QUARTERLY REVIEWS

GRISEOFULVIN

By JOHN FREDERICK GROVE

1. Historical introduction

GRISEOFULVIN (1; R = Cl), $C_{17}H_{17}ClO_6$, m.p. 222°, $[\alpha]_D$ + 340°, a neutral antifungal antibiotic, which since 1958 has found important applications in the systemic treatment of mycoses, both in man and in the veterinary field, was first isolated in 1939 by Raistrick and his collaborators[1] from the mycelium of *Penicillium griseofulvum*; but its unique biological action on other fungi was not then noticed. Subsequently,[2] griseofulvin was obtained from both mycelium and culture filtrate of *P. janczewskii* Zal. [*P. nigricans* (Bainier) Thom] and was observed to cause stunting and malformation of the germ-tubes of *Botrytis allii* and, in lower concentrations, helical curling and waving of the hyphae. More recently,[3] griseofulvin has been recognised as a metabolic product of many species of *Penicillium*; mutant strains of *P. patulum*, which give yields of 3 g./l. in shake culture,[4] are used for the commerical production of the antibiotic in stirred aerated fermenters where yields of 5·8 g./l. have been claimed.[5] In chloride-deficient and to some extent in normal media, both *P. nigricans* and *P. griseofulvum* produced[6] the dechloro-analogue (1; R = H) of griseofulvin: when potassium bromide was added to the chloride-deficient medium, the product[7] contained the bromo-analogue (1; R = Br), the first bromine-containing fungal metabolic product to be characterised. The fluoro-[8] (1; R = F) and the racemic iodo-analogue[9] (1; R = I) have been synthesised.

The chemistry and absolute configuration of griseofulvin were elucidated mainly by the efforts of a group of workers at the Akers Research Laboratories of Imperial Chemical Industries Limited and that work was published between 1952 and 1959. The intense research effort which followed has led to the total synthesis of the antibiotic by four different laboratories.

[1] Oxford, Raistrick, and Simonart, *Biochem. J.*, 1939, **33**, 240.
[2] Brian, Curtis, and Hemming, *Trans. Brit. Mycol. Soc.*, 1946, **29**, 173; Grove and McGowan, *Nature*, 1947, **160**, 574.
[3] Brian, Curtis, and Hemming, *Trans. Brit. Mycol. Soc.*, 1955, **38**, 305.
[4] B.P. 788,118.
[5] B.P. 868,958.
[6] MacMillan, *J.*, 1953, 1697.
[7] MacMillan, *J.*, 1954, 2585.
[8] Taub, Kuo, and Wendler, *Chem. and Ind.*, 1962, 557, 1617.
[9] Gerecke, Kyburz, Planta, and Brossi, *Helv. Chim. Acta*, 1962, **45**, 2241.

2. Mode of Action

Griseofulvin exerts its characteristic, fungistatic effects only on the growing tips of fungal hyphae, presumably by interfering with the synthesis and organisation of cell-wall material,[10,11] and, as a result of the abnormalities produced, the size of colonies growing in the presence of griseofulvin may be severely restricted. Old cell-walls remote from the hyphal tip are not affected.[12] Griseofulvin is not translocated within fungal hyphae,[10,11,13] and only mycelium in intimate contact with a griseofulvin-containing medium is modified. Fungi whose cell-walls are based on cellulose are unaffected by griseofulvin; sensitive fungi all contain chitin as a major cell-wall constituent.[10] Although some chitin-containing fungi do not respond to it,[13,14] and the reported reversal by purines of the griseofulvin-induced inhibition of *Microsporum canis* has led[15] to a proposal that griseofulvin interferes with nucleic acid metabolism, nevertheless, it still seems possible that griseofulvin interferes in some way with the synthesis of cell-wall chitin.

The modified hyphae are less able to penetrate plant cell-walls[11]or animal hairs[16] than are those growing in the absence of griseofulvin, and these observations may account for the activity of griseofulvin *in vivo*.

3. Applications in Medicine

Griseofulvin is active *in vitro* against many fungi, including *Trichophyton* spp. and *M. canis*, responsible for diseases of the hair and skin in man and animals.[16] It is a systemic antifungal compound and has been detected spectrofluorometrically in the hair of animals given griseofulvin by mouth;[16] and this method of treatment was shown to be effective in experimental ringworm infections of guinea-pigs[17,18] and cattle.[19] The recent medical literature contains many reports[20–22] of the successful oral treatment by griseofulvin of fungal infections in man, but no comprehensive review is available. In the rat griseofulvin is absorbed from the alimentary tract into the blood.[23] The amount deposited in the keratin

[10] Brian, *Ann. Bot.*, 1949, **13**, 59.
[11] Aytoun, *Ann. Bot.*, 1956, **20**, 297.
[12] Banbury, *J. Exp. Bot.*, 1952, **3**, 86.
[13] Abbot and Grove, *Exp. Cell. Res.*, 1959, **17**, 105.
[14] Napier, Turner, and Rhodes, *Ann. Bot.*, 1956, **20**, 461.
[15] McNall, *Arch. Dermatol.*, 1960, **81**, 657.
[16] Gentles and Barnes, *Arch. Dermatol.*, 1960, **81**, 703.
[17] Gentles, *Nature*, 1958, **182**, 476.
[18] Martin, *J. Invest. Dermatol.*, 1959, **32**, 525.
[19] Lauder and O'Sullivan, *Vet. Record*, 1958, **70**, 949.
[20] Blank and Roth, *Arch. Dermatol.*, 1959, **79**, 259.
[21] Williams, Marten, and Sarkany, *Lancet*, 1958, **276**, 1212.
[22] Russell, Frain-Bell, Stevenson, Riddell, Djavahiszwili, and Morrison, *Lancet*, 1960, **279**, 1141.
[23] Davis, Child, and Tomich, *J. Pharm. Pharmacol.*, 1961, **13**, 166.

depends on the blood-level achieved and this in turn is related to the particle size of the orally administered antibiotic.[24]

Large doses of griseofulvin injected intravenously in rats cause a transient depression of mitosis, and intraperitoneal administration damages the seminal epithelium;[25] but when it is taken orally toxicity is very low[16,26] and in man only minor side-effects have been observed.

Strains of *M. canis* and *Trichophyton rubrum* resistant to griseofulvin *in vitro* have been isolated[27] but have not yet been encountered *in vivo*.

4. Applications in Agriculture

Griseofulvin is also active *in vitro* against many important fungal pathogens of plants.[10,14] Although early results were encouraging, and griseofulvin was found to control a number of plant diseases attributed to these fungi,[28,29] it has shown no long-term superiority over cheaper fungicides and no practical applications in agriculture or horticulture have developed.

