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**Microbial Transformation of Dehydrogriseofulvin and Griseofulvin :  
<sup>2</sup>H N.m.r. and Mass Spectrometric Studies of Stereochemical Courses  
of Microbial Hydrogenation and Hydroxylation \***

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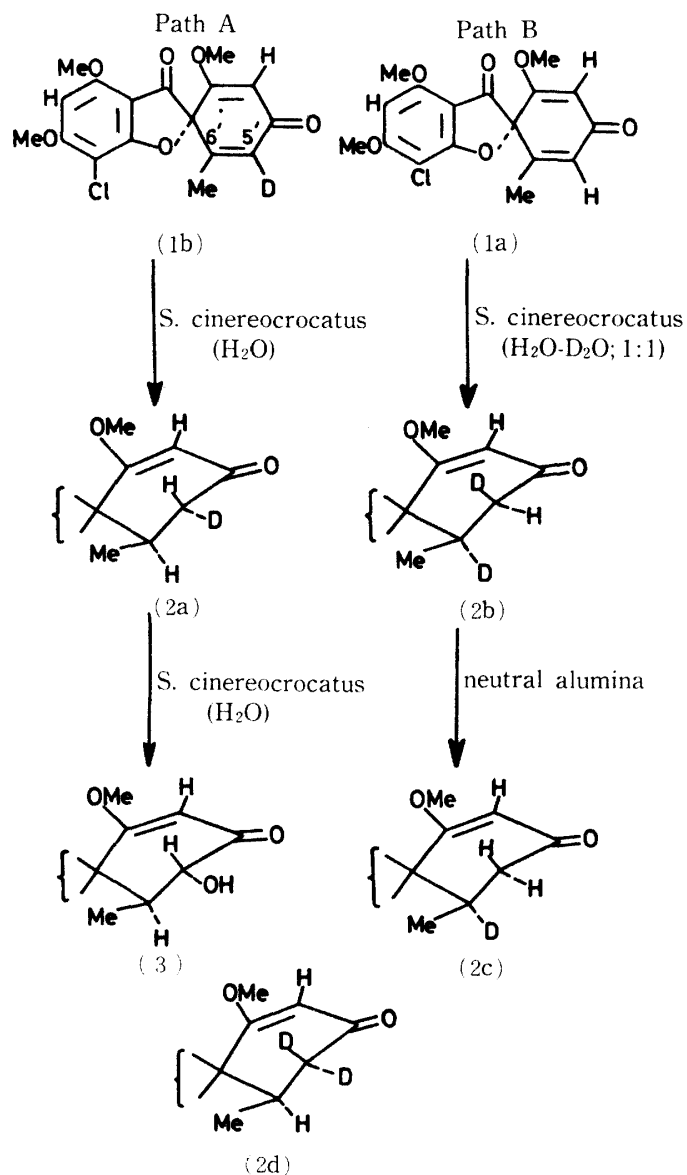
We have recently demonstrated that <sup>2</sup>H n.m.r. spectroscopy provides very powerful method to study biosynthetic pathways involving hydrogen. <sup>2</sup>H N.m.r. chemical shifts, expressed in p.p.m., essentially the same as those of the analogous <sup>1</sup>H isotope. Therefore, <sup>2</sup>H n.m.r. signals of griseofulvin and related compounds can be assigned on the basis of known chemical shifts in the corresponding <sup>1</sup>H n.m.r. spectra. 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 cinereocrocatu*s NRRL 3443 as studied by <sup>2</sup>H n.m.r. and mass spectrometry.

[5'-<sup>2</sup>H] Dehydrogriseofulvin (**1b**) (<sup>2</sup>H<sub>0</sub> 73.9, <sup>2</sup>H<sub>1</sub> 26.1%) was administered to a shaken culture of *S. cinereocrocatu*s on the 4th day of the fermentation period. After 3 days, griseofulvin (**2a**) (<sup>2</sup>H<sub>0</sub> 75.4, <sup>2</sup>H<sub>1</sub> 24.6%) was isolated from the broth. Since the <sup>2</sup>H n.m.r. resonance of (**2a**) is at the same position as that of the 5'α-signal of [5'α,5'β-<sup>2</sup>H] griseofulvin (**2d**) (<sup>2</sup>H<sub>0</sub> 21.5, <sup>2</sup>H<sub>1</sub> 53.6, <sup>2</sup>H<sub>2</sub> 24.9%) prepared by a previously described method (Figure, A and C), the configuration of deuterium was unequivocally ascribed as 5'α. As shown in the Figure, B, the <sup>2</sup>H 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-<sup>2</sup>H] 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.

