Griseofulvin (X; R = Me)...	7-Chloro-4 : 6 : 4′-trimethoxy-6′-methylgris-3′-en-3 : 2′-dione.
Norgriseofulvic acid	7-Chloro-4(or 6)-hydroxy-6(or 4)-methoxy-2′-methylgrisan-3 : 4′ : 6′-trione.
Dihydrogriseofulvin	7-Chloro-4 : 6 : 6′-trimethoxy-2′-methylgrisan-3 : 4′-dione.
Tetrahydrodeoxygriseofulvin	7-Chloro-4 : 6 : 6′-trimethoxy-2′-methylgrisan-3-one.

7-Chloro-4 : 6-dimethoxy-2′-methylgrisan-3 : 4′ : 6′-trione (XII), of which (IX) and (X) are isomeric monoenol ethers, normally exists in the enolic form. As such it may be regarded as a derivative of dihydroresorcinol and the observed pK of 4·5 may be compared with the pK's of dimedone and dihydroresorcinol found by Schwarzenbach and Lutz (*Helv. Chim. Acta*, 1940, **23**, 1162) to fall close to 5·2. Strongly acidic enols are known to react readily with diazomethane with exclusive formation of the enol ether (Eistert, " Newer Methods of Preparative Organic Chemistry," Interscience Publ. Inc., New York, 1948, p. 528). Nevertheless, the method has been little used for the preparation of the enol ethers of cyclic 1 : 3-diketones. We find that the cyclic 1 : 3-diketones dimedone and dihydroresorcinol react smoothly with diazomethane, and the enol ethers obtained are readily hydrolysed (see also Euler and Hasselquist, *Rec. Trav. chim.*, 1950, **69**, 402).

Structure (IX; R = Me) satisfactorily accords with all the known physical and chemical properties of griseofulvin. It is consistent with the ultra-violet and infra-red spectroscopic evidence, and with the high optical rotation of griseofulvin and its hydrolysis products, which contain the αβ-unsaturated ketone system of high polarisability attached to the *spiro*-carbon atom. A second asymmetric carbon atom is also present (in the 6'-position in griseofulvin) and is responsible for the small negative rotations of decarboxygriseofulvic acid and of the C_{14} oxidation products, and finally appears in the periodate fission product (+)-methylsuccinic acid. The non-reactivity of the carbonyl group in ring B may readily be explained in terms of steric hindrance.
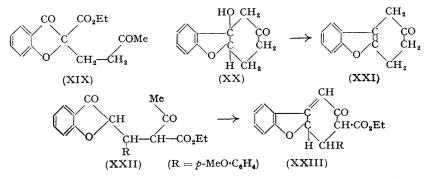
7-Chloro-4 : 6-dimethoxycoumaranone-2-β-butyric acid (VIII; R = H), obtained in the oxidation of griseofulvic acid with alkaline hydrogen peroxide presumably arises by decarboxylation of the intermediate dicarboxylic acid (VIII; R = CO_2H), the expected product of the oxidation by alkaline hydrogen peroxide or hypobromite of the dihydro-resorcinol ring C. 7-Chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid, one of the permanganate oxidation products of griseofulvin, undoubtedly results from a similar reaction sequence, set out in detail in Part II, in which the intermediate product (VIII; R = H) is oxidised at the activated methine group to the corresponding tertiary alcohol (VIII; R = OH); this must also occur in the alkaline hypobromite oxidation (cf. Beckmann, *Ber.*, 1950, **83**, 315).

The structure and mode of formation of decarboxygriseofulvic acid, one product of aqueous alkaline hydrolysis of griseofulvin, may be deduced as follows. Ultra-violet spectroscopy suggests that a gross molecular rearrangement is involved, and in particular that the partial structure (VI) is absent in the latter compound (Part I). Moreover, in Part II it was shown that decarboxygriseofulvic acid is almost certainly a partially hydrogenated dibenzofuran. It is therefore considered that decarboxygriseofulvic acid is formed by the reactions (XII ⟶ XIII ⟶ XV ⟶ XVI) or (XII ⟶ XIV ⟶ XV ⟶ XVI). The alternative intermediates (XIII) or (XIV) undergo internal Knoevenagel condensation

between the otherwise unreactive coumaranone carbonyl group and an activated methylene group, with simultaneous decarboxylation; liberation of carbon dioxide during the reaction has been demonstrated experimentally. The aldol intermediate (XV) may lose a molecule of water in two ways, to give the tetrahydrodibenzofuran (XVI) or (XVII). The ultra-violet spectrum of decarboxygriseofulvic acid (Part I) is consistent only with (XVI). On this basis the phenol $C_{15}H_{13}O_4Cl$ is (XVIII), and its formation from (XII) by yellow mercuric oxide in alkali (Part II) is simply a dehydrogenation of the intermediate substituted tetrahydrodibenzofuran. "*iso*Decarboxygriseofulvic acid," formed in small

