

Title	Microbial transformation of dehydrogriseofulvin and griseofulvin : ^2H N. m. r. and mass spectrometric studies of stereochemical courses of microbial hydrogenation and hydroxylation
Sub Title	
Author	佐藤, 良博(Sato, Yoshihiro) 小田, 泰子(Oda, Taiko) 斉藤, 肇(Saito, Hajime)
Publisher	共立薬科大学
Publication year	1977
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.22 (1977.) ,p.118- 120
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000022-0118

慶應義塾大学学術情報リポジトリ(KOARA)に掲載されているコンテンツの著作権は、それぞれの著作者、学会または出版社/発行者に帰属し、その権利は著作権法によって保護されています。引用にあたっては、著作権法を遵守してご利用ください。

The copyrights of content available on the KeiO Associated Repository of Academic resources (KOARA) belong to the respective authors, academic societies, or publishers/issuers, and these rights are protected by the Japanese Copyright Act. When quoting the content, please follow the Japanese copyright act.

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

YOSHIHIRO SATO, TAIKO ODA and HAZIME SAITO (National Cancer Center Research Institute)

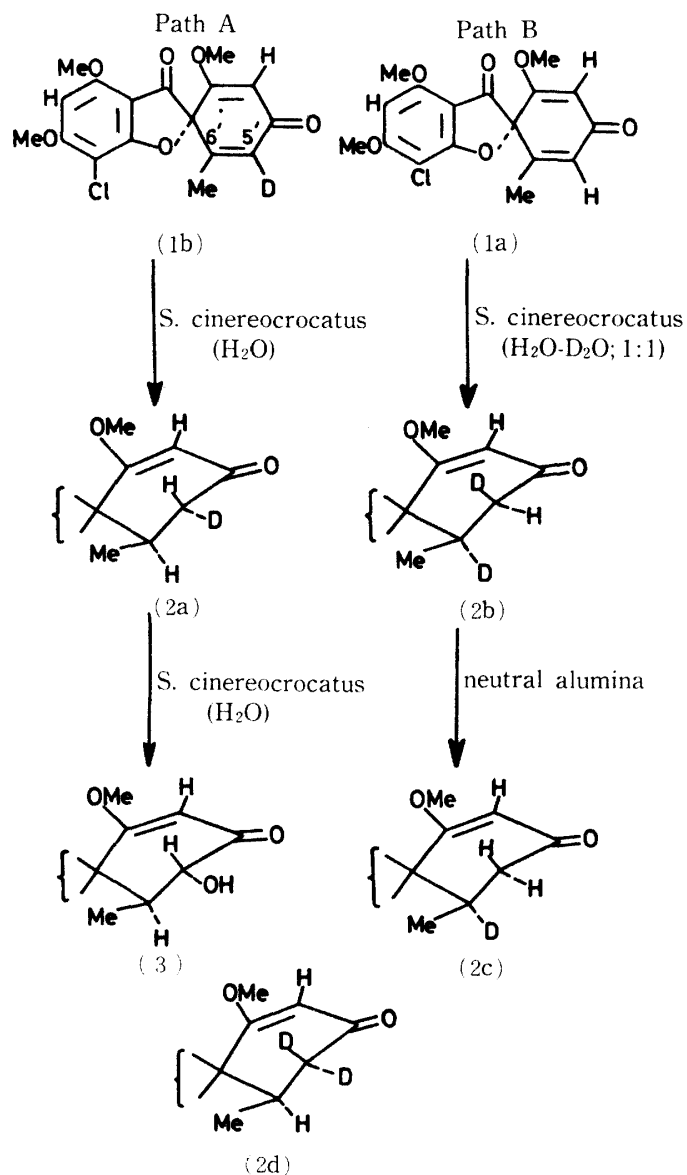
佐藤良博, 小田泰子, 斉藤 肇

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 cinereocrocat* 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. cinereocrocat* 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. cinereocrocat* 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

* 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).

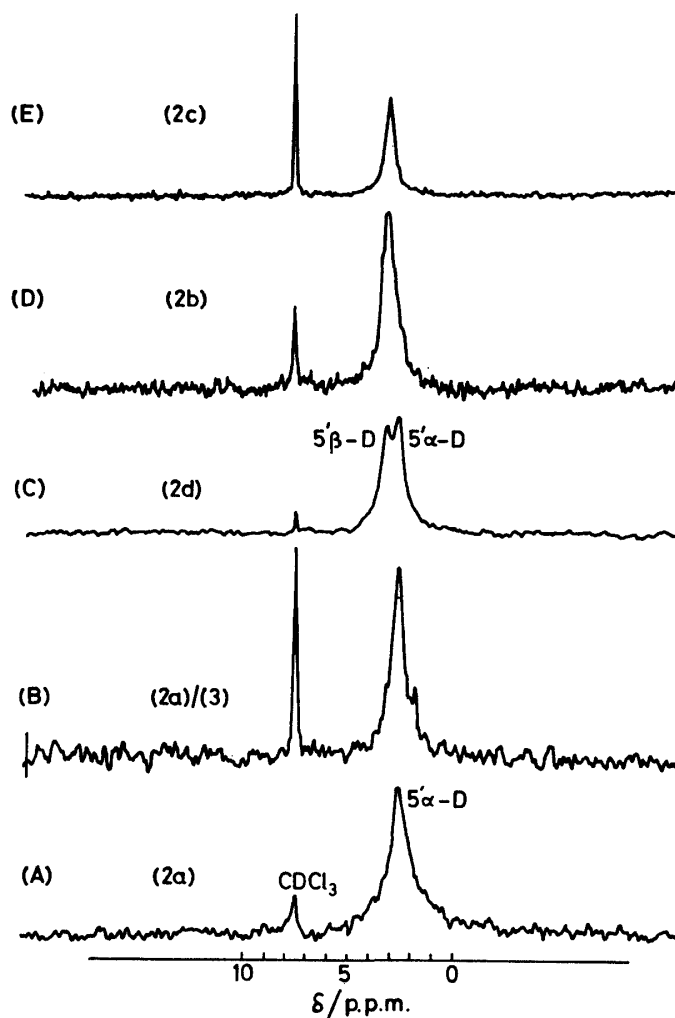


FIGURE. ^1H N.m.r. spectra of microbial transformation products of dehydrogriseofulvin and griseofulvin, and of $[5'\alpha,5'\beta\text{-}^2\text{H}]$ -griseofulvin, in chloroform (C_2F_6 , internal lock) at 15.28 MHz on a JEOL PFT-100/EC-100 spectrometer with proton noise-decoupling.

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.