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GRISEOFULVIN BIOSYNTHESIS: NEW EVIDENCE OF TWO ACETATE-DISPOSITIONS IN THE RING A FROM ^{13}C NUCLEAR MAGNETIC RESONANCE STUDIES

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Griseofulvin (1), the antibiotic produced by Penicillium species, is known to be derived from acetate.[1] Birch et al.[1,2] have revealed the labelling pattern in the skeleton of 1 after incorporation of [1-^{14}C]-acetate using a strain of Penicillium griseofulvum, and have established the general validity of the acetic acid hypothesis.[3] For further biosynthetic studies of 1, Rhodes et al.[4] have demonstrated the incorporation of phenolic intermediates, griseophenone C (2) and B (3). Tanabe et al.[5] have proved that ^{13}C-satellite signals can be applied for the biosynthetic studies of microbial metabolites, giving an example of the [2-^{13}C]-acetate-enriched griseofulvin. We recently examined the incorporation of [2-^{3}H, 2-^{14}C]-acetate and [2-^{2}H$_3$]-acetate into 1 by Penicillium urticae, and demonstrated[6,7] that the positions of tritium and deuterium atoms incorporated are 5 in the ring A, and 3', 5'α, and 6'-methyl in the ring C. And in the experiments in which [2-^{2}H$_3$]-acetate was used as a tracer, it was proved that ^{2}H nmr is very powerful method to study biosynthesis involving hydrogen.[7] In this communication, we report the biosynthetic studies of griseofulvin (1) by ^{13}C nmr using [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C$_2$]-acetate as tracers. In order to elucidate the acetate-disposition especially on the ring A of griseofulvin, first ^{13}C nmr of naturally occuring griseofulvin and two of its derivatives, 5-bromogriseofulvin and dechlorogriseofulvin, were recorded in pentadeuteriopyridine solution with Varian XL-100-12 WG NMR spectrometer equipped with a Varian 620/L computer, operating at 25.2 MHz at 30°C, and ^{13}C chemical shifts were assigned from off-resonance-decoupled, proton-noise-decoupled (p.n.d.) and selective single-frequency-heteronuclear-decoupled spectra.

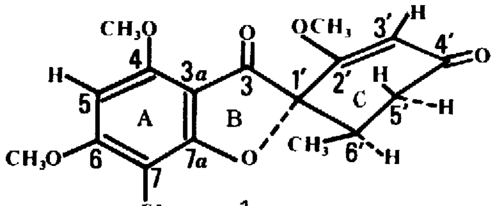

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Table I. ^{13}C-Chemical shifts (δ) of griseofulvin (1); signal patterns of off-resonance-decoupled spectrum of 1; coupling constants (Hz) of [1,2-$^{13}C_2$]-acetate-enriched 1; and signal enhancements observed in a) [2-^{13}C]-acetate-enriched 1 and b) [1-^{13}C]-acetate-enriched 1.

Carbon	δ_{ppm}	J_{c-c} (Hz)	Enrichment** a)	b)
6'-CH_3	14.3 Q*	36	+	
6'	36.8 D	36		+
5'	40.5 T	40	+	
6-OCH_3	56.4 Q	-	+	
2'-OCH_3	56.6 Q	-	+	
4-OCH_3	57.1 Q	-	+	
5	90.7 D	71 73	+	
1'	91.0 S	40	+	
7	96.9 S	81 81	+	
3'	105.4 D	74	+	
3a	105.5 S	75 75	+	
4	158.2 S	76 72		+
6	164.9 S	70 80		+
7a	169.6 S	80 74		+
2'	170.6 S	74		+
3	191.9 S	41		+
4'	195.5 S	41		+

* Q, quartet; T, triplet; D, doublet; S, singlet.

** +, signal enhancement

Table II. Effects (Δδ) of chlorine and bromine substituents on chemical shifts of phloroglucinol trimethylether (4).