yield with decarboxygriseofulvic acid (Part I), is considered to be the alternative dehydration product (XVII); this is confirmed by the ultra-violet absorption curve ($\lambda_{max.}$ 342 mμ; log ε 4·38) which is typical of a compound containing an $\alpha\beta$-unsaturated keto-group conjugated with an aromatic ring, and by the infra-red spectrum which shows the carbonyl group to be conjugated (strong bands at 1670 and 1610 cm.$^{-1}$) [the keto-grouping in (XVI) absorbs at 1710 cm.$^{-1}$ (Part I)]. It may be recalled that (XVII) is the structure proposed for decarboxygriseofulvic acid by Oxford *et al.* (*loc. cit.*). It is formed in small yield when decarboxygriseofulvic acid is heated with methanolic sodium hydroxide in an atmosphere of nitrogen.

(XIX) (XX) (XXI)

(XXII) (R = p-MeO·C$_6$H$_4$) (XXIII)

Considerable support for the postulated rearrangement of (XII) to the substituted tetrahydrodibenzofurans (XVI) and (XVII) comes from Henecka's observation (*Ber.*, 1949, **81**, 197) that ethyl 2-3'-ketobutyl-3-ketocoumaran-2-carboxylate (XIX) affords the aldol condensation product (XX) with dilute mineral acid and the tetrahydrodibenzofuran (XXI) with aqueous sodium hydroxide, and from the report by Panse, Shah, and Wheeler (*J. Indian Chem. Soc.*, 1941, **18**, 453) that (XXII) under the influence of aqueous alkali affords the dibenzofuran derivative (XXIII) although the $\alpha\beta$-unsaturated ketone nature of this product was not proved.
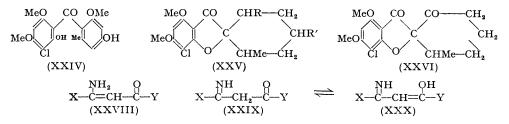
Grove and McGowan's observation (*Nature*, 1947, **160**, 574) that (XVI) showed basic properties, forming a complex with ferric chloride has been re-investigated. It has been found that the complex is not a simple ferrichloride and analytical data suggest that it is a complex of the type [BH]$^+_2$ FeCl$_4^{--}$. It is not hygroscopic but is decomposed by warm water with the formation of (XVII) in high yield. (XVII) does not reform the complex on treatment with ferric chloride in glacial acetic acid and the detailed mechanism involved in the overall rearrangement (XVI $\longrightarrow$ XVII) is not understood.

The extreme lability of one of the aromatic ether groups in griseofulvin towards aqueous 0·5N-sodium hydroxide is surprising but finds an analogy in the hydrolysis of alizarin 1-methyl ether with barium hydroxide (Perkin, *J.*, 1907, **91**, 2069). In view of the well-known reactivity of a methoxyl group in the *ortho*-position to a carbonyl group, it is probably the 4- rather than the 6-methoxyl group which is hydrolysed.
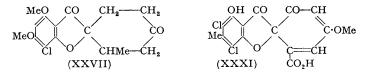
The behaviour of griseofulvin and 7-chloro-4 : 6-dimethoxy-2'-methylgrisan-3 : 4' : 6'-trione towards sodium alkoxides, described above, substantiates this lability of the ether linkages and the compounds C$_{17}$H$_{17}$O$_6$Cl and C$_{19}$H$_{21}$O$_6$Cl are 7-chloro-4(or 6)-ethoxy-6(or 4)-methoxy-2'-methylgrisan-3 : 4' : 6'-trione and 7-chloro-4(or 6) : 2'-diethoxy-6(or 4)-methoxygris-2'-en-3 : 4'-dione, respectively.

" Decarboxynorgriseofulvic acid " has not been obtained from the alkaline hydrolysis of griseofulvin. The compound (XVI) does not contain the ester vinylogue structure present in the 4-methoxycoumaranone portion of griseofulvin; that the rather unusual hydrolysis of the aryl ether group should fail to proceed once the rearrangement to the tetrahydro-dibenzofuran structure has taken place is therefore less surprising than that 7-chloro-4(or 6)-hydroxy-6(or 4)-methoxy-2'-methylgrisan-3 : 4' : 6'-trione should apparently resist rearrangement. It is conceivable that the structure is stabilised by chelation from a hydroxy-substituent in the 4-position; alternatively, it is possible that the break-down products have merely evaded isolation.

Griseofulvin (IX; R = Me) may be regarded as the vinylogue of a 1 : 3-diketone and therefore susceptible to fission under alkaline conditions. In the cleavage of griseofulvin with 2N-sodium methoxide, fission of the $C_{(2)}$–$C_{(3)}$ bond in the coumaranone ring must occur before, or at the same time as, hydrolysis of the ether linkage, otherwise formation of the benzophenone (XXIV) might be expected and no evidence of this was obtained. It is not clear why the sodium salt of the salicylic acid (I) was isolated in this reaction and not the methyl ester, although rigorous exclusion of water was not attempted.

