^{2}H Nuclear Magnetic Resonance Studies on Biosynthesis: Stereochemistry of the 5′-Hydrogen Atoms of Griseofulvin derived from Griseophenone B and 4-Demethyldehydrogriseofulvin

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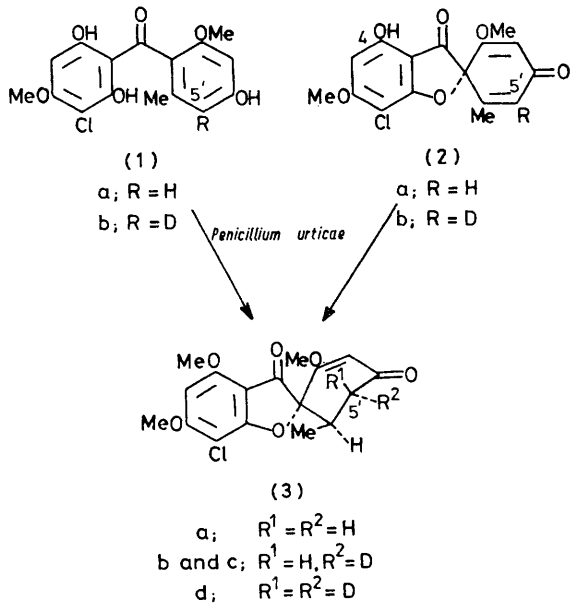
Summary ^{2}H N.m.r. spectroscopy has been used to establish that in *Penicillium urticae*, griseofulvin is biosynthesized with the 5′α-configuration of deuterium from [5′-^{2}H]griseophenone B and 4-demethyl-[5′-^{2}H]-dehydrogriseofulvin.

WE have already demonstrated that ^{2}H n.m.r. spectroscopy[1] is very powerful method for observing the fate of hydrogen atoms in biosynthetic processes[2] and microbial transformations[3] in the griseofulvin series. Rhodes[4] and Harris[5] have established the biosynthetic transformation of griseophenone B (**1a**) and 4-demethyldehydrogriseofulvin (**2a**), respectively, to griseofulvin (**3a**). However, stereochemical studies of the 5′-hydrogen atoms using these precursors (**1a** and **2a**) have not been carried out. We now report the stereochemical fate of the deuterium atom at C-5′ during the biosynthetic transformations of (**1a**) and (**2a**) by *Penicillium urticae* as studied by ^{2}H n.m.r. and mass spectrometry.

The two deuteriated tracers were synthesized as follows. Dehydrogenation of [5′-^{2}H]griseofulvin[3] with selenium oxide followed by demethylation as previously described[6] gave 4-demethyl[5′-^{2}H]dehydrogriseofulvin (**2b**) ($^2H_0$ 66·0, $^2H_1$ 34·0%). Treatment[7] of (**2b**) with zinc-acetic acid afforded [5′-^{2}H]griseophenone B (**1b**) ($^2H_0$ 71·1, $^2H_1$ 29·9%).



SCHEME. R^1 and R^2 correspond to the β- and α-configuration, respectively.

To a suspension† in medium 2 (15 40-ml flasks, 15 ml per flask) of the mycelium obtained from 7-day-old shaken cultures of *Penicillium urticae* in medium 1 (15 40-ml flasks, 20 ml per flask), was administered 15 mg of [5′-^{2}H]griseophenone B (**1b**). After a further 3 days griseofulvin (**3b**) ($^2H_0$ 97·7, $^2H_1$ 2·3%) was isolated from the broth. Similarly a tracer experiment with 4-demethyl[5′-^{2}H]dehydrogriseofulvin (**2b**) afforded another deuteriated griseofulvin (**3c**) ($^2H_0$ 95·8, $^2H_1$ 4·2%) (Scheme). Since the ^{2}H n.m.r. resonances of (**3b**) and (**3c**) are at the same position as that of the 5′α-signal‡ of [5′α, 5′β-^{2}H]griseofulvin (**3d**) (see Figure), the configuration of the deuterium atoms was unequivocally ascribed as 5′α.

Accordingly, the present ^{2}H n.m.r. results confirm our previous conclusion[2] that, in the feeding experiments involving [2-$^2H_3$]acetate, the 5′-deuterium atom in biosynthetically deuteriated griseofulvin has the α-configuration. The isotopic dilutions of (**3b**) and (**3c**), as calculated from mass spectroscopy, were 13 and 8, respectively, consistent with the postulated biosynthetic sequence[5] that (**2b**) is at a closer stage than (**1b**) to the final product. Furthermore, it is interesting that the stereochemical course of the 5′-hydrogen atoms is the same as that in the microbial hydrogenation[3] of dehydrogriseofulvin to griseofulvin (**3a**) by *Streptomyces cinereocrocatus* NRRL 3443, suggesting the existence of similar enzyme systems in both microorganisms.

