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CARBON-13 NUCLEAR MAGNETIC RESONANCE ASSIGNMENTS AND BIOSYNTHESIS OF OCHRATOXIN A

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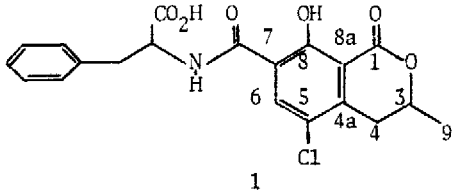
The carbon-13 chemical shifts have been assigned to all the carbons in the isocoumarin portion of ochratoxin A. Incorporation of carbon-13 enriched acetate was used to confirm the biosynthesis of ochratoxin A.

Ochratoxin A (1) is a toxic fungal metabolite of Aspergillus ochraceus Wilh. The toxin is an L-β-phenylalanine derivative of 3,4-dihydro-5-chloro-7-carboxy-8-hydroxy-3R-methylisocoumarin.



1

Following our recent studies[1] of incorporation of ^{14}C into ochratoxin, we wish now to report our findings elucidating the biosynthesis of 1 as studied by ^{13}C NMR.

Previous[2] ^{13}C studies of ochratoxin involved incorporation of ^{13}C from sodium formate solely in the carbonyl carbon of the amide bond. Incomplete ^{13}C chemical shift assignments were reported.

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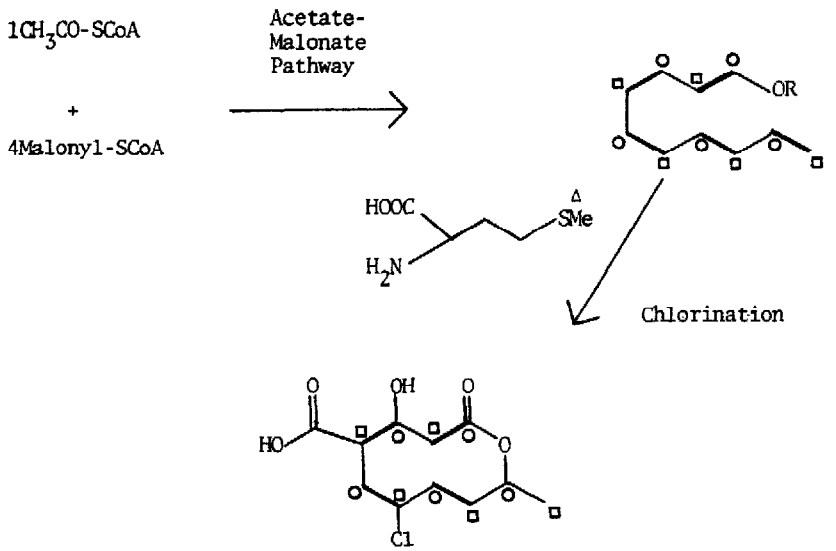
Steyn[3] has studied the biosynthesis of ochratoxin with radioisotope techniques, a scheme presented in his study has been used in the present work to further elucidate the biosynthesis of the isocoumarin portion of 1.

Scheme 1



○ = Atom from C-1 of acetate;

□ = Atom from C-2 of acetate

intact acetate residues shown by heavy bonds

Cultures of A. ochraceus[1] were supplemented with [1-^{13}C]-, [2-^{13}C]-, or [1,2-^{13}C]-acetate (90%) to give ochratoxin A enriched with approximately 1% ^{13}C abundance at each position of incorporation.

Table I lists the ^{13}C chemical shifts and multiplicities we have assigned to the isocoumarin portion of 1. Assignments are based on calculations utilizing values found in the literature[4] and the off-resonance natural abundance spectrum.

Table I[a]

Chemical Shift Assignments for Isocoumarin Portion of Ochratoxin A

Carbon Number	Found	Calculated[4]
1	169.6 s	
3	75.8 d	
4	32.2 t	
4a	140.6 s	145.8
5	123.0 s	128.1
6	138.9 d	136.8
7	109.8 s	116.3
8	159.0 s	156.4
8a	120.6 s	119.9
9	20.7 q	

[a]Spectra were obtained in $CDCl_3$ solutions with TSM as internal reference on a Bruker Fourier Transform spectrometer.

The ^{13}C spectra of [1-^{13}C]- and [2-^{13}C]-acetate enriched samples exhibited enhancements of each resonance, enabling us to establish the origin of each carbon atom as shown in the Scheme; this isocoumarin biosynthesis is analogous to the acetate incorporation pattern proposed by Holker and Young for a related metabolite from Periconia macrospinosa.[5]

Chemical shifts for the phenylalanine portion of ochratoxin A were readily determined when the spectrum of ochratoxin A was compared with the spectrum of N-acetylphenylalanine methyl ester.[6]

References

1. E. B. Lillehoj, O. Aalund, and B. Haed, Appl. Environ. Microbiol. 36, 720 (1978).
2. Y. Maebayashi, K. Miyaki, and M. Yamazaki, Chem. Pharm. Bull. 20, 2172 (1972). M. Yamazaki, Y. Maebayaski, and K. Miyaki, Tetrahedron Lett. 2301 (1971).
3. For a recent review see: Pieter S. Steyn, p. 440 in Mycotoxins edited by J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman, Pathotox, Park Forest South, IL, 1977.
4. G. C. Levy and G. L. Nelson, Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, Wiley-Interscience, New York, 1972.
5. J. S. E. Holker and K. Young, Chem. Commun. 525 (1975).
6. L. F. Johnson and W. C. Jankowski, Carbon-13 NMR Spectra, Wiley-Interscience, New York, 1972.

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