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UTILIZATION OF ^{13}C-^{13}C COUPLING IN STRUCTURAL AND BIOSYNTHETIC STUDIES V[1]

THE ^{13}C FT NMR SPECTRUM OF STERIGMATOCYSTIN

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We wish to report the use of ^{13}C-doubly labeled acetate in studying the biosynthesis of the *Aspergillus versicolor* metabolite, sterigmatocystin. Previously the biosynthesis of this metabolite was studied with ^{14}C and ^{13}C tracers.[2,3] Recent mutant experiments have now firmly established the intermediacy of the polyhydroxyanthraquinone averufin in aflatoxin biosynthesis in *A. parasiticus*.[4] Presumably, sterigmatocystin is formed in *A. versicolor* through this same intermediate.

Sterigmatocystin was isolated from cultures fed 90% enriched $^{13}CH_3{}^{13}CO_2Na$, that had been diluted three-fold with unlabeled acetate. The ^{13}C-^{13}C couplings observed in the ^{13}C NMR spectrum (Fig. 1) of the labeled material established the folding pattern of the polyketide precursor of both averufin and sterigmatocystin. Of the two possible pathways through averufin, (a) and (b), given in Scheme 1, only path (b) would give sterigmatocystin in the ^{13}C feeding experiment which had the proper ^{13}C-^{13}C coupling patterns in the aromatic carbon region of its spectrum.

Very strong ^{13}C-^{13}C couplings were observed with most carbon signals. The ^{13}C chemical shifts δ_c and the coupling constants J obtained are shown in Table 1. The weak signals for carbons 2, 9, and 13 were hidden by the stronger signals for carbons 4, 6, and 16.

The labeling pattern in the dihydrofuran ring of sterigmatocystin was established in the following way. The J value obtained for C_{15} (34 Hz) agreed very well with those reported[5] for sp^3-sp^3 bonds, indicating that this carbon was coupled to C_{14} (34 Hz). The J value obtained for