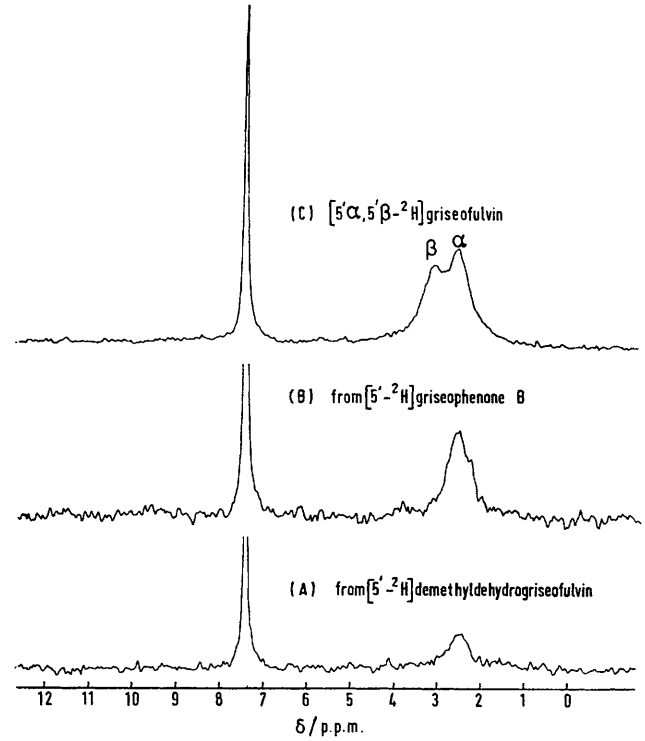


FIGURE. ^{2}H N.m.r. spectra of 5′-deuteriated griseofulvin samples (**3b** and **3c**) biosynthetically obtained from 4-demethyl-[5′-^{2}H]dehydrogriseofulvin and from [5′-^{2}H]griseophenone B, and of [5′α, 5′β-^{2}H]griseofulvin (**3d**). Spectra were recorded on a JEOL PFT-100/EC-100 spectrometer in the pulsed Fourier transform mode at 15·28 MHz in chloroform solution (C_6F_6 internal lock). The sharp signal at lower field is due to natural abundance ^{2}H in the chloroform. Spectral width 500 Hz, 2K data points, 90° pulse (28 μs), repetition time 2·2 s. (A) (**3c**), 30 mg, 24,773 transients; (B) (**3b**), 30 mg, 14,691 transients; (C) (**3d**), 60 mg, 410 transients.

The present experiments, coupled with the results of earlier studies (penicillin G,[8] ovalicin,[9] and pterocarpin[10] biosynthesis, and refs. 2 and 3) indicate that ^{2}H n.m.r. spectroscopy is a valuable method for examining the fate of hydrogen atoms in microbial and plant systems.

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† Medium 1 consists of: corn steep liquor (40 g), lactose (70 g), KH_2PO_4 (6 g), KCl (2 g), $CaCO_3$ (5 g), and H_2O to make 1 l (pH 6·2); medium 2 consists of: glucose (20 g), KH_2PO_4 (5 g), KCl (0·5 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), and H_2O to make 1 l (pH 7·6).

‡ The ^{2}H n.m.r. spectrum of [5′α,5′β-^{2}H]griseofulvin was assigned on the basis of the ^{1}H n.m.r.-chemical shifts reported by Levin, since chemical-shift displacements due to isotope effect are negligible; S. G. Levin and R. E. Hicks, *Tetrahedron Letters*, 1971, 311.

[1] P. Diehl and Th. Leipert, *Helv. Chim. Acta*, 1965, **47**, 545; P. Diehl in 'Nuclear Magnetic Resonance Spectroscopy of Nuclei Other than Protons,' eds. T. Axenrod and G. A. Webb, Wiley-Interscience, New York, 1974, p. 257; H. H. Mantsch, H. Saitô, and I. C. P. Smith, *Progr. N.M.R. Spectroscopy*, 1977, **11**, 211.
[2] Y. Sato, T. Oda, and H. Saitô, *Tetrahedron Letters*, 1976, 2695.
[3] Y. Sato, T. Oda, and H. Saitô, *J.C.S. Chem. Comm.*, 1977, 415.
[4] A. Rhodes, G. A. Somerfield, and M. P. McGonagle, *Biochem. J.*, 1963, **88**, 349.
[5] C. M. Harris, J. S. Roberson, and T. M. Harris, *J. Amer. Chem. Soc.*, 1976 **98**, 5380.
[6] V. Arkley, J. Attenburrow, G. I. Gregory, and T. Walker, *J. Chem. Soc.*, 1962, 1260.
[7] D. Taub, C. H. Kuo, H. L. Slates, and N. L. Wendler, *Tetrahedron*, 1963, **19**, 1.
[8] B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 123.
[9] D. E. Cane and S. L. Buchwald, *J. Amer. Chem. Soc.*, 1977, **99**, 6132.
[10] P. M. Dewick and D. Ward, *J.C.S. Chem. Comm.*, 1977, 338.