Griseofulvin applied through the root system will protect the foliage of plants from infection. This property of translocation was demonstrated initially by bioassay[28,30] and later,[31] conclusively, by the reisolation and chemical identification of griseofulvin from the tissues of treated plants. Uptake of griseofulvin from an aqueous solution by bean roots was a rapid process, probably linked with respiration; but translocation in the shoots was a passive movement in the transpiration stream and the amount taken up by the whole plant was related linearly to the volume of water transpired.[32]

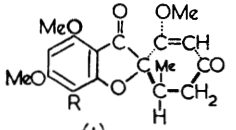
Griseofulvin is relatively non-phytotoxic; some minor modifications of root growth[30] are probably associated with an interference with mitosis.[25] It is slowly degraded in plant tissue[31,32] (see section 6), and the protective action against fungal pathogens is therefore transient. A study[33,34] of the movement of a group of griseofulvin homologues and relatives in broad bean showed that desirable properties included, not only activity *in vitro*, stability, and absence of phytotoxicity, but also the correct water-solubility and oil–water partition ratio to ensure translocation: griseofulvin was the most effective of the compounds tested. In this work griseofulvin was

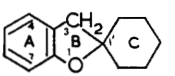
[24] Atkinson, Bedford, Child, and Tomich, *Nature*, 1962, **193**, 588; Duncan, MacDonald, and Thornton, *J. Pharm. Pharmacol.*, 1962, **14**, 217.
[25] Paget and Walpole, *Nature*, 1958, **182**, 1320.
[26] McLeod and Nelson, *Proc. Soc. Exp. Biol. Med.*, 1959, **10**, 259.
[27] Aytoun, Campbell, Napier, and Seiler, *Arch. Dermatol.*, 1960, **81**, 650.
[28] Brian, Wright, Stubbs, and Way, *Nature*, 1951, **167**, 347.
[29] Rhodes, Crosse, McWilliam, Tootill, and Dunn, *Ann. Appl. Biol.*, 1957, **45**, 215; for review see Brian, *Trans. Brit. Mycol. Soc.*, 1960, **43**, 1.
[30] Stokes, *Plant and Soil*, 1954, **5**, 132.
[31] Crowdy, Gardner, Grove, and Pramer, *J. Exp. Bot.*, 1955, **6**, 371.
[32] Crowdy, Grove, Hemming, and Robinson, *J. Exp. Bot.*, 1956, **7**, 42.
[33] Crowdy, Green, Grove, McCloskey, and Morrison, *Biochem. J.*, 1959, **72**, 230.
[34] Crowdy, Grove, and McCloskey, *Biochem. J.*, 1959, **72**, 241.

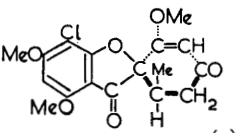
administered through the roots, a method unlikely to be of use in practice since the compound is rapidly degraded in soil.[35]

5. The Chemistry of Griseofulvin

(*a*) **Nomenclature.**—The systematic nomenclature[36] is based on the trivial name grisan for the tricyclic system (2) numbered as shown. The absolute


(1)


(2)


(3)

configuration of griseofulvin, (2*S*, 6′*R*)-7-chloro-4,6,2′-trimethoxy-6′-methylgris-2′-en-3,4′-dione (1; R = Cl), is known:[37] but since correlation of configuration in a series of related compounds is not always evident on the nomenclature system of Cahn, Ingold, and Prelog, the configuration at the two asymmetric centres 2 and 6′ is designated by *d* or *l*. Thus, griseofulvin is prefixed by (*d*,*d*)- and the diastereoisomer (3) by (*l*,*d*), the spiran centre being that first mentioned. In an alternative system,[38] the natural isomer is referred to as (+)-griseofulvin and the diastereoisomer (3) as *epi*-(+)-griseofulvin. This system is less cumbersome than the (*d*,*d*)-notation but, like that of Cahn *et al.*, is confusing when used to correlate transformation products (*e.g.*, dihydrogriseofulvin has $[\alpha]_D - 20°$).

(*b*) **Structure and Absolute Configuration.**—Griseofulvin was shown[1] to contain three methoxyl groups, an ethylenic double bond, and a reactive carbonyl group. One methoxyl group was liable to both dilute aqueous acid and alkali, and catalytic reduction of the resulting acidic product (4; R = Cl), $C_{16}H_{15}ClO_6$(Chart 1), to a neutral alcohol (8; R = R′ = OH), $C_{16}H_{19}ClO_6$, showed[39] that griseofulvin, in confirmation of infrared spectroscopic predictions,[40] was the methyl enol ether of a 1,3-diketone. The presence of a second, unreactive, carbonyl group was shown by the infrared ($\lambda_{max.}$1685 cm.$^{-1}$) and ultraviolet ($\lambda_{max.}$288, 323 mμ; logϵ 4·32, 3·71) spectra of the alcohol (8; R = R′ = OH)[39] in which the reactive carbonyl group had been removed but the characteristic phloracetophenone chromophore of griseofulvin[40] had been retained.

Oxidative degradation of ring C of the acidic trione (4; R = Cl) with alkaline hydrogen peroxide[41] gave the acid (7; R = Cl, R′ = H) which

[35] Wright and Grove, *Ann. Appl. Biol.*, 1955, **43**, 288.
[36] Grove, MacMillan, Mulholland, and Rogers, *J.*, 1952, 3977.
[37] MacMillan, *J.*, 1959, 1823.
[38] Brossi, Baumann, Gerecke, and Kyburz, *Helv. Chim. Acta*, 1960, **43**, 1444.
[39] Grove, MacMillan, Mulholland, and Rogers, *J.*, 1952, 3949.
[40] Grove and McGowan, *Chem. and Ind.*, 1949, 647.
[41] Grove, Ismay, MacMillan, Mulholland, and Rogers, *J.*, 1952, 3958.

was oxidised further by permanganate to the hydroxy-acid (7; R = Cl, R′ = OH). The latter was cleaved by periodate to (+)-methylsuccinic acid (11) and the salicylic acid (10), which was decarboxylated to 2-chloro-3,5-dimethoxyphenol whose structure was proved by unambiguous synthesis.[42] The existence of a second six-membered carbocyclic ring in griseofulvin was shown by the isolation of orcinol (9; R= H) after fusion with potassium hydroxide[1] and, finally, by the fission by boiling 2N-sodium methoxide of griseofulvin into orcinol monomethyl ether (9; R = Me) and the salicylic acid (10), fragments which contain all the carbon atoms of the parent molecule.[36] The manner in which the two partial structures (9) and (10) are linked in griseofulvin is unequivocally determined by the structures of the oxidation products (7; R = Cl, R′ = H and OH); the formation of 3-methoxy-2,5-toluquinone (6) on chromic

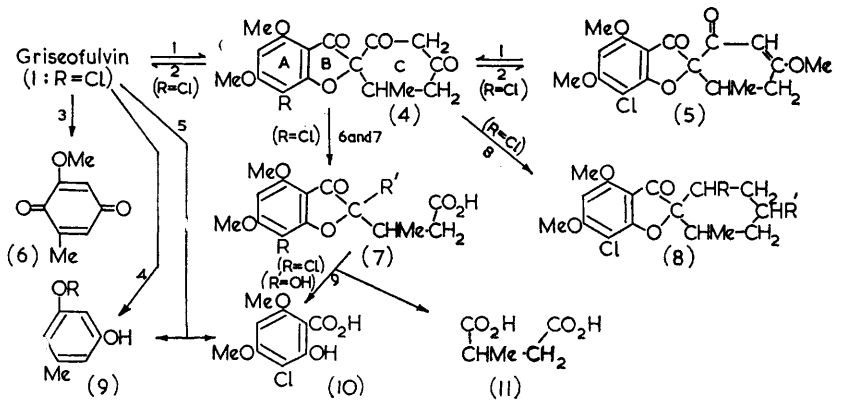


CHART 1.