	4-Cl	2-Br	2-Br,4-Cl
C(1)	-2.1	-4.2	-6.3
C(3)	-5.1	-4.2	-8.6
C(5)	-5.1	-1.1	-6.3

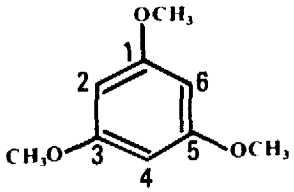

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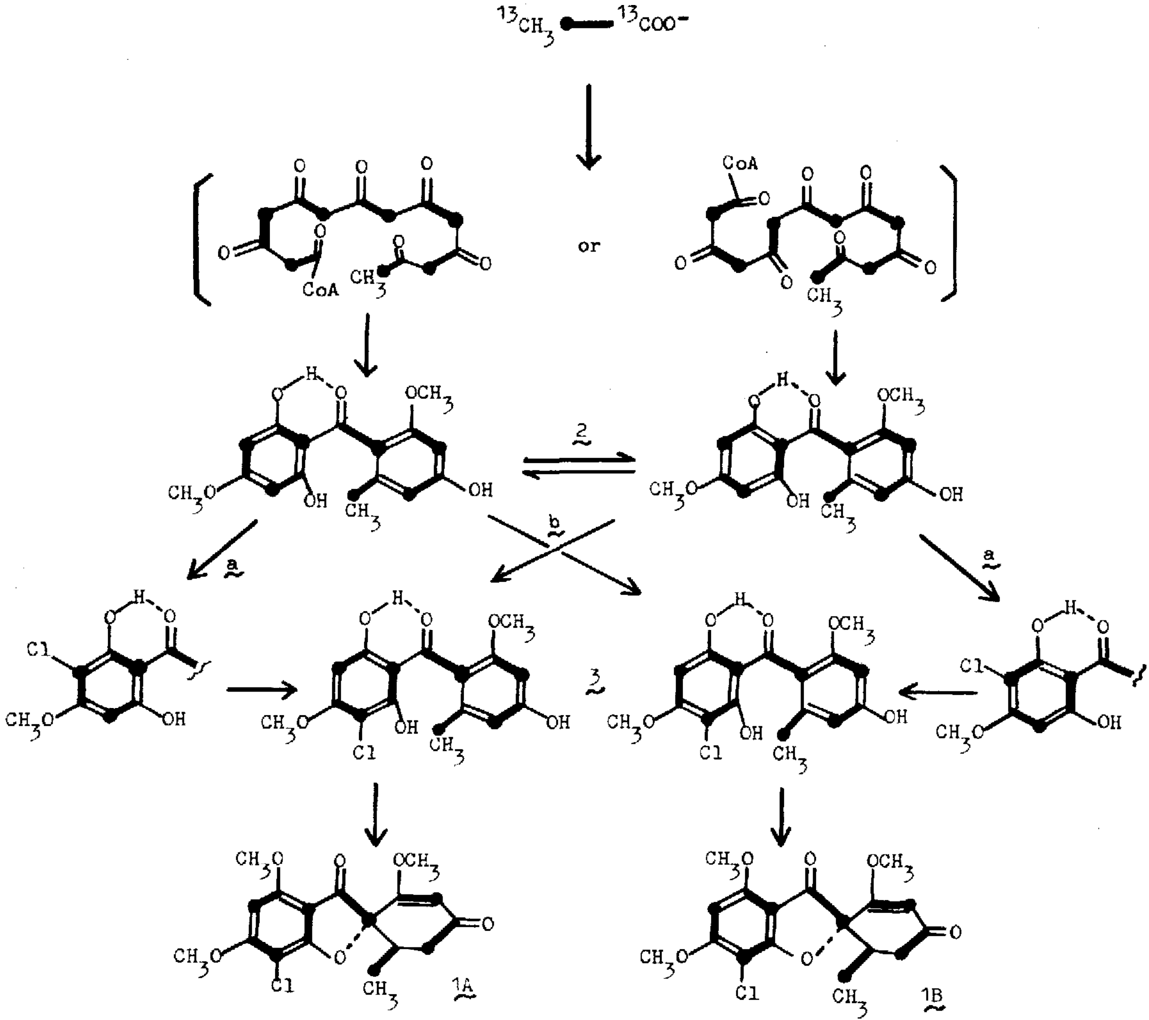
Table III. Chemical shifts of 7-dechlorogriseofulvin, griseofulvin, and 5-bromogriseofulvin and effects of chlorine and bromine substituents on chemical shift.