Aqueous alkaline hydrolysis does not yield the salicylic acid (I) by fission of the 1 : 3-diketone vinylogue structure because after hydrolysis of the enolic ether system (IV; R = Me) the product (XII) is a 1 : 3 : 5-triketone and fission takes place more easily elsewhere.

(XXIV)   (XXV)   (XXVI)

(XXVIII)   (XXIX)   (XXX)

Products A and C of the catalytic reduction of (XII) (Part I) are 7-chloro-4' : 6'-dihydroxy-4 : 6-dimethoxy-2'-methylgrisan-3-one (XXV; R = R' = OH) and 7-chloro-4 : 6-dimethoxy-2'-methylgrisan-3-one (XXV; R = R' = H) respectively, while product B is considered to be (XXV; R = OH, R' = H) rather than (XXV; R = H, R' = OH) since the ketone obtained by chromic oxide oxidation is shown in Part VI to be the 1 : 3-diketone (XXVI) and not (XXVII). The reduction of 1 : 3-diketones to the corresponding dihydric and monohydric secondary alcohols and finally to the saturated hydrocarbon has many analogies in the literature.

(XXVII)   (XXXI)

The reaction of griseofulvin with methanolic ammonia (Part I) is typical of αβ-unsaturated β-alkoxy-ketones and 1 : 3-diketones (see Cromwell, *Chem. Reviews*, 1946, **38**, 83). The basic product, $C_{16}H_{16}O_5NCl,H_2O$, may possess either the amino- (XXVIII) or the imino-structure (XXIX ⇌ XXX), both of which have been proposed for the amino-derivatives of open-chain 1 : 3-diketones (Combes and Combes, *Bull. Soc. chim.*, 1892, **7**, 778; Rugheimer, *Ber.*, 1916, **47**, 2759). However, the wine-red ferric chloride colour given by the griseofulvin derivative and the amino-derivatives of several *cyclo*hexane-1 : 3-diones, prepared for comparison, is not readily accounted for by incorporating either (XXVIII), (XXIX), or (XXX) into the more rigid *cyclo*hexane ring. Moreover, the infra-red absorption spectra of the cyclic 1 : 3-diketone derivatives are difficult to interpret (cf. Cromwell *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 3337). This aspect is at present being investigated in these laboratories.

In conclusion, it is interesting that the spiran structures (IX and X; R = Me) proposed for griseofulvin and *iso*griseofulvin respectively, closely resemble one of the structures (XXXI) suggested for the chlorine-containing mould product, erdin, by Calam, Clutterbuck, Oxford, and Raistrick (*Biochem. J.*, 1947, **41**, 458). However, the properties of griseofulvin and *iso*griseofulvin on the one hand and erdin on the other are dissimilar. Catalytic reduction of erdin splits the oxygen bridge with the formation of a substituted benzophenone, while 80% sulphuric acid (as in the case of many hydroxybenzophenones) splits the carbonyl link. The ether linkage in the coumaranone ring of griseofulvin is stable to catalytic reduction. Treatment of griseofulvin with 80% sulphuric acid gives, first,

7-chloro-4 : 6-dimethoxy-2′-methylgrisan-3 : 4′ : 6′-trione and then at higher temperatures 2 mols. of carbon dioxide are evolved with the production of much tar.

Structure (IX; R = Me) possesses two features which are rather uncommon in a natural product, namely, the aromatic chlorine substituent and the spiran structure. Since the isolation by Raistrick and co-workers of griseofulvin, geodin, erdin, and caldariomycin (Clutterbuck, Mukhopadhyay, Oxford, and Raistrick, *Biochem. J.*, 1940, **34**, 664) a number of chlorine-containing mould metabolic products have been described including sclerotiorine (Curtin and Reilly, *Biochem. J.*, 1940, **34**, 1419), a series of compounds from *Aspergillus ustus* (Hogeboom and Craig, *J. Biol. Chem.*, 1946, **162**, 363; Doering, Dubos, Noyce, and Dreyfus, *J. Amer. Chem. Soc.*, 1946, **68**, 725), and the antibiotics chloramphenicol and aureomycin (Broschard *et al.*, *Science*, 1949, **109**, 199), the latter giving 5-chlorosalicylic acid on degradation (Kühn and Dury, *Ber.*, 1951, **84**, 563). Naturally occurring spirans are somewhat rarer, but the steroidal sapogenins diosgenin and sarsasapogenin and the alkaloid gelsemine (Gibson and Robinson, *Chem. and Ind.*, 1951, 93) may be mentioned. 4 : 6 : 2′-Trimethoxy-6′-methylgris-2′-en-3 : 4′-dione, the dechloro-analogue of griseofulvin, produces the same type of biological response in *Botrytis allii* though at higher concentrations (MacMillan, *Chem. and Ind.*, 1951, 719). The importance of the spiro-structure in conferring " curling " activity on the molecule is under investigation.

