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A ^2H NMR Study on Biosynthesis. Evidence of Hydrogen Exchange at the Chain-starter Methyl Group of Griseofulvin Manifested by ^2H Incorporation from Deuterium Oxide in Medium and from Sodium Acetates*

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In order to prove the origin of hydrogen atom in the griseofulvin biosynthesis, we attempted to analyze ^2H NMR spectra of griseofulvin samples supplemented with: (a) sodium [2-^{13}C, 2-$^2H_3$]acetate; (b) sodium acetate and deuterium oxide in medium; (c) sodium [2-^{13}C]acetate and deuterium oxide. Here we report evidence of participation of medium water into the methyl group of chain-starter, with use of various kinds of acetate precursors, as a result of hydrogen exchange.

The biosynthetically deuterated griseofulvin samples were obtained in buffers replacing the original culture solution. The mycelium obtained from 7-day-old shaken cultures of *Penicillium urticae* in medium 1 was suspended in medium 2, 3, 4 or 5 for the biosynthetic experiments, respectively. The deuterated griseofulvin samples thus obtained were used for ^2H NMR analysis. ^2H NMR spectra were recorded with a JEOL PFT-100/EC spectrometer operated at 15.28 MHz with proton-decoupled Fourier transform mode.

The observed ^2H NMR peak-intensity of [^2H]griseofulvin is 48, 25, 19 and 8% for 6′-methyl, 5′α-D, 3′-D and 5-D, respectively. The extent of the deuterium incorporation is thus obtained as 1.9(3), 1(1), 0.7(1) and 0.3(1), respectively, when deuterium for 5′α-D is considered to be fully incorporated and the theoretical values are shown in the parentheses. Thus, we noted that ~1 deuterium atom is replaced for 6′-methyl group by hydrogen in [^2H]- and [^2H,^{13}C]griseofulvin samples during biosynthesis from the labelled acetate. Further, we observed direct incorporation of deuterium from medium water containing 50% deuterium oxide. These results strongly support that hydrogen exchange occurs in the 6′-methyl group, which has proven to be the chain-starter group for the heptaketide formation.

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