Reagents: 1, H^+. 2, CH_2N_2. 3, CrO_3. 4, KOH. 5, NaOMe. 6, H_2O_2–NaOH. 7, $KMnO_4$. 8, Pt–H_2. 9, $NaIO_4$.

oxide oxidation[41] indicates that griseofulvin has the structure (1) rather than the alternative methyl enol ether structure (5) which would be expected to yield an unstable quinone under these conditions. Structure (5) was then allocated to an isomer of griseofulvin formed on acid-catalysed methylation of the trione (4; R = Cl)[39,43] and as one product of the remethylation of the trione with diazomethane. This allocation was confirmed by reductive transformation of the enol ether system[44,45] according to the sequence in Chart 2: the diketone (12), derived from griseofulvin

[42] Grove, MacMillan, Mulholland, and Zealley, *J.*, 1952, 3967.
[43] Duncanson, Grove, and Jeffs, *J.*, 1958, 2929.
[44] Mulholland, *J.*, 1952, 3987.
[45] Mulholland, *J.*, 1952, 3994.

was stable to alkali, but the 3,2′-diketone (13) derived from the isomer (5) underwent hydrolytic cleavage to the acid (14).

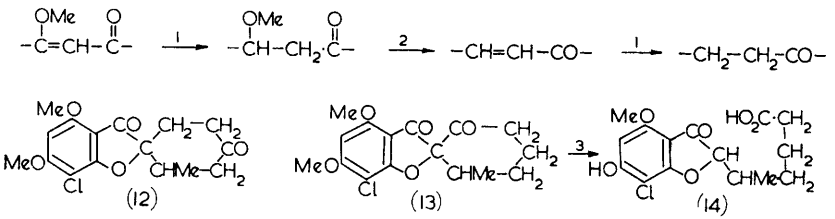


CHART 2.

Reagents: 1, H_2–Pd. 2, H^+. 3, OH^-.

An equilibrium mixture, separable into its components by chromatography, of griseofulvin (40%) and the diastereoisomer (3), m.p. 214-216°, $[\alpha]_D$ + 88° (60%), resulted from the action of boiling 0·5N-sodium methoxide on griseofulvin.[37] The mechanism of the epimerisation can proceed as indicated in route (a) of Chart 3. An alternative route (b),

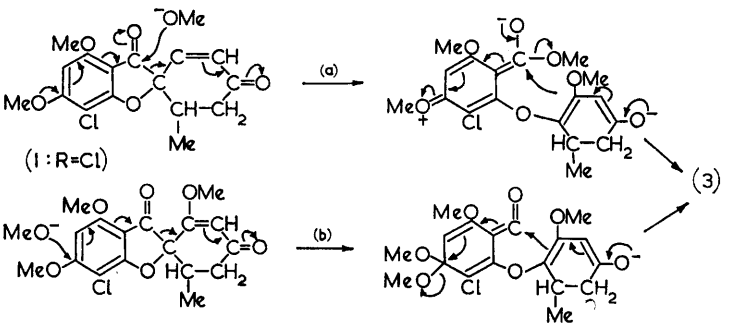


CHART 3.

involving a keten intermediate analogous to that suggested for the epimerisation of geodin[46] (63; R = Cl), is also possible but the stability, under the same conditions, of the acidic trione (4; R = Cl), which bears a formal negative charge on ring C, favours mechanism (a). As with griseofulvin, oxidative degradation of the diastereoisomer (3) gave (+)-methylsuccinic acid (11) and it follows that the spiran centre of griseofulvin and not the 6′-centre was inverted in the formation of the diastereoisomer (3). Since (+)-methylsuccinic acid has been related to D-glyceraldehyde the absolute configuration at position 6′ in griseofulvin is fixed as in (1). The configuration at the spiran centre may be deduced from the observation that the

[46] Barton and Scott, *J.*, 1958, 1767.

equilibrium mixture of griseofulvin and its diastereoisomer contains only 40% of griseofulvin; the latter is assigned the sterically less favoured configuration (1) in which the bulky 3-carbonyl and 6′-methyl groups are *cis*-related.[37] This configuration has been confirmed by *X*-ray crystallographic examination of the 5-bromo-derivative.[46a]

(*c*) **General Chemical Reactions of Griseofulvin and its Related Compounds.**—(i) *Molecular rearrangement leading to substituted dibenzofurans.* The triones (4; R = Cl or H) with dilute aqueous alkali (Chart 4) undergo[1,6,39,36] fission of ring C, followed by loss of carbon dioxide and internal Knoevenagel condensation (4) → (16; R′ = H) → (17) → (20) → (19), to give the (−)-ketones (19) (together with, when R = Cl, the $\Delta^{10(4)}$-isomer) which were shown to be tetrahydrodibenzofuran derivatives on spectroscopic evidence[40] and by aerial oxidation to the dibenzofurans (18) also obtained directly from the triones (4) by oxidation with alkaline mercuric oxide.[41] The structures of the degradation products (18) and (19) were confirmed by synthesis:[47] Michael condensation of 7-chloro-4,6-dimethoxycoumaranone with pent-3-en-2-one gave the racemic ketone (17; R= Cl) which was cyclised to the racemic ketone (19; R= Cl); the latter was resolved by means of (−)-menthyl *N*-aminocarbamate. Formation of the 2′,4′-diketal of the trione (4; R = Cl) blocked the rearrangement.[48]

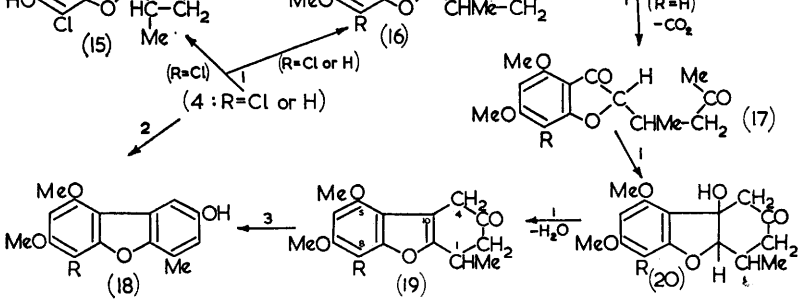


CHART 4.

Reagents: 1, OH^-. 2, HgO–NaOH. 3, NaOH–O_2.

(ii) *Hydrolysis and alcoholysis of ether linkages.*[36,37,49] Conditions for hydrolysis of the 2′-ether link have been given in section 5*b*. The 6-ether linkage is also labile to aqueous alkali, though stable to acid, in those griseofulvin derivatives and degradation products which retain the

[46a] Brown and Sim, *J.* 1963, 1050.
[47] MacMillan, Mulholland, Dawkins, and Ward, *J.*, 1954, 429.
[48] Arkley, Attenburrow, Gregory, and Walker, *J.*, 1962, 1260.
[49] Duncanson, Grove, MacMillan, and Mulholland, *J.*, 1957, 3555.

