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THE USE OF CARBON-13 NUCLEAR MAGNETIC RESONANCE TO ESTABLISH THAT THE BIOSYNTHESIS OF TENELLIN INVOLVES AN INTRAMOLECULAR REARRANGEMENT OF PHENYLALANINE

Edward Leete* and Nicholas Kowanko[1]
Department of Chemistry, University of Minnesota

Richard A. Newmark
3M Company, St. Paul, Minnesota

Leo C. Vining*
Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

A. Gavin McInnes and Jeffrey L. C. Wright
Atlantic Regional Laboratory, National Research Council of Canada,
Halifax, Nova Scotia, Canada

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It has been previously established[2] that tenellin (4), a yellow pigment produced by the insect pathogenic fungi *Beauveria tenella* (Delacroix) Siem., and *Beauveria bassiana* (Bals.) Vuill. is derived from acetate, phenylalanine, and methionine as illustrated schematically in Figure 1. It was shown that the carboxyl carbon of phenylalanine (1) migrates, ultimately becoming C-4 of tenellin. A plausible intermediate in the biosynthesis of tenellin would be α-formylphenylacetyl coenzyme A (2) which would yield the required carbon skeleton by condensation with the poly-β-keto acid derived from acetate, extra methyl groups being derived from methionine.

This type of rearrangement of the phenylalanine side chain occurs in the biosynthesis of tropic acid (3), the acid moiety of the *Datura* alkaloids, scopolamine and hyoscyamine.[3] It has been recently shown that the formation of tropic acid involves an intramolecular rearrangement of the carboxyl group.[4] The nature of this rearrangement in the biosynthesis of tenellin has now been investigated by carrying out a feeding experiment with [1,3-$^{13}C_2$]phenylalanine, in which the majority of labelled molecules contained two ^{13}C atoms. If the rearrangement of the side chain is intramolecular, the resultant tenellin will contain two contiguous ^{13}C atoms which would afford satellite peaks in the ^{13}C-NMR, due to spin-spin coupling, symmetrically located about the corresponding singlet peaks arising from C-4 and C-5. An intermolecular migration of the car-

boxyl group would afford no such satellite peaks, but would show enrichment of the singlet peaks at C-4 and C-5.

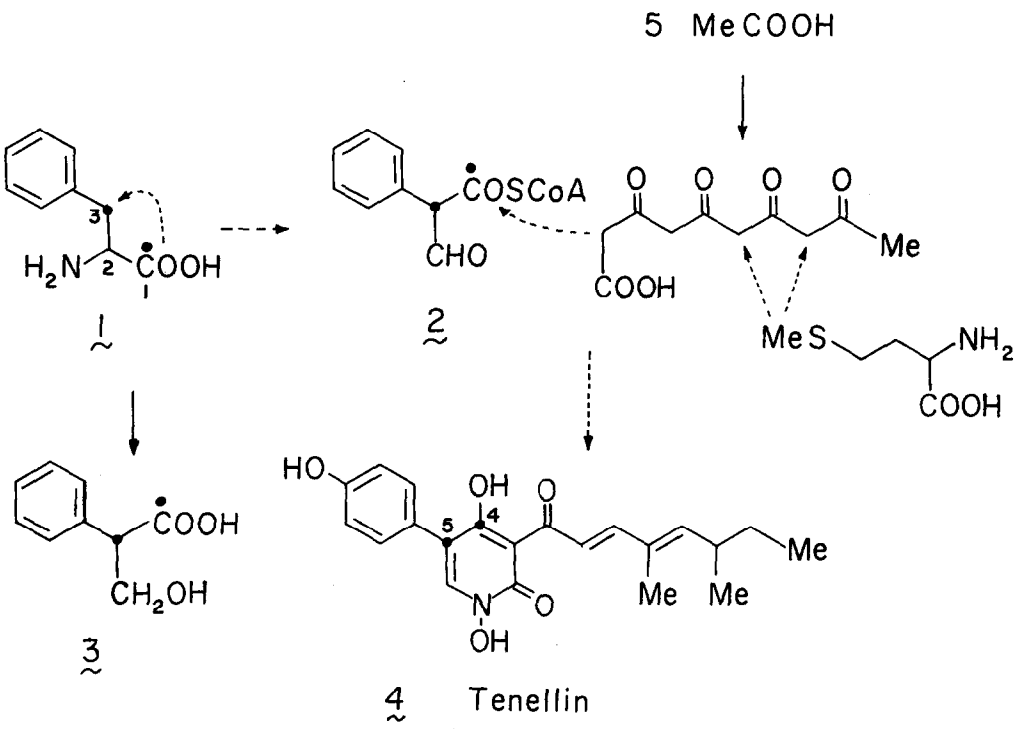

Figure 1. Biosynthesis of Tenellin

DL-[1-^{14}C,1,3-$^{13}C_2$]Phenylalanine (81% Ph^{13}CH$_2$CH(NH$_2$)^{13}COOH, 9% Ph^{13}CH$_2$CH(NH$_2$)COOH, 9% PhCH$_2$CH(NH$_2$)^{13}COOH, 8.77 x 10^7 dpm/mM, 102.5 mg) was added to a culture of *B. bassiana* which was just starting to show some yellow color. After 7 days tenellin was isolated and purified by chromatography on silicic acid.[5] Tenellin (207 mg) having an activity of 6.37 x 10^6 dpm/mM (7.3% specific incorporation) was obtained. The proton-noise decoupled ^{13}C-NMR spectrum of this enriched material is illustrated in Figure 2. The chemical shifts were identical (± 0.1 ppm) with those previously reported.[6] Satellite peaks are apparent at C-4 and C-5 (^{1}J 62 Hz). The specific incorporation was calculated by measurement of the integrated areas of I_c (the central singlet peak) and I_s (sum of the satellite peaks) using the following derivation:

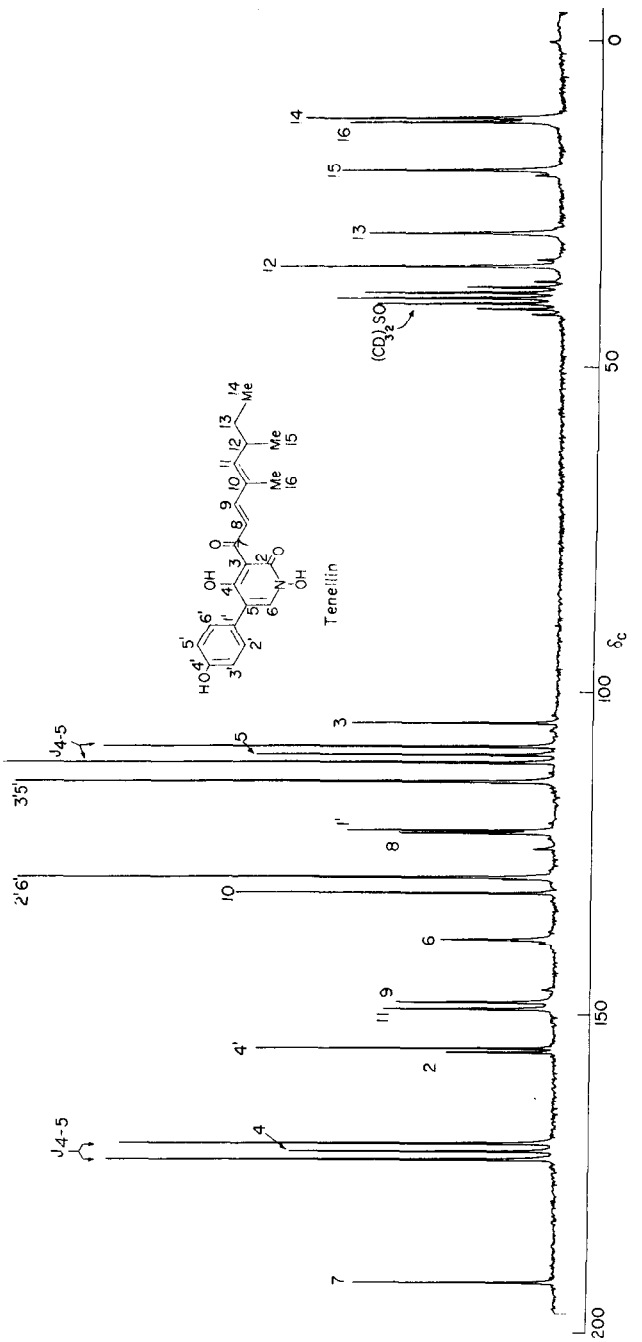

Figure 2. The proton-noise decoupled ^{13}C-NMR spectrum of tenellin (100 mg in 0.4 ml $(CD_3)_2SO$) derived from [1,3-$^{13}C_2$]phenylalanine.

$$\text{Specific inc.}(x) = \frac{\text{fraction of doubly labelled molecules in tenellin}}{\text{fraction of doubly labelled molecules in phenylalanine}} \qquad (A)$$

$$\text{also } x = \frac{\text{fraction of singly labelled molecules in tenellin}}{\text{fraction of singly labelled molecules in phenylalanine}} \qquad (B)$$

$$\text{Thus } \frac{I_s}{I_c} = r = \frac{Ax}{(1-x)0.011 + Bx}$$

(where $(1-x)0.011$ is the contribution due to natural abundance)

$$\text{Solving: } x = \frac{0.011r}{A - r(B - 0.011)} \qquad (1)$$

Analysis of the triplets at C-4 and C-5 yielded an average value of $r = 3.45$. Substituting in equation (1) where $A = 0.81$ and $B = 0.09$, a value of $x = 0.071$ (7.1% specific incorporation) is obtained. This is in excellent agreement with the specific incorporation obtained from the ^{14}C assay, indicating that essentially all the rearrangement of the phenylalanine is intramolecular

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References and Footnotes

1) On sabbatical leave from Moorhead State University, Minnesota.

2) A. G. McInnes, D. G. Smith, J. A. Walter, L. C. Vining, and J. L. C. Wright, J. Chem. Soc. Chem. Comm., 282 (1974).

3) This rearrangement has been reviewed: E. Leete, 'Biosynthesis', Specialist Periodical Report of the Chemical Society, Ed. T. A. Geissman, Vol. 2, pp 115-120 (1973).

4) E. Leete, N. Kowanko, and R. A. Newmark, J. Amer. Chem. Soc., in press.

5) S. H. El. Basyouni, D. Brewer, and L. C. Vining, Canad. J. Bot., 46, 441 (1968).

6) A. G. McInnes, D. G. Smith, C.-K. Wat, L. C. Vining, and J. L. C. Wright, J. Chem. Soc. Chem. Comm., 281 (1974). By error, Figure 1 of reference 2 has the assignments for C-1' and C-8 reversed.