

Title	Incorporation of [2- <sup>3</sup> H]- and [2- <sup>2</sup> H <sub>3</sub> ]-acetate by penicillium urticae into griseofulvin and determination of the stereochemistry of isotopes at C-5'
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Author	佐藤, 良博(Sato, Yoshihiro) 町田(関), 敏子( Machida(Seki), Toshiko) 小田, 泰子( Oda, Taiko)
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INCORPORATION OF  $[2-^3\text{H}]$ - AND  $[2-^2\text{H}_3]$ -ACETATE BY  
*PENICILLIUM URTICAE* INTO GRISEOFULVIN AND DETERMINATION  
 OF THE STEREOCHEMISTRY OF ISOTOPES AT C-5' \*

Yoshihiro Sato, Toshiko Machida (Seki) and Taiko Oda

佐藤良博, 町田(関)敏子, 小田泰子

Griseofulvin is known as a typical example of polyketomethylene antibiotics. The biosynthetic pathway of griseofulvin from acetate-malonate units has already been established by Birch's and Rhode's groups using  $^{14}\text{C}$  or  $^{36}\text{Cl}$ -precursors. However, problems regarding the extent of incorporation of tritiums or deuteriums from  $[2-^3\text{H}]$ - or  $[2-^2\text{H}_3]$ -acetate into griseofulvin have remained to be solved.

In this communication we wish to report the incorporation of tritium from  $[2-^3\text{H}]$ -acetate into the griseofulvin molecule by *Penicillium urticae*. The labelled precursor consisting of  $1.08 \times 10^7$  dpm of  $[2-^{14}\text{C}]$ -acetate and  $6.77 \times 10^7$  dpm of  $[2-^3\text{H}]$ -acetate ( $^3\text{H}/^{14}\text{C}$  ratio, 6.27) was added to the shaking culture of *P. urticae* on the 8th day of the fermentation period. After 2 or 24 hr of fermentation, broth from each flask was filtered to separate the mycelium and filtrate, both of which were subsequently separated into neutral and acidic fractions. Griseofulvin was then obtained from the neutral fraction. The result is shown in Table I.

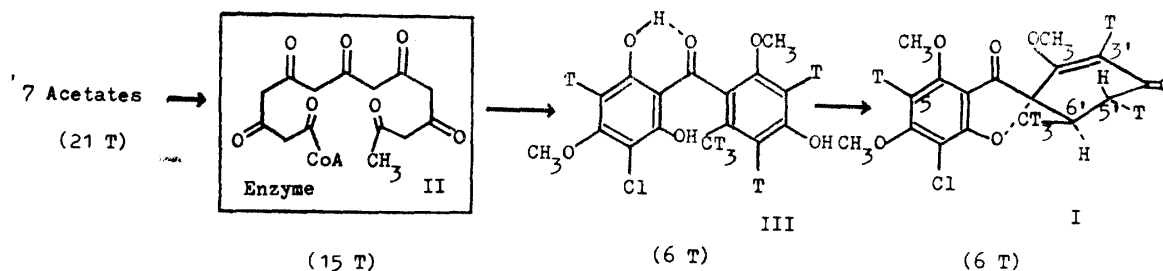
TABLE I. Radioactivity of Griseofulvin obtained from the Filtrate

Fermentation time (hr)	Isotope	dpm/mg	% of Incorp.	$^3\text{H}/^{14}\text{C}$
2	$^3\text{H}$	$7.99 \times 10^3$	0.46	1.85
	$^{14}\text{C}$	$4.32 \times 10^3$	1.43	
24	$^3\text{H}$	$1.29 \times 10^4$	0.55	1.77
	$^{14}\text{C}$	$7.27 \times 10^3$	1.78	

The results indicate that, during the biosynthesis of griseofulvin through polyketomethylene intermediate (II), all the tritium atoms on griseophenone B (III) were retained in the griseofulvin molecule (Chart 1).

The positions of tritium atoms incorporated into griseofulvin were proved by chemical means: Hydrolysis of the doubly labelled griseofulvin ( $^3\text{H}/^{14}\text{C}$  ratio, 2.97) with  $\text{H}_2\text{SO}_4$  in acetic acid gave griseofulvic acid (IV), whose  $^3\text{H}/^{14}\text{C}$  ratio was reduced to 2.56. This shows that one tritium atom is lost from position 3'. Adsorption of a