7-chlorocoumaranone nucleus: it is stable in derivatives of dechlorogriseofulvin.[6] Simultaneously with undergoing the rearrangement in (i) above, the trione (4; R = Cl) gave a phenol (15), oriented on spectroscopic evidence and by oxidative degradation of the ethyl ether to 3-chloro-4-ethoxy-2-hydroxy-6-methoxybenzoic acid, whose structure was established by unambiguous synthesis. The phenol (15) was stable to aqueous alkali and was not rearranged to a tetrahydrodibenzofuran. Selective acid-catalysed *O*-alkylation of the phenol (15) in ring C was successful,[48] the phenolic hydroxyl group being unaffected. The latter was readily alkylated by the methods outlined in section (iv) below.

Selective fission of the 4-ether group in griseofulvin was effected by the magnesium iodide–ether complex in benzene.[48] The resulting phenol (26; R = OMe, R′ = H, R″ = Me) was readily alkylated: rearrangement of the 4-allyl ether provided a route to 5-substituted griseofulvin analogues.

All the ether linkages in griseofulvin were labile to alkoxide,[37] ether exchange to give, *e.g.*, the triethyl ether (26; R = OEt, R′ = R″ = Et) being accompanied by racemisation at the spiran centre and in some instances (see Chart 1) by fission of the molecule into fragments derived from rings A and C.

(iii) *Catalytic reduction.* Hydrogenolysis of the 2′,4′-dione enol ether system[44] occurs simultaneously with hydrogenation, and the deoxo-compounds (23; R = H) and (24; R = Cl, R′ = H) were formed from griseofulvin in addition to the dihydro- (Chart 2) and tetrahydro-derivative (24; R = Cl, R′ = OH); and compounds (8; R = OH or H, R′ = H) were obtained[39] from the trione (4; R = Cl) in addition to the diol (8; R = R′ = OH). Reductive dechlorination also occurred giving, from griseofulvin, the alcohol (24; R = H, R′ = OH) and providing a link which established the structure of dechlorogriseofulvin (1; R = H).

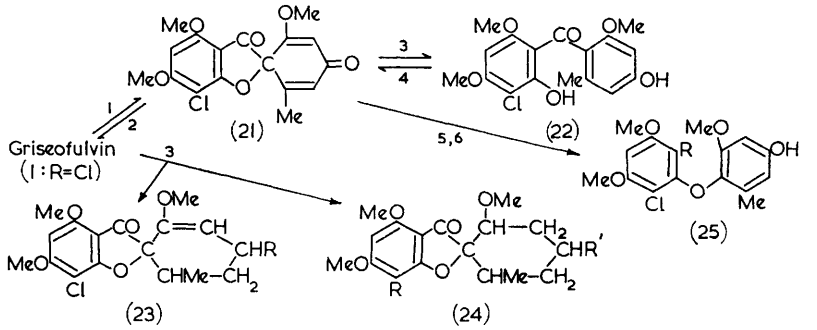
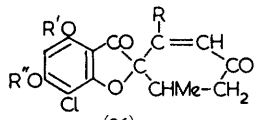


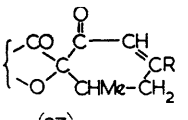
CHART 5.

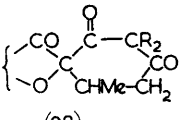
Reagents: 1, Se. 2, H_2–Rh–C(Se). 3, H_2–Pd. 4, $K_3Fe(CN)_6$. 5, NaOMe. 6, H_2SO_4.

(iv) *Other reactions of ring* C. In general, ring C undergoes the reactions expected for a cyclohexane-1,3-dione and its enol ethers. With

methanolic ammonia griseofulvin gave the 2′-amine (26; R = NH_2, R′ = R″ = Me).[39] With diazoalkanes, *O*-alkylation of the trione (4; R = Cl) predominated,[43] giving homologues (26; R = OAlkyl, R′ = R″ = Me) of griseofulvin and of the 4′-ether (5), but some *C*-alkylation occurred, giving 3′-alkyl derivatives. The latter were more readily obtained by using alkyl halides in aqueous acetone in the presence of potassium acetate or silver oxide.[50] Further base-catalysed alkylation afforded neutral 3′,3′-dialkyl derivatives (28).


(26)


(27)


(28)

3′-Halogeno-derivatives of the trione (4; R = Cl) were obtained by using 1 mol. of halogen in dimethylformamide.[51] More drastic conditions with 3 mol. of chlorine in carbon tetrachloride or bromine in acetic acid in the presence of an excess of mercuric acetate, gave, ultimately, 5,3′,3′-trihalogeno-derivatives from which the 3′-halogen atoms could frequently be removed with potassium iodide in acetic acid, leading to 5-halogeno-derivatives.[51] The 3′-(3-oxobutyl) derivative of the trione (4; R = Cl) was obtained by base-catalysed Michael condensation.[51] The 3′-substituted triones could be converted by standard methods into the 2′- and the 4′-ethers.

With phosphoryl chloride in the presence of water and lithium chloride the trione (4; R = Cl) was converted into a mixture of the 2′- and 4′-chloride (26; R = Cl, R′ = R″ = Me) and (27; R = Cl), respectively;[52] 3′-substituted triones gave predominantly 4′-chlorides. Under the same conditions, 4′-amines (27; R = NH_2) or ethers gave 2′-chlorides by a mechanism in which attack on the 2′-carbonyl group, giving a phosphate ester with concomitant elimination of the 4′ substituent, is followed by replacement of the phosphate ester by Cl^-. Similarly, 2′-amines (26; R = NH_2, R′ = R″ = Me) gave 4′-chlorides. The chlorides of both series reacted with ammonia and other bases to give the corresponding amines, with thiols under alkaline conditions to give *S*-alkyl compounds, and with phenols and lower alcohols to give ethers.[52]

Reduction of griseofulvin with alkali-metal hydrides gave the alcohol (23; R = OH).[53]

(v) *The isomer* (5). This behaved similarly to griseofulvin in most of its reactions except that hydrogenolysis of the 2′,4′-dione enol ether system did not take place during catalytic reduction[44] and, owing to steric hindrance, the 2′-oxo-group was unreactive.[39] Consequently, Girard's reagent

[50] Gregory, Holten, Robinson, and Walker, *J.*, 1962, 1269.
[51] Walker, Warburton, and Webb, *J.*, 1962, 1277.
[52] Stephenson, Walker, Warburton, and Webb, *J.*, 1962, 1282.
[53] Kyburz, Geleick, Frey, and Brossi, *Helv. Chim. Acta*, 1960, **43**, 2083.

P can be used to separate mixtures of 2′- and 4′-ethers.[48] Interaction between the coumaranone and 3′-en-2′-one chromophores[49] gives to the 4′-ether (5) a characteristic ultraviolet absorption which serves, in the majority of cases,[43] to distinguish homologues of the isomer (5) from the corresponding compounds in the griseofulvin series. Infrared spectra can also be used[54] to distinguish between 2′- and 4′-ethers.