EXPERIMENTAL

M. p.s are corrected. Microanalyses are by Drs. Strauss and Weiler, Oxford (who also obtained some of the ultra-violet absorption spectra) and by Mr. W. Brown. Infra-red spectra were obtained as described in Part I.

Ethylation of 7-Chloro-4 : 6-dimethoxy-2′-methylgrisan-3 : 4′ : 6′-trione (Griseofulvic Acid).— (a) *With ethanolic hydrogen chloride.* A suspension of griseofulvin (2·5 g.) in absolute ethanol (200 ml.) was saturated with dry hydrogen chloride and then heated under reflux for 2 hours. After removal of most of the solvent *in vacuo*, the residue was poured into ice-water. The precipitate was collected, washed with sodium carbonate and water, dried, and crystallised from alcohol, giving a solid (0·4 g.), m. p. 164—166°. The residue obtained from the mother-liquor on evaporation was extracted with hot benzene (60 ml.). The benzene solution was separated from insoluble material and chromatographed on acid-washed alumina (20 × 2·5 cm.). Elution with ether +1% of methanol gave the following bands (identified in ultra-violet light) : (i) violet, which gave a colourless solid (30 mg.), m. p. 182—185°, raised by crystallisation from methanol to 197—205°; (ii) violet, not completely separated from (i); and (iii) blue-violet, giving an oil which yielded a solid (0·07 g.), m. p. 202—203°. Fraction (ii) yielded a colourless solid (0·6 g.), m. p. 166°, and a colourless solid which after crystallisation from ethanol had m. p. 147—180° and was rechromatographed giving two further fractions (0·15 g.), m. p. 163—165° and (0·06 g.), m. p. 200°. The combined fractions of m. p. 163—166° crystallised from ethanol in colourless prisms or short needles, m. p. 167—168°, of *7-chloro-4′-ethoxy-4 : 6-dimethoxy-6′-methylgris-3′-en-3 : 2′-dione* (X; R = Et). When seeded with the higher-melting form [see (b) below], it crystallised in long needles, m. p. 192—193°, $[\alpha]_D^{20}$ +214° (c, 0·98 in acetone) (Found : C, 58·8; H, 5·0; Cl, 9·8. $C_{18}H_{19}O_6Cl$ requires C, 58·8; H, 5·2; Cl, 9·7%).

The fractions of m. p. 200—203°, *7-chloro-2′-ethoxy-4 : 6-dimethoxy-6′-methylgris-2′-en-3 : 4′-dione* (IX; R = Et), crystallised from ethanol as colourless needles or prisms, m. p. 205—206°, $[\alpha]_D^{20}$ +316° (c, 0·98 in acetone) (Found : C, 59·0; H, 5·2; Cl, 10·1%). It was less soluble in ethanol than its isomer.

(b) *With diazoethane.* 7-Chloro-4 : 6-dimethoxy-2′-methylgrisan-3 : 4′ : 6′-trione (3·0 g.), suspended in ether, was treated with excess of ethereal diazoethane (Adamson and Kenner, *J.*, 1937, 1551). After 24 hours at 0°, unchanged material (1·15 g.) was removed by filtration. The gum obtained on evaporation was chromatographed in benzene (20 ml.) on acid-washed alumina (20·0 × 2·5 cm.). Elution was followed in ultra-violet light and gave two violet bands. Elution of the first with ether +0·5% of methanol gave 7-chloro-4′-ethoxy-4 : 6-dimethoxy-6′-methylgris-3′-en-3 : 2′-dione (X; R = Et) (0·73 g.), long blunt-ended needles (from ethanol), m. p. 192°. A mixed m. p. with the form, m. p. 168°, obtained as in (a) above, melted sharply at 192°. Elution of the second with ether +2% of methanol gave the isomer (IX; R = Et) (0·54 g.), m. p. and mixed m. p. with a specimen obtained as in (a), 203°.

(c) *With ethanol.* 7-Chloro-4 : 6-dimethoxy-2′-methylgrisan-3 : 4′ : 6′-trione (102 mg.), heated under reflux for 6 hours with absolute ethanol (10 ml.), gave unchanged starting material

(50 mg.) and a little 7-chloro-4′-ethoxy-4 : 6-dimethoxy-6′-methylgris-3′-en-3 : 2′-dione (X; R = Et), m. p. 188°. None of the 2′-ethoxy-isomer (IX; R = Et) was isolated in this experiment.

Oxidation of 7-Chloro-2′-ethoxy-4 : 6-dimethoxy-6′-methylgris-2′-en-3 : 4′-dione (IX ; R = Et).
(a) *With chromic oxide.* A warm solution of (IX; R = Et) (366 mg.) in acetic acid (8 ml.) was treated in portions with chromic oxide (1·0 g.) in acetic acid (7 ml.) and water (2·2 ml.). After the vigorous reaction had moderated, the mixture was heated under reflux for 20 minutes, cooled, diluted with water (25 ml.), and extracted with benzene. The brown oil, obtained on recovery, was sublimed *in vacuo* at room temperature, giving a yellow solid with a trace of gum. After being pressed on a porous plate, it crystallised from ether–light petroleum in long yellow needles, m. p. and mixed m. p. with a synthetic specimen of 3-ethoxy-2 : 5-toluquinone, 54—56°. Sublimation at 45° gave a little more of the quinone (total, 5 mg.), greatly contaminated with brown oil.

