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**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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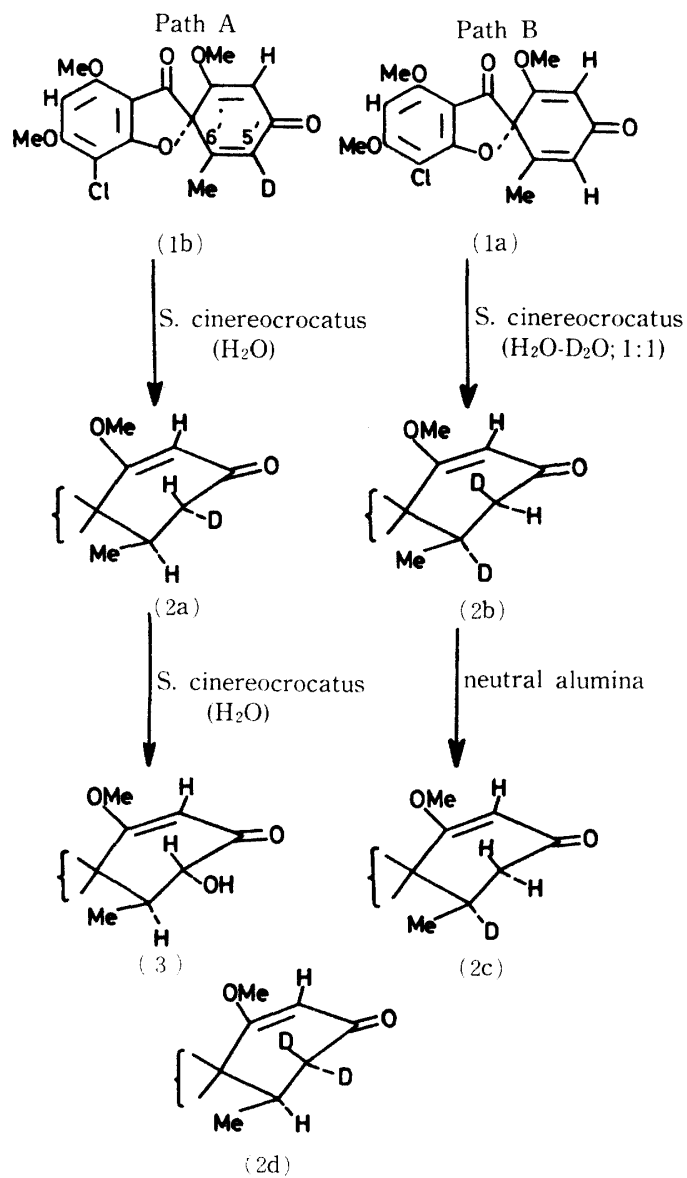
佐藤良博, 小田泰子, 齊藤 肇

We have recently demonstrated that ²H n.m.r. spectroscopy provides very powerful method to study biosynthetic pathways involving hydrogen. ²H N.m.r. chemical shifts, expressed in p.p.m., essentially the same as those of the analogous ¹H isotope. 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. 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 ²H n.m.r. and mass spectrometry.

[5'-²H] Dehydrogriseofulvin (**1b**) (²H₀ 73.9, ²H₁ 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**) (²H₀ 75.4, ²H₁ 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**) (²H₀ 21.5, ²H₁ 53.6, ²H₂ 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 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.

The above results were further confirmed by an alternative study of deuteration at the 5'-position by D₂O in the fermentation medium *via* microbial hydrogenation (Scheme, B). A medium containing 50% D₂O and undeuteriated dehydrogriseofulvin (**1a**) was inoculated with a culture of *S. cinereocrocatu*s 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 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).

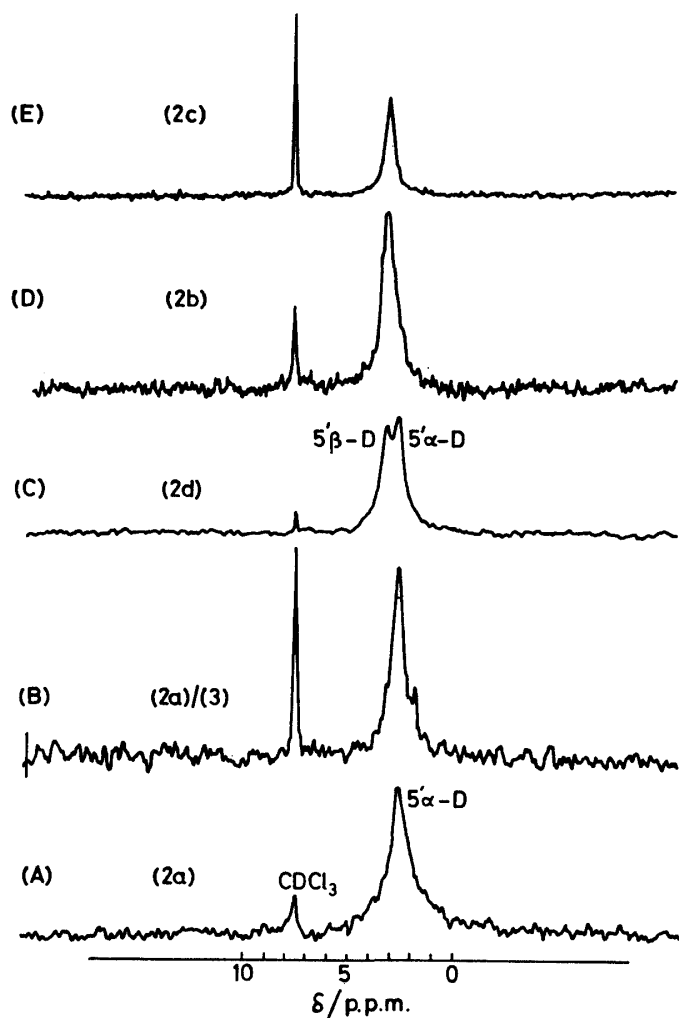


FIGURE. ^2H N.m.r. spectra of microbial transformation products of dehydrogriseofulvin and griseofulvin, and of $[5'\alpha,5'\beta\text{-}^2\text{H}]$ -griseofulvin, in chloroform (C_2F_6 , internal lock) at 15.28 MHz on a JEOL PFT-100/EC-100 spectrometer with proton noise-decoupling.

Treatment of **(2c)** with neutral alumina for a further 24 h showed that the deuterium content was 0.11 ^2H /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.