SCHEME I

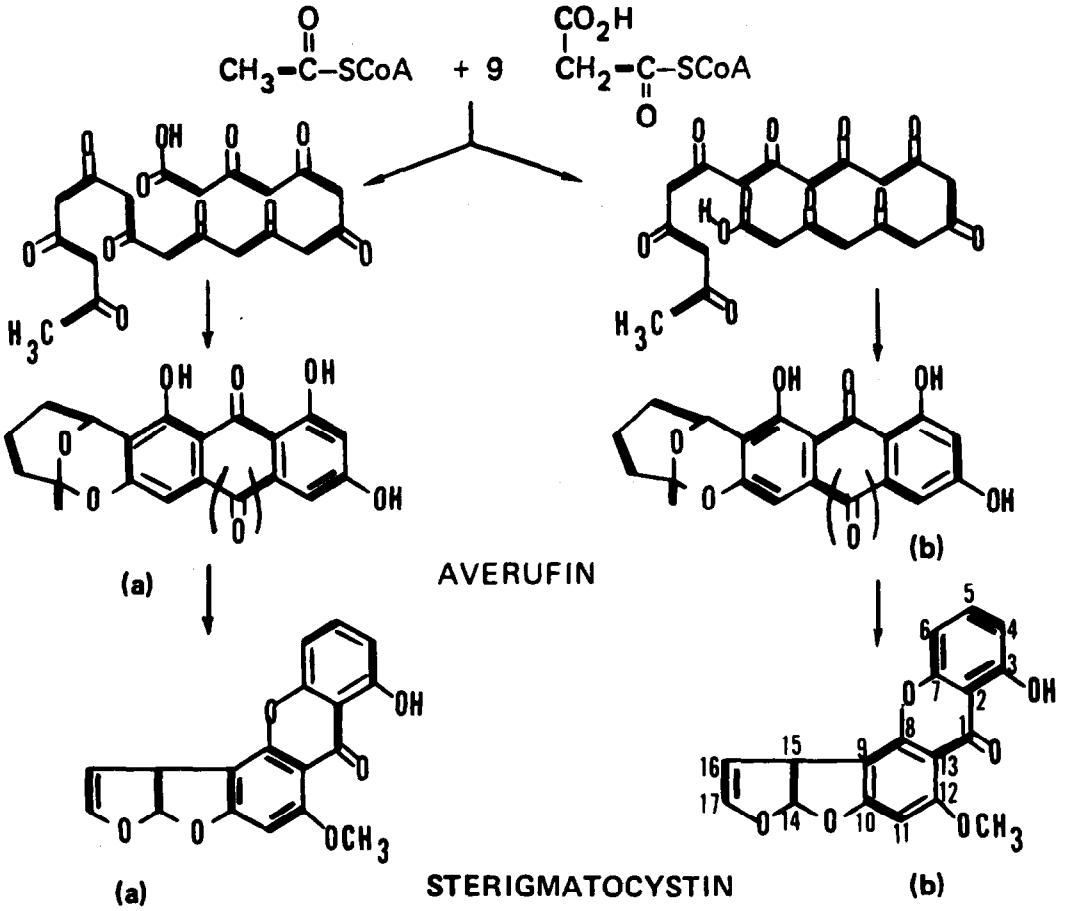

FIGURE I

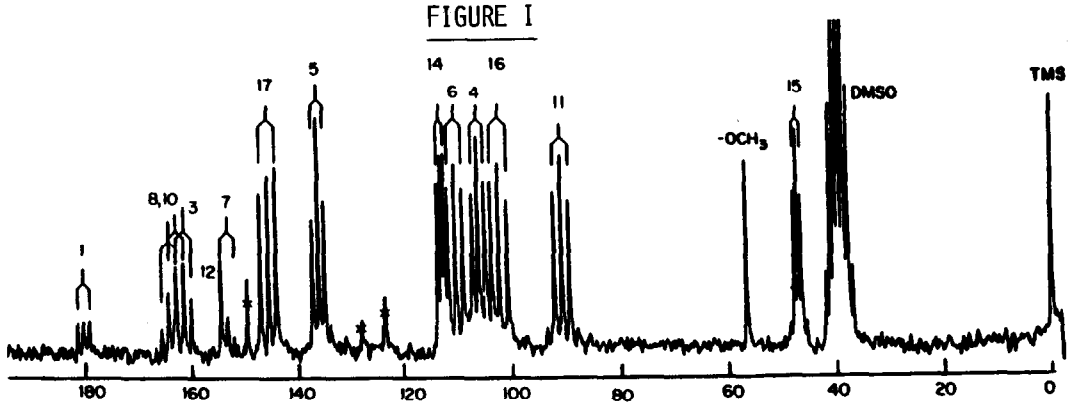

TABLE I

carbon	sterigmatocystine δ_C	sterigmatocystine J_{C-C}(Hz)	6-methoxysterigmatocystin δ_C	calcd.
15	47.2	34	47.2	
OCH_3	56.7		56.7	
11	90.9	72	90.9	
16[a]	102.5	76	102.2	
4	106.4	58	109.0	108.8
6	110.7	70	139.0	142.0
14	113.3	34	113.3	
5	136.1	59	120.6	121.1
17	145.5	76	145.6	
7	153.3	(b)	143.8	144.5
12	154.5	singlet	154.0	
3	161.4	(b)	153.1	154.6
8,10	162.9	(b)	162.7	
	164.2	(b)	164.2	
2,9,13	-		105.2	
	-		106.4	
	-		108.7	
1	180.3	58	180.1	
6-OCH_3			57.3	

Determined on a Varian XL-100 nmr spectrometer at 25.2 MHz in d_6-DMSO, relative to internal TMS, saturated solution. spectral width; 5120 Hz, pulse width; 20 μsec, acquisition time; 0.8 sec, data points; 8192, accumulation; 203,000 times.
(a) This assignment was confirmed by selective decoupling at δ 5.5 in the ^1H NMR spectrum; therefore, the previous assignment[3] given for these carbons (C_{14} and C_{16}) should be reversed.
(b) Owing to the overlap of thsese signals, the magnitude of the coupling constant could not be determined.

C_{16} (76 Hz) was considerably larger than that for ethylene[5] (67.2 Hz), indicating bonding to C_{17} (76 Hz) which bears an electronegative group. It is, therefore, apparent that the bonds between C_{14} and C_{15}, and C_{16} and C_{17} were formed from intact acetic acid molecules without cleavage of the C-C bond.

In order to distinguish pathway (b) from (a), it was necessary to establish the ^{13}C-^{13}C coupling relationships of either of carbons C_1, C_5, or C_{11}. Unambiguous chemical-shift assignments between the environmentally similar carbons C_2 and C_{13}, C_4 and C_6, and C_{10} and C_{12} were required for determining these ^{13}C-^{13}C coupling relationships.

These signal assignments were made with the aid of the ^{13}C NMR spectrum of an additional member of this series, 6-methoxysterigmatocystin,[6,7] the signal assignments of which are given in Table 1. The effects of a methoxy substituent on aromatic-carbon chemical shifts were reported as follows[8]: a para carbon signal is shifted by -6.8 ppm, a meta carbon signal by +2.4 ppm, an unsubstituted ortho carbon signal by -15.0 ppm, and a substituted ortho carbon signal by -8.8 ppm relative to δ_C of the unmethoxylated aromatic-ring carbons. The methoxylated carbon signal itself is shifted by +31.3 ppm. By use of these substituent parameters, the calculated and observed δ_C values for 6-methoxysterigmatocystin have been compared in Table 1, being in very good agreement; the fact established the assignments.

The C_4-C_5 and C_6-C_7 ^{13}C-^{13}C couplings observed in sterigmatocystin obtained from the feeding experiment of doubly-labeled acetate clearly established

that the polyketide chain in sterigmatocystin biosynthesis is folded in a manner shown in pathway (b). The lack of ^{13}C-^{13}C coupling observed with C_{12} can also be taken as an evidence which supports this biosynthetic conclusion.

Thomas[9] has recently proposed a mechanism for sterigmatocystin biosynthesis *via* the intermediacy of averufin that can be accomodated by the present labeling results.

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