Trituration of the non-volatile residue with ether gave starting material, m. p. and mixed m. p. 203°.

(b) *With potassium permanganate.* Treatment of (IX; R = Et) (150 mg.) in acetone (50 ml.) with powdered potassium permanganate (0·6 g.) as described by Oxford *et al.* (*loc. cit.*) for the permanganate oxidation of griseofulvin gave 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (31 mg.), m. p. and mixed m. p. 220—222°, together with 7-chloro-2-hydroxy-4 : 6-dimethoxy-coumaranone-2-β-butyric acid (8 mg.), m. p. 186—189° (decomp.), identified by mixed m. p. and infra-red spectra.

Oxidation of 7-Chloro-4′-ethoxy-4 : 6-dimethoxy-6′-methylgris-3′-en-3 : 2′-dione (X ; R = Et).—
(a) *With chromic oxide.* A warm solution of (X; R = Et) (379 mg., 1 millimol.) in acetic acid (8 ml.) was treated in portions with chromic acid (1·05 g.) in acetic acid (7 ml.) and water (2·2 ml.), as described above. Working up in the same manner gave a colourless sublimate melting indefinitely at *ca.* 130° and a little unchanged starting material, m. p. and mixed m. p. 189°. Further extraction of the diluted reaction mixture, continuously with benzene for 3 days, gave a dark gum (60 mg.) which was sublimed *in vacuo* at 50°. Some brown oil was obtained which very slowly crystallised in ether. The crystals consisted mainly of small colourless needles of starting material but included one diamond-shaped, yellow crystal, m. p. 117—119°. No 3-ethoxy-2 : 5-toluquinone was obtained.

(b) *With potassium permanganate.* Treatment of (X; R = Et) (230 mg.) in acetone (60 ml.) with powdered potassium permanganate (0·95 g.) as above gave 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (37 mg.), m. p. and mixed m. p. 220—222°, and 7-chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid (2 mg.), m. p. 172—175°, identified by mixed m. p. and comparison of the infra-red spectra.

Iron Complex formed by 8-Chloro-1 : 2 : 3 : 4-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyl-dibenzofuran (XVI).—8-Chloro-1 : 2 : 3 : 4-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyldibenzo-furan (XVI) (10 mg.) was dissolved in glacial acetic acid (5 ml.), and anhydrous ferric chloride (30 mg.) in concentrated hydrochloric acid (0·5 ml.) added. After a few minutes the *complex* crystallised and was filtered, washed with a little glacial acetic acid, and dried *in vacuo* at room temperature for 1 week over phosphoric oxide and potassium hydroxide [Found : C, 45·7; H, 4·1; Fe, 7·3; Cl (total), 27·9; Cl (ionic), 17·7. $(C_{15}H_{15}O_4Cl)_2,FeCl_2,2HCl$ requires C, 45·6; H, 4·1; Fe, 7·1; Cl (total), 27·0; Cl (ionic), 18·0%]. The complex sintered at 160° but did not melt at 300°. It was readily soluble in glacial acetic acid. It was decomposed by warm water with the production of a colourless solid (see below) ; the aqueous mother-liquor contained both Fe^{+++} and Fe^{++}.

8-Chloro-1 : 2 : 3 : 9-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyldibenzofuran (XVII).—The iron complex from the tetrahydrodibenzfuran (XVI) (90 mg.) was decomposed by hot water, and the crude product (72 mg.), m. p. 152—170°, in benzene (5 ml.) was chromatographed on alumina (pH 4; Grade II). Elution with benzene was followed in ultra-violet light and gave (i) a lower greyish-green fluorescent band which gave starting material (XVI) (20 mg.), m. p. and mixed m. p. 136°, and (ii) a yellow fluorescent band which yielded a colourless solid (45 mg.). After crystallisation from benzene the latter formed *8-chloro-1 : 2 : 3 : 9-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyldibenzofuran* as prisms, m. p. 209—210°, undepressed on admixture with the " *iso*decarboxygriseofulvic acid " obtained in the alkaline hydrolysis of griseofulvin in Part I, and depressed below 180° by the phenol (XVIII) [Found : C, 61·3; H, 5·2; Cl, 12·7%; *M* (Rast), 277. $C_{15}H_{15}O_4Cl$ requires C, 61·1; H, 5·1; Cl, 12·0%; *M*, 295). Ultra-violet absorption max. in methanol : 342, 250 mμ (log ε 4·38, 4·00). It did not re-form the complex on treatment with ferric chloride and gave an orange colour, unchanged at 100°, with concentrated sulphuric acid.