(vi) *Fission of ring* B. Hydrogenolysis over a noble-metal catalyst of (−)-dehydrogriseofulvin (21), obtained[55] by dehydrogenation of griseofulvin with selenium in t-butyl alcohol and acetic acid, gave the benzophenone (22). This reductive scission was also effected by zinc in acetic acid.[56] (−)-Dehydrogriseofulvin was converted into the diphenyl ether derivatives (25; R = H and CO_2Me) by the action of acid and base, respectively.[57] With base the reaction mechanism paralleled that in Chart 3, route (a).

(vii) *The* (l,d)-*diastereoisomer* (3). The chemistry of the diastereoisomer (3) was similar to that of griseofulvin apart from the action of dilute aqueous alkali, which gave the salicylic acid (10) instead of the ketone (19; R = Cl),[37] and some small differences in the chemistry of the products of reduction of ring C.[58]

(viii) *Ring* A *analogues.* Supplies of the bromo-analogue (1; R = Br) have been limited and no detailed study of its chemistry has been possible. Like griseofulvin it gave the toluquinone (6) on chromic oxide oxidation, and the substitution pattern of ring A was confirmed by permanganate oxidation to 3-bromo-2-hydroxy-4,6-dimethoxybenzoic acid whose structure was proved by synthesis. On oxidative degradation of dechlorogriseofulvin the intermediate half-ester (29) was obtained in addition to the acids (7; R = H, R′ = H and OH). Dechlorogriseofulvin and its degradation products with ring A intact gave an intense blue-violet colour with nitric acid (*d* 1·42), a useful diagnostic test for a free 7-position. Nitration of dechlorogriseofulvin[59] gave 5- and 7-nitro-compounds, also obtained synthetically[9] from the appropriate coumaranones. Partial synthesis of the fluoro-analogue (1; R = F) has been achieved by fluorination of the 6-phenol (15) with perchloryl fluoride (after protection of ring C), followed by reductive dechlorination.[8] Conditions for the specific chlorination of ring A have been described.[8]

(*d*) **Synthesis.**—Reactions carried out under equilibrium conditions lead to racemates in the biologically inactive, thermodynamically more stable, (*l*,*d*)-series derived from (3). Synthetic racemic griseofulvin has been produced in two ways from the (*l*,*d*)-racemate:

1. Inversion at the spiran centre with sodium methoxide (see section 5*c*),

[54] Page and Staniforth, *J.*, 1962, 1292.
[55] Day, Nabney, and Scott, *J.*, 1961, 4067.
[56] Kuo, Hoffsommer, Slates, Taub, and Wendler, *Chem. and Ind.*, 1960, 1627.
[57] Kyburz, Wursch, and Brossi, *Helv. Chim. Acta*, 1962, **45**, 813.
[58] Dawkins and Mulholland, *J.*, 1959, 1830.
[59] Arkley, Gregory, and Walker, *J.* 1963, 1603.

followed by chromatographic separation of the resulting equilibrium mixture.

2. *Via* a 5′,6′-unsaturated derivative, *e.g.*, (±)-dehydrogriseofulvin (21) (Chart 5), catalytic reduction (steric control) of which gives predominantly racemic griseofulvin.

Hydrolysis, resolution of the racemic trione (4; R = Cl) by means of the brucine[60] or quinine metho-salt,[55] and remethylation gave griseofulvin.

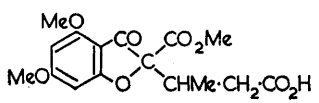
The most satisfactory route to the (*l*,*l*)-isomer (I; R = Cl), the optical antipode of griseofulvin, is by epimerisation of the (*d*,*l*)-isomer (3), obtained by resolution of the synthetic racemic (*l*,*d*)-trione.[61]

Five routes to the 3-oxocoumaranspirocycloalkane system have been developed, and products with a 5- and a 6-membered ring C and with alkyl-, alkoxy-, nitro-, and halogen-substituted ring A have been made.

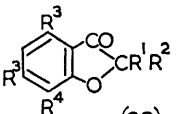
(i) *Condensation of coumaranones* (30; R = R′ = H) *with dihalogenoalkanes in the presence of potassium t-butoxide.*[62] By this general method the racemic (*l*,*d*)-diastereoisomer of compound (8; R = R′ = H) was prepared from 7-chloro-4,6-dimethoxycoumaranone and 1,5-dibromohexane.

(ii) *Double Michael addition of ethynyl vinyl ketones to coumaranones.*[63] The product (obtained in 5% yield) from 7-chloro-4,6-dimethoxycoumaranone and methoxyethynyl propenyl ketone was racemic griseofulvin, indicating that the second stage of the condensation took place under kinetic control.

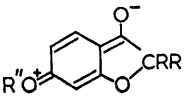
(iii) *Cyclisation of* 2,2-*disubstituted coumaranones* (*ester synthesis*). Preparation of the intermediates (30; R^1 and $R^2 \neq H$) from the parent coumaranones (30; $R^1 = R^2 = H$) is more difficult when R^3 is a 4- or 6-alkoxyl group since participation of the molecular species (31; R = R′ = H) reduces the nucleophilic character at position 2. This difficulty was overcome[62,64,9] by use of intermediates of type (31; R = COX, R′ = H), obtained by cyclisation of esters of type (32), which were then submitted to Michael condensation with methyl vinyl ketones.



(29)



(30)

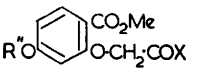


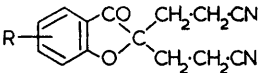
(31)

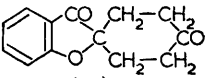
Nevertheless, the nature of the ring A substituents exercises a subtle and little understood influence over the course of the subsequent cyclisations. In the simplest case,[65] grisan-3,4′-dione (34) was prepared from the

[60] Brossi, Baumann, Gerecke, and Kyburz, *Helv. Chim. Acta*, 1960, **43**, 2071.
[61] Brossi, Baumann and Burkhardt, *Helv. Chim. Acta*, 1962, **45**, 1292.
[62] Dawkins and Mulholland, *J.*, 1959, 2203.
[63] Stork and Tomasz, *J. Amer. Chem. Soc.*, 1962, **84**, 310.
[64] Dean and Manunapichu, *J.*, 1957, 3112.
[65] McCloskey, *J.*, 1958, 4732.