Carbon	7-Dechloro-griseofulvin ppm	Griseofulvin ppm	$\Delta\delta_{Cl}$ a)	5-Bromo-griseofulvin ppm	$\Delta\delta_{Br}$ b)	$\Delta\delta_{Cl,Br}$ c)
4	159.3	158.2	-1.1	154.6	-3.6	-4.7
6	171.4	164.9	-6.5	162.3	-2.6	-9.1
7a	176.3	169.6	-6.7	169.3	-0.2	-7.0
2'	170.6	170.6	0.0	169.6	-1.0	-1.0

a) (Chemical shift of griseofulvin) - (Chemical shift of 7-dechlorogriseofulvin)

b) (Chemical shift of 5-bromogriseofulvin) - (Chemical shift of griseofulvin)

c) (Chemical shift of 5-bromogriseofulvin) - (Chemical shift of 7-dechlorogriseofulvin)



Scheme 1

The chemical shifts of griseofulvin are shown in Table I,[8] with enhancements observed in [1-^{13}C]- and [2-^{13}C]-acetate-enriched samples and coupling constants of [1,2-$^{13}C_2$]-acetate-enriched 1. Recently Levine et al.[9] describe ^{13}C nmr of griseofulvin, four of its derivatives and several model compounds in hexadeuteriodimethyl sulfoxide solution. Their assignments agree with those of ours except for the interchange of the C(6) and C(7a) resonances. Accordingly ^{13}C nmr spectra of phloroglucinol trimethylether (4) and three of its halogen derivatives were measured and effects of halogen substituents on chemical shift were examined (Table II). These values due to halogen substitution were in accord with those of griseofulvin derivatives as shown in Table III. Furthermore, on bromination of griseofulvin at C-5, the signal intensities at α-positions, C(4) and C(6), were decreased by a factor of about 4 compared with those of griseofulvin, supporting the validity of the chemical shift assignment on the ring A. Then, *Penicillium urticae* was fermented for 7 days and the mycelium thus obtained was used for the biosynthetic experiments in the medium[10] containing sodium [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate as tracers.[11] The p.n.d. ^{13}C nmr spectra of the [1-^{13}C]- and [2-^{13}C]-acetate enriched samples showed the enhancements at the anticipated positions in the skeleton (Table I). Furthermore, the enhancements were observed in the three methoxyl groups[7] in the spectrum of griseofulvin enriched by feeding [2-^{13}C]-acetate. The p.n.d. ^{13}C nmr spectrum of the [1,2-$^{13}C_2$]-acetate-derived sample showed ten pairs of ^{13}C-^{13}C couplings indicating that C(6'-CH_3)-C(6'), C(5')-C(4'), C(3')-C(2') and C(1')-C(3) in the rings C and B, and C(3a)-C(4), C(5)-C(6), C(7)-C(7a), C(3a)-C(7a), C(7)-C(6) and C(5)-C(4) in the ring A originate from intact acetate units. Thus the grisan ring system of griseofulvin will be formed by condensation of a heptaketide chain as shown in Scheme 1. This observation suggests that, through a polyketomethylene intermediate, griseophenone C (**2**) is formed and its phloroglucinol moiety makes a rotation to an equilibrium, and a chlorination to give griseophenone B (**3**) by course **a** or **b**, followed by several steps of reactions will afford griseofulvin. This evidence clearly indicates that griseofulvin is composed of **1A** and **1B** shown in Scheme 1.

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