The above results were further confirmed by an alternative study of deuteration at the 5'-position by D<sub>2</sub>O in the fermentation medium *via* microbial hydrogenation (Scheme, B). A medium containing 50% D<sub>2</sub>O and undeuteriated dehydrogriseofulvin (**1a**) was inoculated with a culture of *S. cinereocrocatu*s which had been fermenting for 3 days. The <sup>2</sup>H 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 condition, 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 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

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\* J.C.S. Chem. Comm., 1977, 415.



SCHEME

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 peak intensity of **(2c)**, 19mg decreased considerably in comparison with of **(2b)**, 22mg (Figure, D and E).

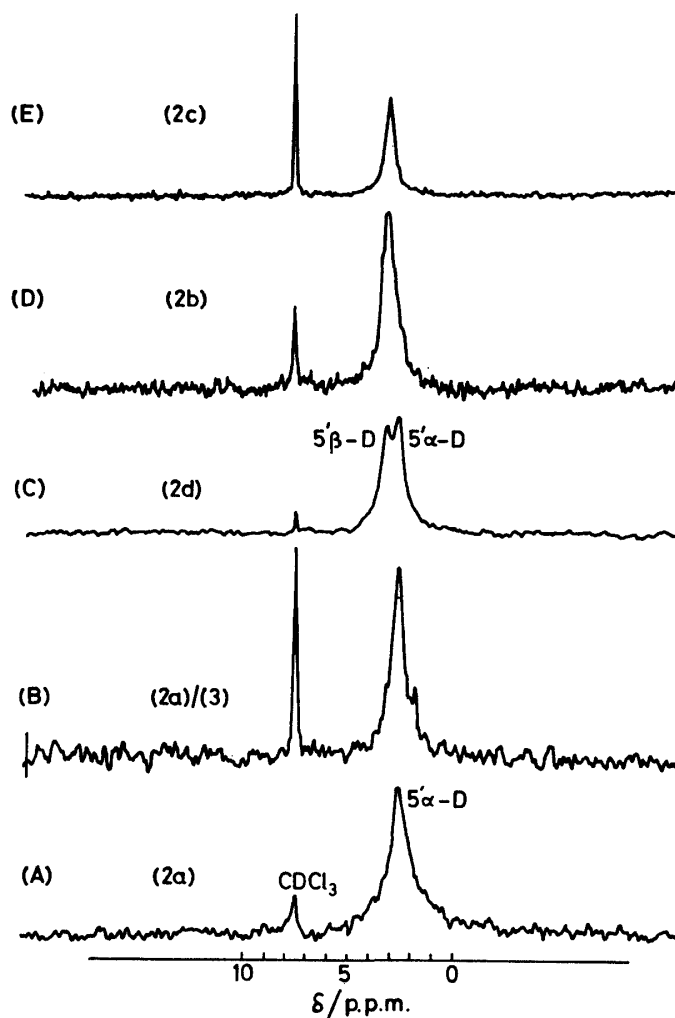


FIGURE.  $^1\text{H}$  N.m.r. spectra of microbial transformation products of dehydrogriseofulvin and griseofulvin, and of  $[5'\alpha,5'\beta\text{-}^2\text{H}]$ -griseofulvin, in chloroform ( $\text{C}_2\text{F}_6$ , internal lock) at 15.28 MHz on a JEOL PFT-100/EC-100 spectrometer with proton noise-decoupling.

Treatment of **(2c)** with neutral alumina for a further 24 h showed that the deuterium content was 0.11  $^2\text{H}$ /molecule ( $^2\text{H}_0$  89.1,  $^2\text{H}_1$  10.9%). These results indicate that during the course of the microbial hydrogenation, **(1a)** was transformed to  $[5'\beta,6'\text{-}^2\text{H}]$ griseofulvin in which deuteriums are incorporated at the  $5'\beta$ - and  $6'\alpha$ -position in ca 2:1 ratio. Finally, the above results were also supported by mass spectrometric studies of the  $5'\alpha$ -hydroxylation products from  $[5'\beta\text{-}^2\text{H}]$ - and  $[5'\alpha,5'\beta\text{-}^2\text{H}]$ -griseofulvin samples.