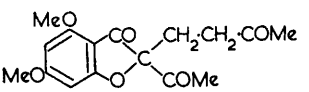
dinitrile (33; R = H), obtained by Michael condensation of coumaranone and vinyl cyanide, by hydrolysis and Dieckmann cyclisation of the diester: but the cyclisation step failed with coumaranones where R was alkyl or alkoxyl.[64] Base-catalysed cyclisation of coumaranones (30; R^1 =

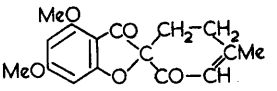

(32)

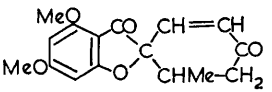

(33)


(34)

$CH_2{\cdot}CH_2{\cdot}COMe$, $R^2 = CO_2Me$) gave high yields,[9] but for configuration of an α-methyl group in compounds of type (30; R^1 = $CHMe{\cdot}CH_2{\cdot}COMe$, R^2 = CO_2Me) was of importance in determining the course of the cyclisation step. Of the two synthetic racemates (30; R^1 = $CHMe{\cdot}CH_2{\cdot}COMe$, R^2 = CO_2Me, R^3 = OMe, R^4 = Cl or H) the less stable (16; R = Cl or H, R′ = Me) (α-methyl and 3-oxo-group *cis*-related) gave only low or negligible yields of the racemic triones (4; R = Cl or H) and the reverse Michael reaction predominated; but the more stable isomer underwent cyclisation in fair yield with alkoxide at room temperature, to give the racemic (*l*,*d*)-diastereoisomers of the triones (4; R = Cl or H).[60,9] This route has been used in preparing a large number of ring A analogues of griseofulvin.[9] The stereochemical requirements of the reaction precluded the use of the degradation product (16; R = Cl, R′ = Me) as a "relay." Only one racemate (30; R^1 = $CHMe{\cdot}CH_2{\cdot}COMe$, R^2 = CO_2Et) was obtained from coumaranone and from the 4,6-dimethoxy-analogue. These racemates were presumably in the more stable series but cyclisation failed[62] with a wide variety of reagents; in general, the reverse Michael reaction occurred in basic conditions whilst acidic reagents were ineffective.


(35)


(36)

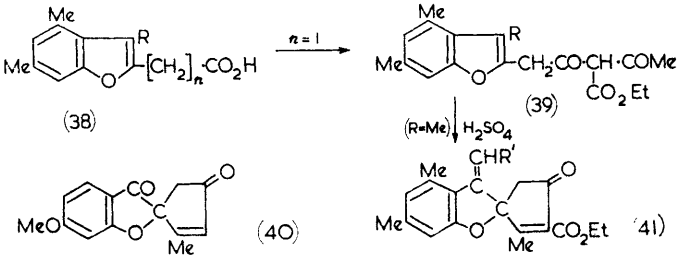

(37)

Because of the ubiquity of the reverse Michael reaction in these cyclisations and the possibility of loss of the 2-acetyl residue, basic conditions were not used to effect cyclisation of the trione (35):[64] acidic reagents gave the 4′-methylgris-3′-en-2′-one (36), and not the hoped-for racemic dione (37).[66]

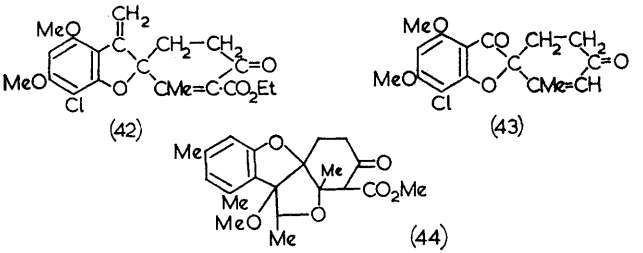
(iv) *Usnolic acid route.* The synthesis[67] of the usnolic acid analogue (41; R′ = H), by acid cyclisation of the ester (39; R = Me), obtained from

[66] MacMillan and Suter, *J.*, 1957, 3124.
[67] Dean, Halewood, Mongkolsuk, Robertson, and Whalley, *J.*, 1953, 1250.

the benzofuran-2-ylacetic acid (38; $n = 1$, R = Me) and ethyl ethoxymagnesioacetoacetate, was adapted,[68] by means of an ozonolysis step, to the preparation of analogues such as the coumaranonespirocyclopentenone (40), and then extended to the synthesis of grisans. Propionic acids



of type (38; $n = 2$; R = Me) led[68] to 3-methylene-4′-oxogrisen esters of type (42): ozonolysis and removal of the ethoxycarbonyl residue then gave the coumaranone (43) which on catalytic reduction furnished,



inter alia, racemates of the (*d*,*d*)-ketone (12) and of the (*l*,*d*)-diastereoisomer of (8; R = R′ = H). Although ozonolysis of the 3-alkylidene derivatives (41; R′ = H or Me), with a 5-membered ring C, gave the expected coumaranones, the 3-ethylidenegrisen derived from (38; $n = 2$, R = Et) gave anomalous results and difuranoid structures of type (44) were obtained.[69]

(v) *Intramolecular phenol radical coupling.* Oxidation of the benzophenone (22) with alkaline potassium ferricyanide[55,56] gave racemic dehydrogriseofulvin (21), reduction of which over 10% palladium–charcoal in a non-hydroxylic solvent[56] or on a selenium-poisoned rhodium–charcoal[55] gave racemic griseofulvin. This method, which has been used[8] to make the fluoro-analogue (I; R = F) as well as dechlorogriseofulvin, seems likely to be a general one for preparing grisadienones from the appropriate phenolic ketones.

[68] Dawkins and Mulholland, *J.*, 1959, 2211.
[69] Dean, Francis, and Manunapichu, *J.*, 1958, 4551; Dean, Deorha, Knight, and Francis, *J.*, 1961, 327.

(*e*) **Detection and Estimation.**—The bioassay,[2] involving observation of morphological changes in the germ-tubes of *B. allii*, is reliable and widely used but suffers from the degree of imprecision inherent in all $\times 2$ serial dilution bioassays. It is not specific for griseofulvin, and many derivatives and relatives of griseofulvin can interfere (see section 8). Many investigators have preferred to use physical methods, particularly ultraviolet spectrophotometry,[31] by utilising the strong absorption band in griseofulvin at 291 mμ, and the more sensitive but temperature-dependent spectrophotofluorimetry,[70] by measuring the emission at 450 mμ. The spectrophotometric method is relatively non-specific since all relatives containing the phloroacetophenone chromophore absorb strongly near 285 mμ; depending on the accuracy required, various mathematical procedures have been adopted for correcting for the background absorption due to the impurities present when griseofulvin is estimated in fermentation broths[71–73] or plant tissue extracts.[33] The fluorometric method is rather more specific and, coupled with a suitable extraction procedure, has been used extensively for detecting and estimating griseofulvin in biological fluids.

An isotope dilution assay involving griseofulvin labelled with ^{35}Cl has also been described.[74]

6. Biosynthesis and Metabolism

Biosynthesis.—*P. griseofulvum* and *P. patulum* give rise to a similar series of metabolic products and, in addition to griseofulvin and 6-methysalicylic acid (45), patulin (46) and gentisic acid and a series of simple phenolic acids, which presumably arise from glucose by the shikimic acid route, have been isolated from both organisms.[75,76]

Mycelianamide (47)[77] and fulvic acid (57)[78] have been identified as metabolic products of *P. griseofulvum*; and the benzophenones (51; R = Cl, R′ = Me; R = Cl, R′ = H; R = R′ = H) and (50), the xanthone (49), (−)-dehydrogriseofulvin (21), and the dechloro-trione (4; R = H) have been isolated from fermentation broths of *P. patulum*[79,80] although some of these were obtained by using chloride-deficient media.

