Microbial Transformation of Dehydrogriseofulvin and Griseofulvin: ^2H N.m.r. and Mass Spectrometric Studies of Stereochemical Courses of Microbial Hydrogenation and Hydroxylation

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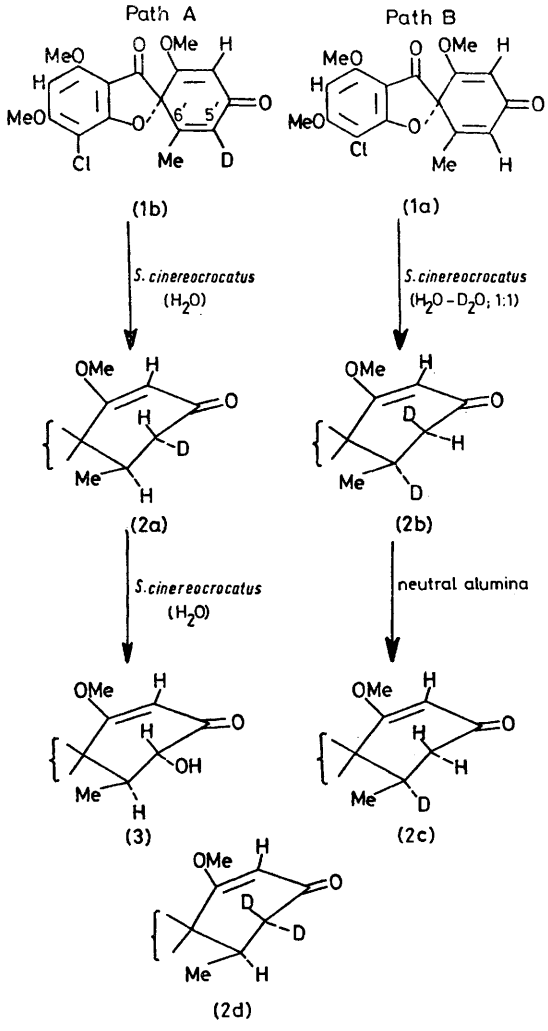
Summary ^2H N.m.r. spectroscopy has been used to determine the stereochemistry of microbial hydrogenation of dehydrogriseofulvin to griseofulvin and microbial hydroxylation of the latter to 5′α-hydroxygriseofulvin, respectively.

WE have recently demonstrated that ^2H n.m.r. spectroscopy provides very powerful method to study biosynthetic pathways involving hydrogen.[1] ^2H N.m.r. chemical shifts, expressed in p.p.m., are essentially the same as those of the analogous ^1H isotope.[2] Therefore, ^2H n.m.r. signals of griseofulvin and related compounds can be assigned on the basis of known chemical shifts in the corresponding ^1H n.m.r. spectra.[3] We now report on the stereochemical courses of the deuterium atom(s) at C-5′ during the microbial transformation of dehydrogriseofulvin (**1a**) and griseofulvin by *Streptomyces cinereocrocatus* NRRL 3443 as studied by ^2H n.m.r. and mass spectrometry. The microbial transformation of (**1a**) to griseofulvin and further conversion of the latter into 5′α-hydroxygriseofulvin (**3**) were initially investigated by Andres and his co-workers.[4]

[5′-^2H]Dehydrogriseofulvin (**1b**) ($^2H_0$ 73·9, $^2H_1$ 26·1%) was administered to a shaken culture of *S. cinereocrocatus* on the 4th day of the fermentation period. After 3 days, griseo-

fulvin (**2a**) ($^2H_0$ 75·4, $^2H_1$ 24·6%) was isolated from the broth. Since the ^2H n.m.r. resonance of (**2a**) is at the same position as that of the 5′α-signal of [5′α,5′β-^2H]griseofulvin (**2d**) ($^2H_0$ 21·5, $^2H_1$ 53·6, $^2H_2$ 24·9%) prepared by a previously described method[1,3b] (Figure, A and C), the configuration of the deuterium was unequivocally ascribed as 5′α. As



SCHEME

shown in the Figure, B, the ^2H n.m.r. spectrum of the mixture of (**2a**) and (**3**) (4·2 : 1) exhibits only one signal, corresponding to the 5′α-signal of [5′α-^2H]griseofulvin (**2a**). Accordingly, it is concluded that the hydroxylation occurs at the 5′α-position of (**2a**) without any configurational change of the deuterium, as summarized in the Scheme, A. These results are in accord with the hydroxylation mechanism in the steroid series.[5]

The above results were further confirmed by an alternative study of deuteriation at the 5′-position by D_2O in the fermentation medium *via* microbial hydrogenation (Scheme, B). A medium containing 50% D_2O and undeuteriated dehydrogriseofulvin (**1a**) was inoculated with a culture of *S. cinereocrocatus* which had been fermenting for 3 days. The ^2H n.m.r. spectrum of the purified griseofulvin (**2b**), which was obtained after 3 days fermentation, is shown in the Figure, D. Because the deuterium atom could be simultaneously incorporated at the 6′-position also under these conditions, the deuterium peak, the position of which is in agreement with that of the 5′β-signal, may be a superposition of 5′β- and 6′-signals. However, it was possible to prove that some incorporation of deuterium had occurred at the 6′-position by treatment[3c] with neutral alumina (Woelm, activity II) for 48 h, which removes the deuterium at the 5′β-position selectively. The decrease in deuterium content was as follows: (**2b**); 0·33 ^2H/molecule, $^2H_0$ 69·3, $^2H_1$ 28·6, and $^2H_2$ 2·1%, and (**2c**); 0·13 ^2H/molecule, $^2H_0$ 86·6 and $^2H_1$ 13·4%. In harmony with this, the

