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Microbial Transformation of Dehydrogriseofulvin and Griseofulvin : ^{2}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 ^{2}H n.m.r. spectroscopy provides very powerful method to study biosynthetic pathways involving hydrogen. ^{2}H N.m.r. chemical shifts, expressed in p.p.m., essentially the same as those of the analogous ^{1}H isotope. Therefore, ^{2}H n.m.r. signals of griseofulvin and related compounds can be assigned on the basis of known chemical shifts in the corresponding ^{1}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 cinereocrocatus* NRRL 3443 as studied by ^{2}H n.m.r. and mass spectrometry.

〔5′-^{2}H〕 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, griseofulvin **(2a)** ($^2H_0$ 75.4, $^2H_1$ 24.6%) was isolated from the broth. Since the ^{2}H n.m.r. resonance of **(2a)** is at the same position as that of the 5′α-signal of 〔5′α,5′β-^{2}H〕 griseofulvin **(2d)** ($^2H_0$ 21.5, $^2H_1$ 53.6, $^2H_2$ 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 ^{2}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-^{2}H〕 griseoflvin **(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_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 ^{2}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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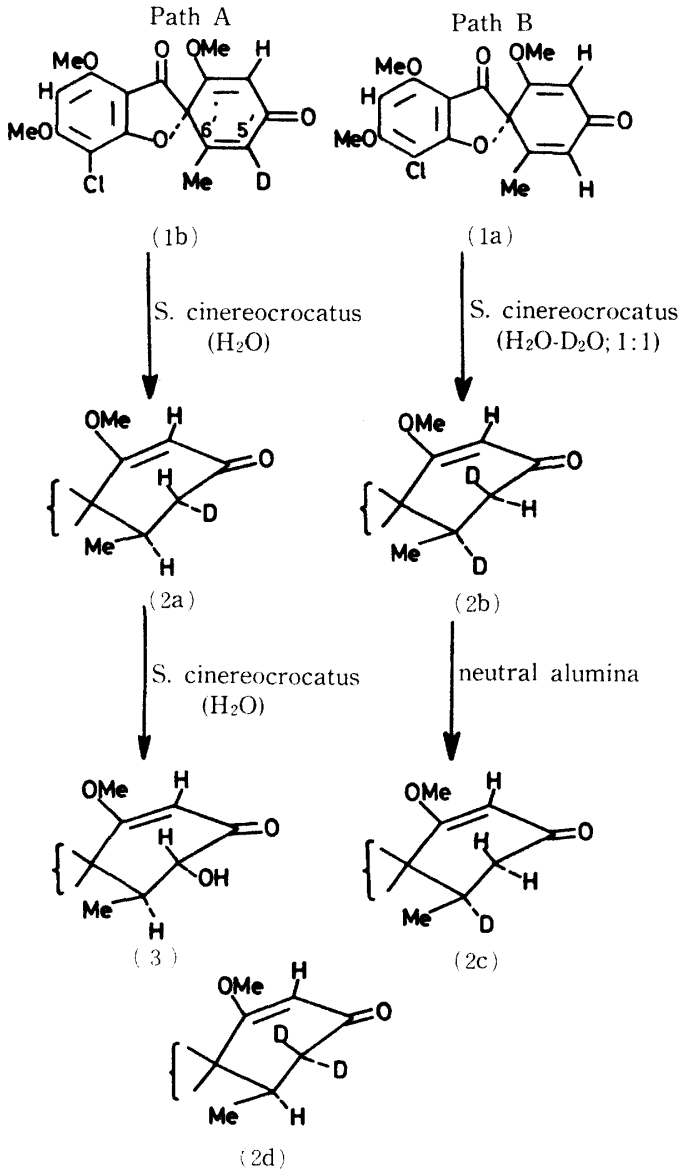

SCHEME

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).

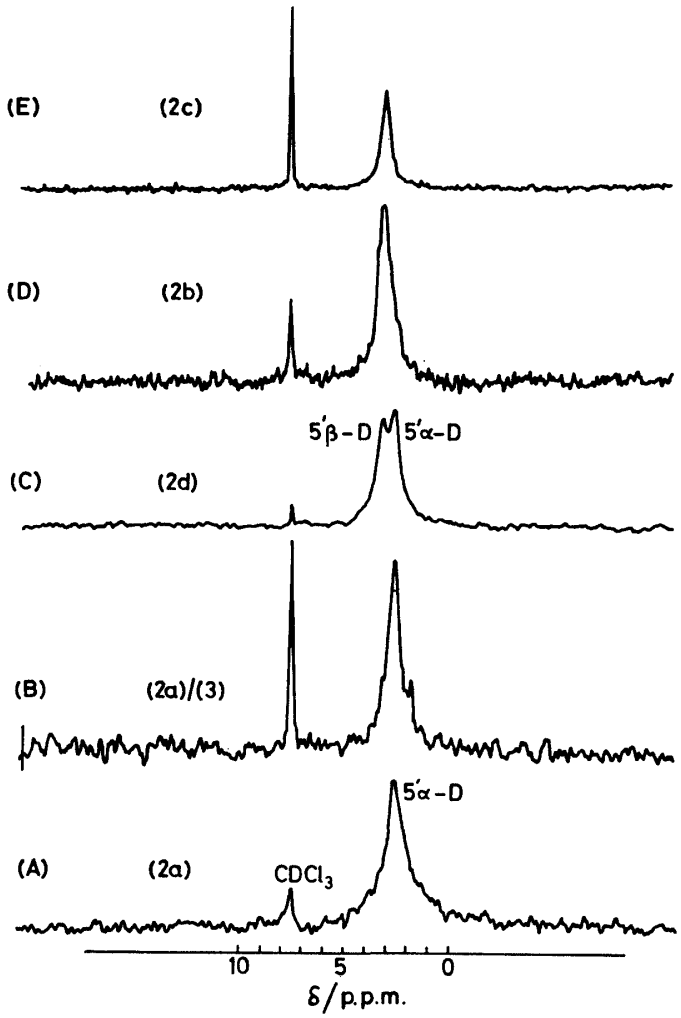

FIGURE. **^{2}H N.m.r. spectra of microbial transformation products of dehydrogriseofulvin and griseofulvin, and of [5'α,5'β-^{2}H]-griseofulvin, in chloroform (C_6F_6, internal lock) at 15·28 MHz on a JEOL PFT-100/EC-100 spectrometer with proton noise-decoupling.**

Treatment of **(2c)** with neutral alumina for a further 24 h showed that the deuterium content was 0.11 ^{2}H/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'-^{2}H]griseofulvin in which deuteriums are incorporated at the 5'β- and 6'α-position 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.