The biosynthesis of many fungal metabolites by head-to-tail linkage of "acetate units" is firmly established and the most direct route to griseofulvin involves two stages [(a) and (c) of Chart 6]. First, direct cyclisation

[70] Bedford, Child, and Tomich, *Nature*, 1959, **184**, 364.
[71] Ashton and Brown, *Analyst*, 1956, **81**, 220.
[72] Ashton and Toothill, *Analyst*, 1956, **81**, 225.
[73] Daly, *Analyst*, 1961, **86**, 129.
[74] Ashton, *Analyst*, 1956, **81**, 228.
[75] Bassett and Tanenbaum, *Experientia*, 1958, **14**, 38.
[76] Simonart, Wiaux, and Verachtert, *Bull. Soc. Chim. biol.*, 1959, **41**, 541.
[77] Birch, Massy-Westropp, and Rickards, *J.*, 1956, 3717.
[78] Dean, Eade, Moubasher, and Robertson, *J.*, 1957, 3497.
[79] McMaster, Scott, and Trippett, *J.*, 1960, 4628.
[80] Rhodes, Boothroyd, McGonagle, and Somerfield, *Biochem. J.*, 1961, **81**, 28.

of the polyoxomethylene intermediate (52)[81] is coupled with methylation[82] (by transfer through the biochemical C_1 donor systems[83]) and chlorination steps in the sequence[80] (51; R = R′ = H) → (51; R = Cl, R′ = H), giving the benzophenone (51; R = Cl, R′ = Me), although proof of this sequence by the use of labelled materials is still awaited. Next, in stage (c), it has been suggested that (−)-dehydrogriseofulvin (21) is formed from (51; R = Cl, R′ = Me) by oxidative radical coupling[55] and is then reduced stereospecifically to griseofulvin which has the labelling pattern (48) when $Me\cdot{}^{14}CO_2H$ is used as substrate.[81] The xanthone (49) could arise from the alternative elimination. Although there is evidence that chlorination takes place at the benzophenone step, chlorination of a grisan intermediate occurs[84] in the biogenesis of geodin (63; R = Cl).

Less direct biosynthetic routes to some fungal metabolites may also exist and a hypothetical sequence which involves ring-closure of an orsellinic acid relative of type (53) followed by fission as shown in (56) has been suggested for the biogenesis of citromycetin [labelling pattern from $Me\cdot{}^{14}CO_2H$ as shown in (58)] which is produced together with palitantin (53) by *Penicillium frequentans*. The intermediate (55) can also give rise to fulvic acid (57) and to the benzophenones (51) by closure of the second ring; and the production of both griseofulvin and fulvic acid by the same organism suggests that this less direct route (b) could be involved in griseofulvin biogenesis.[85]

A decision between these two routes is theoretically possible. "Acetate-derived" carbon skeletons are initiated by the condensation of molecules of malonylcoenzyme A and acetylcoenzyme A with simultaneous decarboxylation; and this step is followed by further condensation with malonylcoenzyme A. By route (a) of Chart 6 the acetate-derived unit becomes the 6′-methyl substituent in griseofulvin; but by route (b) it becomes part of ring A [shown by dotted lines in structure (54)] and the carbon atom marked * at 6′ comes from the fourth unit of the oxomethylene chain (52). Present evidence[86] suggests that the acetic acid derived by Kuhn–Roth oxidation of the 6′-methyl group in labelled griseofulvin carries an excess of activity over that required for uniform labelling of the molecule and that route (a) is correct.

Metabolism.—The slow disappearance of griseofulvin from media supporting growth of *B. allii* and *Mucor ramannianus* has been attributed to enzymic degradation on the hyphal surface.[13] Although a phenolic metabolic product remained unidentified,[87] demethylation is known

[81] Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360.
[82] Birch, *Proc. Chem. Soc.*, 1962, 3.
[83] Hockenhull and Faulds, *Chem. and Ind.*, 1955, 1390.
[84] Rhodes, McGonagle, and Somerfield, *Chem. and Ind.*, 1962, 611.
[85] Whalley, "Chemistry of Natural Phenolic Compounds," ed. Ollis, Pergamon Press, Oxford, 1961, p. 20.
[86] Birch, Cassera, and Rickards, *Chem and Ind.*, 1961, 792.
[87] Grove, Jeffs, and Rustidge, *J.*, 1956, 1956.

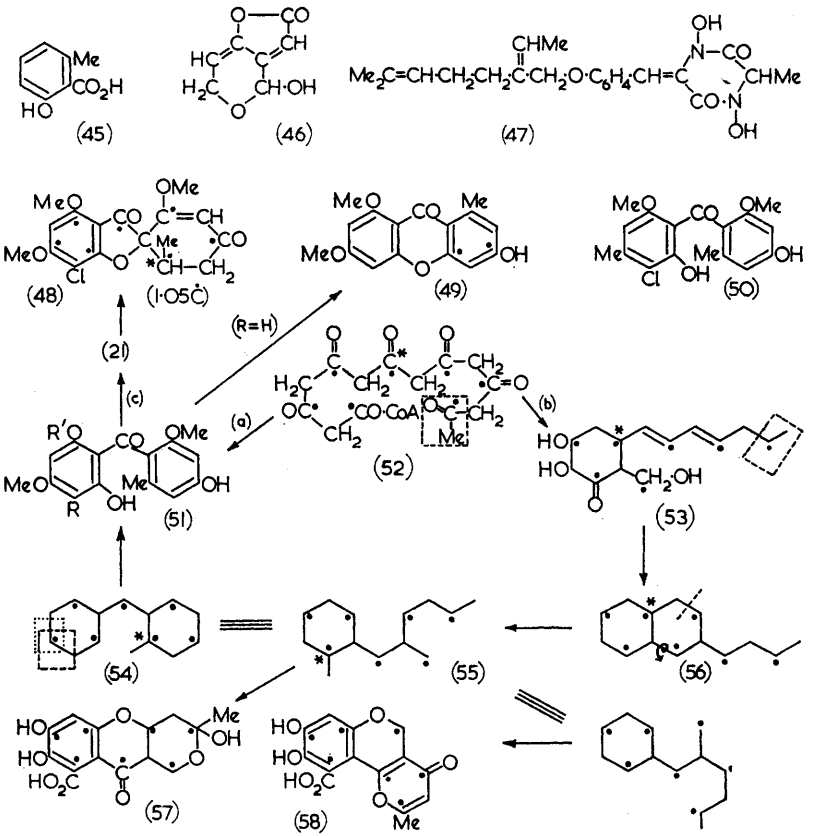


CHART 6.

• denotes atom derived from CO_2H of an "acetate" unit

to be the first step in the degradative sequence since the trione (4; R = Cl) and the phenols (26; R = OMe, R′ = Me, R″ = H; and R = OMe, R′ = H; R″ = Me) have been isolated[88] after destruction of griseofulvin by *B. allii*, *Cercospora melonis*, and *M. canis*, respectively. In this work, although the amounts of demethylated products did not account for all the griseofulvin metabolised, no other metabolic product was detected. The quantitative liberation of chloride ion during the decomposition of griseofulvin by a *Pseudomonas* species[35] suggested that rupture of the aromatic ring is a feature of microbial degradation.