Hydrolysis of Griseofulvin with Barium Hydroxide. Griseofulvin (1·0 g.) and saturated barium hydroxide solution (100 ml.) were heated under reflux for 5 hours (soda-lime guard tube). The crystals gradually disappeared, and a microcrystalline precipitate was formed. This precipitate was rapidly filtered, washed, and extracted with ethanol in a Soxhlet apparatus. The residue was identified as barium carbonate. The extract, on evaporation, gave 8-chloro-1 : 2 : 3 : 4-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyldibenzofuran (XVI) (0·12 g.), long needles (from 50% methanol), m. p. 135·5—137°.

Hydrolysis of 8-Chloro-1 : 2 : 3 : 4-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyldibenzofuran.— The ketone (XVI), m. p. 137° (22 mg.), and boiled N-sodium hydroxide (0·4 ml.) were heated under reflux for 3 hours in nitrogen with enough methanol to yield a homogeneous solution. The solid (15 mg.), m. p. 142—158°, which separated at 0°, was chromatographed in benzene on alumina (pH 4 ; Grade II) and eluted with benzene. The lower band, fluorescing greenish-grey (ultra-violet), yielded unchanged starting material, m. p. 137° (7 mg.). The upper band, fluorescing yellow, yielded a colourless solid, m. p. 209—210° (0·6 mg.) not depressed by admixture with 8-chloro-1 : 2 : 3 : 9-tetrahydro-3-keto-5 : 7-dimethoxy-1-methyldibenzofuran (XVII).

Orcinol Monoethyl Ether.—This was prepared from orcinol (72 g.), ethyl sulphate, and alkali as described by Henrich and Nachtigall for the monomethyl ether (*Ber.*, 1903, **36**, 889). The alkali-insoluble fraction was distilled at 95—110°/4 × 10⁻² mm., giving orcinol diethyl ether (11·5 g.). The alkali-soluble product was distilled at 110—120°/10⁻² mm., giving a yellow oil (46 g.) which was absorbed on alumina (pH 4 ; Grade II) (21·0 × 3 cm.) from its ether solution (450 ml.). Elution with ether, followed in ultra-violet light, gave a narrow yellow band not completely separated from a pale blue fluorescent band. These were collected together (eluate I) and an upper, blue fluorescent band collected separately (eluate II). Eluate I gave a yellow oil which was redistilled at 117—124°/5 × 10⁻² mm., giving a viscous yellow oil (40 g.) which partly crystallised on long storage. A specimen, pressed on a porous tile, crystallised from light petroleum (b. p. 40—60°) in long colourless needles, m. p. 47°. (Found : C, 71·0 ; H, 7·85. Calc. for $C_9H_{12}O_2$: C, 71·0 ; H, 7·9%). Orcinol monoethyl ether is described as an oil, b. p. 265—270°, by Henrich (*Monatsh.*, 1901, **22**, 251). The oily, partly crystalline distillate was used directly in the nitration (see below).

Eluate II gave unchanged orcinol (3·0 g.), m. p. and mixed m. p. 103—106°.

Nitration of Orcinol Monoethyl Ether.—The monoethyl ether (20 g.) in ether (600 ml.) was stirred mechanically and treated dropwise at −4° with nitric acid (10 g.; *d* 1·5). The mixture was then stirred for 1 hour, heated under reflux for 40 minutes, cooled, and washed with water. Recovery of the ethereal layer yielded an oil which was steam-distilled. The volatile fraction, recovered in ether, was absorbed on alumina (pH 4 ; Grade II) (16 × 2·0 cm.) from its benzene solution (100 ml.) and elution with benzene continued until a dark-brown band reached the bottom of the column. Evaporation of the eluate gave an oil which after several crystallisations from light petroleum (b. p. 40—60°) gave 5-ethoxy-3-hydroxy-2-nitrotoluene in yellow needles (1·3 g.), m. p. 52—53° (Found : C, 54·55 ; H, 5·6 ; N, 7·1. Calc. for $C_9H_{11}O_4N$: C, 54·8 ; H, 5·6 ; N, 7·1%). Weselsky and Benedikt (*Monatsh.*, 1881, **2**, 370) give m. p. 54°.

The non-volatile residue was extracted with hot benzene and the cooled extract chromatographed on alumina (28 × 3·0 cm.) (pH 4 ; grade II). Elution with benzene gave the following fractions : (i) Dark yellow-red eluate. Recovery gave a dark oil which after repeated crystallisation from benzene gave 3-ethoxy-5-hydroxy-2-nitrotoluene in yellow needles (1·1 g.), m. p. 114—115° (Found : C, 55·0 ; H, 5·6 ; N, 7·1%). It gave no colour with ferric chloride or Gibbs' reagent. Weselsky and Benedikt (*loc. cit.*) give m. p. 103° ; the lower value may be due to contamination of their product with a second non-steam-volatile product, described below. (ii) Clear yellow eluate. Crystallisation of the recovered gum from benzene gave yellow needles (0·3 g.), m. p. 118—119°, depressed on admixture with 3-ethoxy-5-hydroxy-2-nitrotoluene (Found : C, 49·8 ; H, 4·05 ; N, 7·1. Calc. for $C_7H_7O_4N$: C, 49·7 ; H, 4·2 ; N, 8·3%). Analyses and properties suggested this product was 2-nitro-orcinol, described by Henrich and Meyer (*Ber.*, 1903, **36**, 886) as a non-steam-volatile compound, m. p. 122°.