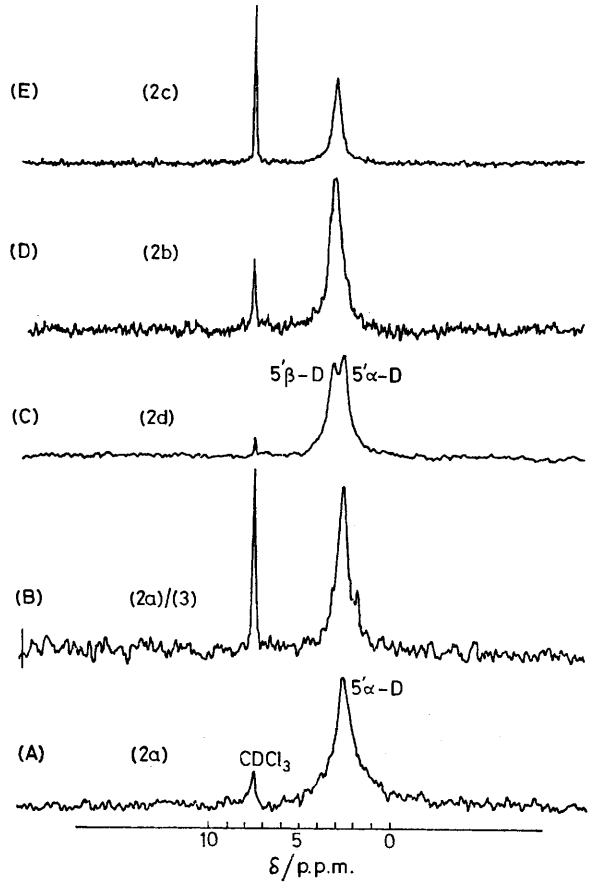


FIGURE. ^2H N.m.r. spectra of microbial transformation products of dehydrogriseofulvin and griseofulvin, and of [5′α,5′β-^2H]-griseofulvin, in chloroform (C_6F_6, internal lock) at 15·28 MHz on a JEOL PFT-100/EC-100 spectrometer with proton noise-decoupling. The sharp signal at lower field is due to the ^2H signal of natural abundance $CDCl_3$. (A) (**2a**), 22 mg, 300 transients; (B) Mixture of (**2a**) and (**3**) (4.2 : 1), 9 mg, 2652 transients; (C) (**2d**), 22 mg, 706 transients; (D) (**2b**), 22 mg, 1076 transients; (E) (**2c**), 19 mg, 22,000 transients.

peak intensity of (**2c**, 19 mg) decreased considerably in comparison with that of (**2b**, 22 mg) (Figure, D and E). Treatment of (**2c**) with neutral alumina for a further 24 h showed that the deuterium content was 0·11 ^2H/molecule ($^2H_0$ 89·1, $^2H_1$ 10·9%). These results indicate that during the course of the microbial hydrogenation, (**1a**) was transformed to [5′β,6′-^2H]griseofulvin in which deuteriums are

incorporated at the 5′β- and 6′α-positions in *ca.* 2:1 ratio. Finally, the above results were also supported by mass spectrometric studies of the 5′α-hydroxylation products from [5′β-^{2}H]- and [5′α,5′β-^{2}H]-griseofulvin samples.

In conclusion, these ^{2}H n.m.r. and mass spectrometric studies clearly showed that the microbial hydrogenation of dehydrogriseofulvin proceeds with *trans* diaxial reduction at the 5′- and 6′-positions and the microbial hydroxylation of griseofulvin proceeds with direct replacement of the 5′α-proton by a hydroxy-group.

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[1] Y. Sato, T. Oda, and H. Saitô, *Tetrahedron Letters*, 1976, 2695.

[2] P. Diehl and Th. Leipert, *Helv. Chim. Acta*, 1964, **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, in 'Progress in Nuclear Magnetic Resonance Spectroscopy,' Pergamon, Oxford, in the press.

[3] (a) M. Gerecke, E. Kyburz, C. V. Planta, and A. Brossi, *Helv. Chim. Acta*, 1962, **45**, 2241; (b) B. H. Arison and N. L. Wendler, *J. Amer. Chem. Soc.*, 1963, **85**, 627; (c) S. G. Levin and R. E. Hicks, *Tetrahedron Letters*, 1971, 311.

[4] W. W. Andres, W. J. McGahren, and M. P. Kunstman, *Tetrahedron Letters*, 1969, 3777.

[5] M. Hayano, M. Gut, R. I. Dorfman, O. K. Sebek, and D. H. Peterson, *J. Amer. Chem. Soc.*, 1958, **80**, 2336; E. J. Corey, G. A. Gregoriou, and D. H. Peterson, *ibid.*, p. 2338; M. Hayano, M. Gut, R. I. Dorfman, A. Schubert, and R. Siebert, *Biochim. Biophys. Acta*, 1959, **32**, 269; S. Baba, H. J. Broidie, M.Hayano, D. H. Peterson, and O. K. Sebek, *Steroids*, 1963, **1**, 151.