The salicylic acid (10)[89] and the phenol (26; R = OMe, R′ = Me, R″ = H)[90] have been isolated from mammalian urine after oral adminis-

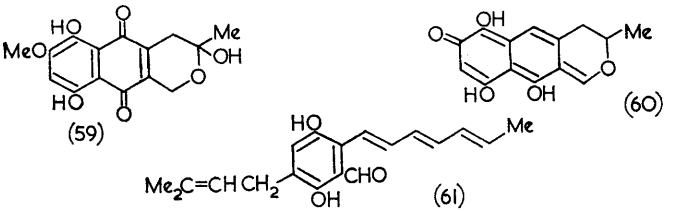
[88] Boothroyd, Napier, and Somerfield, *Biochem. J.*, 1961, **80**, 34.
[89] Tomomatsu and Kitamura, *Chem. Pharm. Bull.*, 1960, **8**, 755.
[90] Barnes and Boothroyd, *Biochem. J.*, 1961, **78**, 41.

tration of griseofulvin: in man the amount of griseofulvin in the urine was less than 1% of that administered, but large amounts of the phenol (26; R = OMe, R′ = Me, R″ = H) were present.[90]

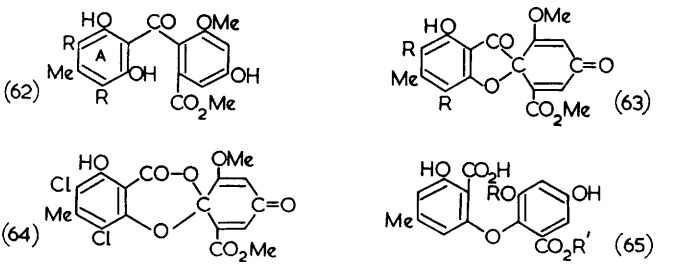
Griseofulvin was slowly degraded in higher plants to unidentified acidic and phenolic compounds, the half-life in bean tissue being about four days.[32] It was relatively stable in roots, but the isomer (5) was rapidly degraded, the salicylic acid (10) being a major product.[33]

7. The Grisans in Mould-product Chemistry

Examples of mould-product structures, in addition to those already mentioned in Section 6, which are formally, though not necessarily, derived by head-to-tail linkage of seven "acetate" units are found in javanicin (59) and purpurgenone (60).[91] In auroglaucin (61) the precursor



of type (53) has been alkylated by an isoprenoid C_5 unit derived from mevalonate.[92] Chlorination of the oxomethylene chain is not uncommon, but *C*-methylation apparently also occurs and the *P. patulum* metabolic product (50) is related to the group of compounds derived from sulochrin[93] (62; R = H), from *Oospora sulphurea-ochracea*, and dihydrogeodin (62; R = Cl).[94] This group, which has been interconverted synthetically[95] and *in vivo*[84] includes the grisadienones (63; R = H)[95] and geodin (63; R = Cl),[46] the depsidone analogue geodoxin (64),[96] and the group[95] of



[91] Whalley, *Chem. and Ind.*, 1958, 131.
[92] Birch, Schofield, and Smith, *Chem. and Ind.*, 1958, 1321.
[93] Nishikawa, *Acta Phytochim. Japan*, 1939, **11**, 167.
[94] Komatsu, *J. Agr. Chem. Soc. Japan*, 1957, **31**, 909, and earlier papers.
[95] Curtis, Hassall, Natori, and Nishikawa, *Chem. and Ind.*, 1961, 1360.
[96] Hassall and McMorris, *J.*, 1959, 2831.

diphenyl ethers related to osoic (65; R = R′ = H) and asterric acid[97] (65; R = R′ = Me) from *Aspergillus terreus*. However, other biosynthetic pathways to (62) involving the cyclisation of a chain made up of eight "acetate" units followed by the loss of one carbon atom and fission (cf. Chart 6) of a tricyclic structure[98] to give a ring A based on 3,5-dihydroxy-*p*-toluic acid have still to be excluded. Proof of a biosynthetic pathway common to *Penicillium*, *Oospora*, and *Aspergillus* spp. would be of interest.

8. Structure–Activity Relations

The ability to produce the morphological changes characteristic of griseofulvin in *B. allii* hyphae is not confined to griseofulvin. The fluoro-analogue (1; R = F) equals griseofulvin in activity;[8] the dechloro- (1; R = H), bromo- (1; R = Br), and other ring A analogues also produce the effect, as do many of the griseofulvin ring C transformation products, *e.g.*, the isomer (5) and the ketone (12), but at higher concentrations.[34] Compounds more active than griseofulvin can be obtained by increasing the oil–water partition coefficient, and the homologues (26; R = OPr^n and OBu^n, R′ = R″ = Me) had 20–50 times the activity of griseofulvin *in vitro*.[34] A polar group in ring C may be necessary as the transformation products (8; R = R′ = H) and (23; R = H) were inactive. This group may be at position 2′ as in the ketone (13), and not necessarily at position 4′; the relation[15] between biological activity and the 4,4′-oxygen–oxygen distance in appropriately substituted grisans, which can be correlated with certain oxygen–nitrogen distances in purine ribosides, is likely to prove fortuitous. Activity is only present in neutral molecules: the acidic trione (4; R = Cl) and the basic griseofulvamine (26; R = NH_2, R′ = R″ = Me) were without activity.[34]

Stereochemistry has a decisive influence and, of the four stereoisomers, only griseofulvin (1; R = Cl) is active:[61] racemic griseofulvin had half the activity of griseofulvin,[56] and the racemic (*l*,*d*)-diastereoisomer (3) together with all the transformation products with the (*l*,*d*)-stereochemistry,[58] were inactive.[34] The synthetic racemic ketone (12)[68] had half the activity of the (*d*,*d*)-isomer. On elimination of the asymmetric 6′-centre some activity is retained and dehydrogriseofulvin (21) and the synthetic grisenone (43) showed typical (weak) griseofulvin-like activity. Grisan-3,4′-dione (34) was inactive. A halogen substituent in the 5- instead of the 7-position reduced activity;[9] and although geodin (63; R = Cl), in common with many dienones, showed fungistatic activity, the typical griseofulvin-like effects were lacking both in the natural product and in its methyl ether. A free 5-position on the aromatic ring is therefore advantageous. Activity is also confined to compounds with the grisan nucleus: synthetic compounds with a 5-membered ring C, *e.g.*, (40), were inactive.

[97] Curtis, Hassall, Jones, and Williams, *J.*, 1960, 4838.
[98] Gatenbeck, *Svensk. kem. Tidskr.*, 1960, **72**, 188.

Apart from a study[33,34] of the activity of some griseofulvin relatives in broad-bean plants (see Section 4) little detailed information has been published on the activity of griseofulvin analogues and transformation products *in vivo*. None of the ring A or ring C analogues tested had systemic activity in the guinea pig greater than that of griseofulvin;[9] esters of the alcohol (23; R = OH) were found to be of comparable activity, but since they were inactive *in vitro* it seems likely that they were converted, *in vivo*, by hydrolysis and oxidation, into griseofulvin.[53]