3-Ethoxy-2 : 5-toluquinone.—3-Ethoxy-5-hydroxy-2-nitrotoluene (0·2 g.) in ethanol (20 ml.) was reduced with Raney nickel at room temperature and pressure in the usual way. On the addition of 3N-hydrochloric acid (5 ml.) to the crude product, an almost colourless solid separated but was not isolated. Sulphuric acid (1·5 g.) in water (10 ml.) was added and the mixture treated dropwise with a solution of sodium dichromate (0·6 g.) in water (8 ml.) at 0°. After 12 hours at 0°, a trace of tar was filtered off. Extraction of the filtrate and recovery gave a dark oil which solidified in light petroleum at 0° (50 mg.). Several crystallisations from light

11 Y

petroleum (b. p. 40—60°) afforded 3-*ethoxy*-2 : 5-*toluquinone*, yellow needles, m. p. 56—58°, which did not depress the m. p. of the quinone of m. p. 54—56° obtained by oxidation of 7-chloro-2′-ethoxy-4 : 6-dimethoxy-6′-methylgris-2′-en-3 : 4′-dione (IX; R = Et) (Found : C, 64·95; H, 5·9. $C_9H_{10}O_3$ requires : C, 65·0; H, 6·1%). 3-Ethoxy-2 : 5-toluquinone gave a green colour with concentrated sulphuric acid. It gradually discoloured.

Hydrolysis of Griseofulvin with 80% *Sulphuric Acid.*—Griseofulvin (500 mg.) in concentrated sulphuric acid (10 ml.) and water (5 ml.) was heated at 150—160° in a stream of carbon dioxide-free nitrogen until evolution of carbon dioxide ceased (40 minutes). The evolved carbon dioxide, trapped in standard baryta, corresponded to 2 mols. per mol. of griseofulvin. The cooled reaction mixture, diluted with water (10 ml.), gave a black solid (330 mg.) which could not be purified.

Alcoholysis of 7-*Chloro*-4 : 6-*dimethoxy*-2′-*methylgrisan*-3 : 4′ : 6′-*trione*.—(a) *Sodium methoxide.* The trione (0·5 g.) was recovered unchanged after 2 hours' heating under reflux with sodium (2·3 g.) in methanol (50 ml.). (b) *Sodium ethoxide.* The trione (0·5 g.) was heated under reflux for 5 hours with sodium (0·55 g.) in absolute ethanol (50 ml.). Dilution of the cooled solution with water, removal of the alcohol *in vacuo*, and acidification gave 7-*chloro*-4(or 6)-*ethoxy*-6(or 4)-*methoxy*-2′-*methylgrisan*-3 : 4′ : 6′-*trione* (0·46 g.), m. p. 214—215° (decomp.) (softening at 206°); it crystallised from methanol in colourless prisms, m. p. (dependent on the rate of heating) 234—236° (decomp.) [Found, on a sample dried at 110° over P_2O_5 : C, 57·8, 58·4; H, 4·95, 4·9; Cl, 9·95; (OMe)(OEt), 21·1. $C_{17}H_{17}O_6Cl$ requires C, 57·85; H, 4·85; Cl, 10·06; (OMe)(OEt), 21·6%]. Ultra-violet absorption max. in methanol : ~330, 289 mμ (log ε 3·81, 4·67).

Action of Sodium Ethoxide on Griseofulvin.—(a) *With ca.* N/25-*sodium ethoxide.* Griseofulvin (1·0 g.) was heated under reflux for 5 hours with sodium (0·4 g.) in absolute ethanol (400 ml.). Dilution with water, acidification, and removal of the ethanol *in vacuo* gave a solid which was adsorbed on acid-washed alumina (activated at 250°) from its benzene solution. The band fluorescing violet in ultra-violet light was eluted with benzene + 1% of methanol. Recovery gave 7-*chloro*-4 : 2′(or 6 : 2′)-*diethoxy*-6(or 4)-*methoxy*-6′-*methylgris*-2′-en-3 : 4′-*dione* which crystallised from methanol and had m. p. 198—203·5°. It was dried at 110° for 1½ hours over phosphoric oxide [Found : C, 59·45; H, 5·3; Cl, 9·02; (OMe)(OEt)₂, 31·7. $C_{19}H_{21}O_6Cl$ requires C, 59·9; H, 5·5; Cl, 9·35; (OMe)(OEt)₂, 31·8%]. Ultra-violet absorption max. in methanol : 325, 291, ~254, 237 mμ (log ε 3·82, 4·39, 4·20, 4·42).