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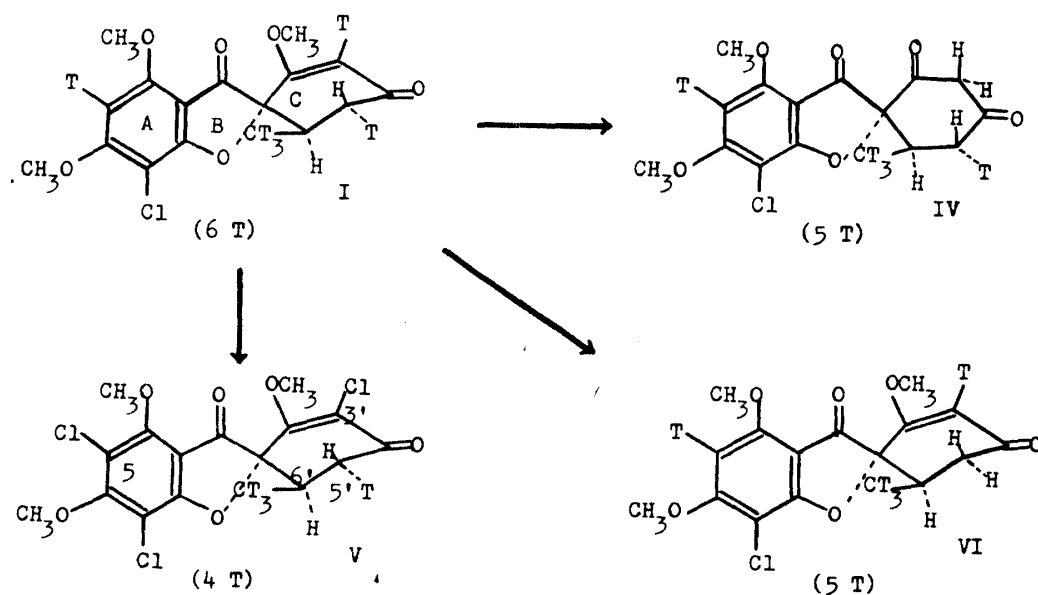
Chart 1. Biosynthesis of Griseofulvin from  $[2-^3\text{H}]\text{-Acetate}$ 

(Tritium is abbreviated as T in the chart)

chloroform solution of griseofulvin ( $^3\text{H}/^{14}\text{C}$  ratio, 2.77) on neutral alumina (Woelm, activity II) overnight afforded 5,3'-dichlorogriseofulvin (V), whose radioactivity ratio (1.87) corresponds to the loss of tritiums from positions 5 and 3'. In other experiment, a mixture of griseofulvin and neutral alumina was stirred in chloroform for 16 hr according to the Levine's method. This procedure is known to effect stereoselective partial replacement of  $5'\beta$ -deuterium of griseofulvin-5',  $5'\text{-d}_2$  by hydrogen.

The product actually obtained by this experiment, however, showed no change of radioactivity ratio, excluding the possibility of  $\beta$ -orientation of tritium at position 5'. Next, griseofulvin ( $^3\text{H}/^{14}\text{C}$  ratio, 2.57) was heated under reflux for 16 hr in  $\text{CH}_3\text{CH}_2\text{-CH}_2\text{C}(\text{CH}_3)_2\text{OH}$  containing  $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)_2\text{ONa}$ , conditions known to effect some exchange of both of the C-5' protons. The product (VI,  $^3\text{H}/^{14}\text{C}$  ratio, 2.45) indicated a loss of radioactivity corresponding to about 30% of one tritium atom (Chart 2).

This considerably small value (30%) was proved to be effective for  $\alpha$ -orientation of tritium at C-5', because the deuteration rates at  $5'\alpha$  of griseofulvin in parallel ex-



periments were about 30-50% of theoretical value on  $^1\text{H}$  nmr and mass spectrometric analyses.

In a separate experiment  $[2\text{-}^2\text{H}_3]\text{-acetate}$  was fermented with the mycelium of *P. urticae* in the medium consisting of 5.0g of KCl, 0.5g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 15.0g of sodium  $[2\text{-}^2\text{H}_3]\text{-acetate} \cdot 3\text{H}_2\text{O}$  (obtained from acetic acid- $\text{d}_4$ , 99.5 Atom% D) and  $\text{H}_2\text{O}$  to a total volume of 1l (pH 7.9). An isotopic composition of  $[^2\text{H}]\text{-griseofulvin}$  thus obtained was proved mass spectrometrically to consist of a deuterated mixture:  $\text{d}_0$  61.4,  $\text{d}_1$  4.3,  $\text{d}_2$  5.9,  $\text{d}_3$  8.6,  $\text{d}_4$  10.6,  $\text{d}_5$  6.4,  $\text{d}_6$  1.4, and  $\text{d}_7$  and  $\text{d}_8 < 1\%$ . Using  $\text{CH}_3\text{O}$  signal as a standard,  $^1\text{H}$  nmr spectrum was examined for the decrease in each  $^1\text{H}$  signal intensity. The positions of deuteriums were 5, 3', 5' $\alpha$  and 6'- $\text{CH}_3$ , and the incorporation ratio at each positions of tritium or deuterium atoms incorporated into griseofulvin are 5 in the A ring, and 3', 5' $\alpha$ , and 6'-methyl in the C ring. This result indicates the *trans* diaxial type reduction on C ring during the courses of griseofulvin biosynthesis.