(b) *With ca.* 0·5N-*sodium ethoxide.* Griseofulvin (2·0 g.) was heated under reflux for 5 hours with sodium (2·28 g.) in absolute ethanol (200 ml.). Dilution with water (200 ml.) and acidification gave a sticky precipitate which was recovered in ether, giving some gummy crystals (0·23 g.). Removal of the gum by washing with methanol followed by recrystallisation from aqueous methanol gave an impure solid, m. p. 212—214° (decomp.) which resisted further purification. The X-ray powder pattern showed it to contain 7-chloro-4 (or 6)-ethoxy-6 (or 4)-methoxy-2′-methylgrisan-3 : 4′ : 6′-trione and was consistent with the presence of some 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid which would account for its violet ferric chloride colour reaction.

The gum, obtained by evaporation of the methanol solution, was dissolved in chloroform and extracted with potassium hydrogen carbonate solution. Acidification gave a resinous solid (1·2 g.), m. p. 125—145° (decomp.), which gave a violet colour with ferric chloride but resisted all attempts at purification.

The reaction was much cleaner when conducted in nitrogen although the only compound isolated was 7-chloro-4(or 6)-ethoxy-6(or 4)-methoxy-2′-methylgrisan-3 : 4′ : 6′-trione, identified by the X-ray powder pattern.

Action of 2N-*Sodium Methoxide on Griseofulvin.*—Griseofulvin (0·20 g.) and 2N-sodium methoxide in methanol (20 ml.) were heated under reflux for 5 hours (separation of crystalline material). The cooled mixture was poured into water, and the clear aqueous solution was acidified, precipitating 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (100 mg.) as a white crystalline solid, m. p. 207—208°, raised to 212° (decomp.) by crystallisation from ethyl acetate, and identical with an authentic specimen. Extraction of the aqueous mother-liquors with ether and recovery gave an oil which was distilled *in vacuo*. The oily distillate, crystallised from 1 : 9-benzene–light petroleum, had m. p. 54—59° (*ca.* 8 mg., 10%) and did not depress the m. p. of an authentic sample of orcinol monomethyl ether (Found : C, 69·6; H, 7·05%).

Action of 0·5N-*Sodium Methoxide on Griseofulvin.*—Griseofulvin (2·0 g.) and a solution of sodium (2·28 g.) in methanol (200 ml.) were heated under reflux for 5 hours, cooled, diluted with ice-water (200 ml.), and kept for several hours. The precipitate was dried at 100° (1·76 g.), m. p. 238—240°; it formed clusters of small hard prisms (from toluene or toluene–light petroleum), m. p. 239—240°, $[\alpha]_D^{23}$ +155° (*c,* 1·378 in chloroform) [Found : C, 56·8, 57·0, 56·95; H, 4·4, 4·5,

4·9; Cl, 9·55, 9·4; OMe, 24·4, 24·0%; M (Rast), 348. $C_{18}H_{19}O_7Cl$ requires C, 56·45; H, 4·95; Cl, 9·3; 3OMe, 24·3%; M, 382·5. $C_{18}H_{21}O_7Cl$ requires C, 56·2; H, 5·45; Cl, 9·25; 3OMe, 24·2%; M, 384·5). Ultra-violet absorption max. in methanol : 330, 289, 236 mμ (log ε 3·73, 4·37, 4·42). It resembled griseofulvin in many respects, but was rather more soluble in cold chloroform or acetone; it gave a yellow colour in concentrated sulphuric acid and a brick-red precipitate with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid, and slowly absorbed bromine in chloroform solution with evolution of hydrogen bromide on warming. With Girard's reagent P under standard conditions it gave a soluble complex from which there was recovered the starting material on acidification. Oxidation with chromic acid gave (II; R = Me), and with potassium permanganate gave (I). Alkaline hydrolysis gave 8-chloro-1 : 2 : 3 : 4-tetra-hydro-3-keto-5 : 7-dimethoxy-1-methyldibenzofuran but no 7-chloro-4(or 6)-hydroxy-6(or 4)-methoxy-2'-methylgrisan-3 : 4' : 6'-trione (cf. behaviour of griseofulvin). Acid hydrolysis gave an *acid*, not identical with 7-chloro-4 : 6-dimethoxy-2'-methylgrisan-3 : 4' : 6'-trione, colourless prisms (from aqueous methanol), m. p. 253—255° (decomp.), $[\alpha]_D^{21}$ +237° (c, 0·096 as sodium salt in aqueous methanol) (Found : C, 55·3, 55·7; H, 4·9, 4·5; Cl, 10·2; OMe, 17·72. $C_{17}H_{17}O_7Cl$ requires C, 55·35; H, 4·6; Cl, 9·65; 2OMe, 16·8. $C_{17}H_{19}O_7Cl$ requires C, 55·05; H, 5·15; Cl, 9·6; 2OMe, 16·75%. Ultra-violet absorption max. in methanol : 330, 286 mμ (log ε, 3·87, 4·67). Oxidation of the acidic material with yellow mercuric oxide gave (XVIII).