New Techniques in Biosynthetic Studies using ^{13}C Nuclear Magnetic Resonance Spectroscopy. The Biosynthesis of Tenellin Enriched from Singly and Doubly Labelled Precursors

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Summary ^{13}C n.m.r. spectroscopy was used for the dual purpose of assessing the relative merits of singly and doubly labelled precursors while establishing the biosynthesis of tenellin from ^{13}C-labelled acetate, phenylalanine, and methionine; precursors in which two adjacent sites are labelled can provide superior information, and may prove useful for obtaining enrichment data at low incorporations.

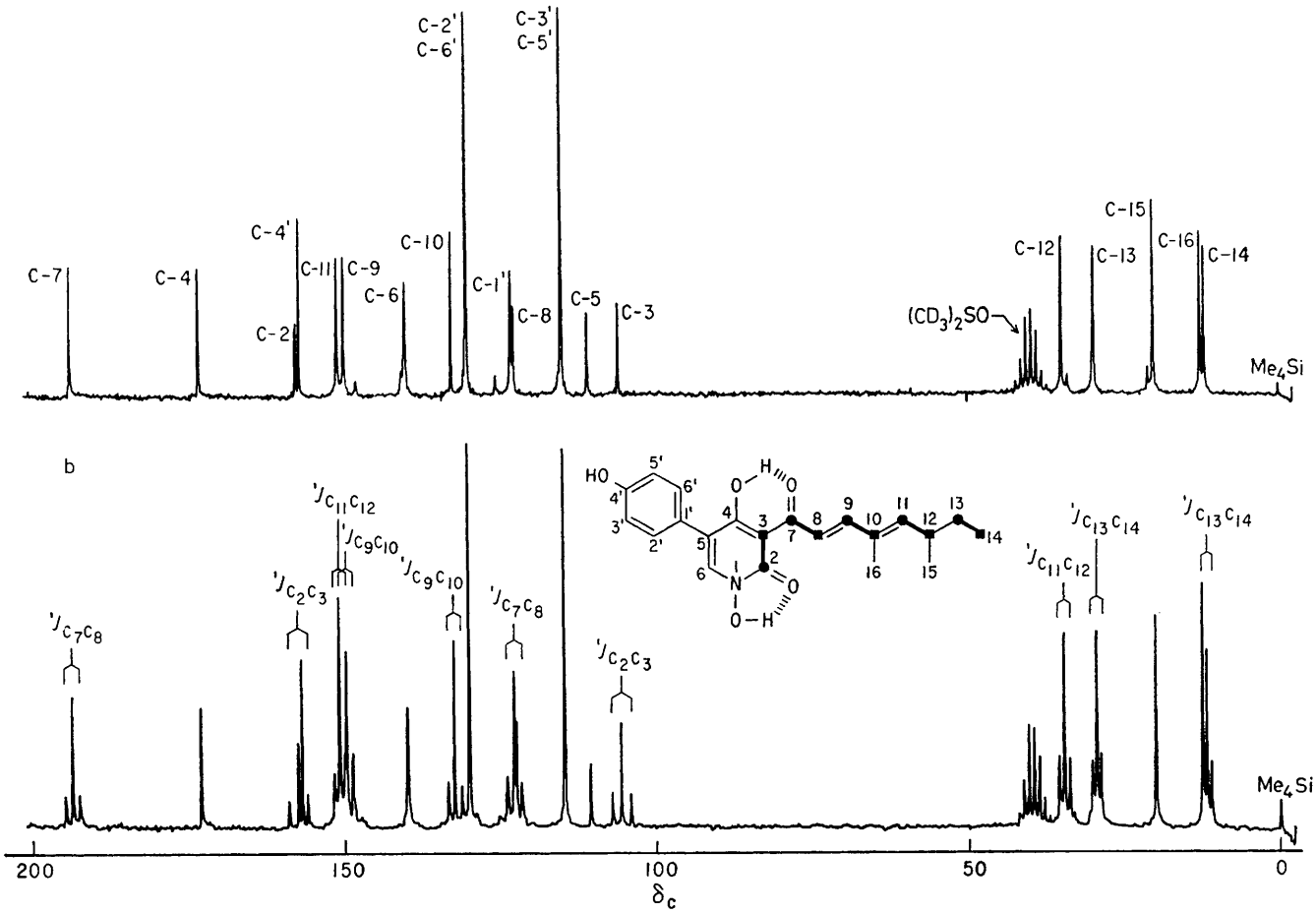


FIGURE 1. The p.n.d. PFT ^{13}C n.m.r. spectra of tenellin in $(CD_3)_2SO$; (a) natural abundance, (b) labelled with 90% enriched [1,2-^{13}C]acetate. Percentage enrichments, due to incorporation of intact two-carbon units into tenellin, calculated (see text for formula) from spectrum b are as follows: 1·0 (C-2), 0·6 (C-3); 0·5 (C-7), 0·7 (C-8); 1·0 (C-9), 0·6 (C-10); 0·9 (C-11), 0·8 (C-12); 0·9 (C-13), 0·9 (C-14).

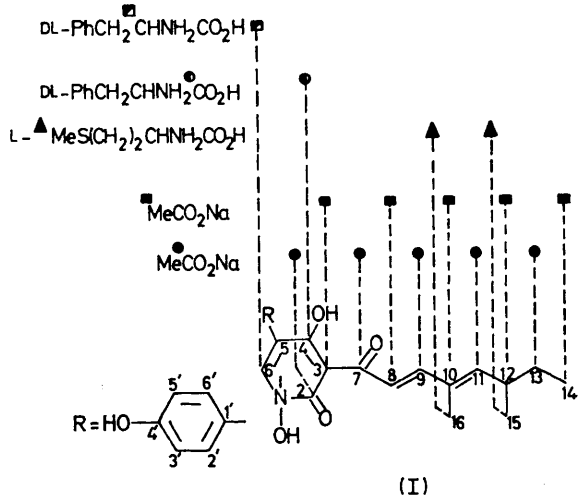
TENELLIN is produced by cultures of *Beauveria bassiana* (Bals.) Vuill. and has structure (I).[1] Preliminary ^{14}C-labelling experiments indicated that acetate, methionine, and phenylalanine were efficient precursors, and that the latter was incorporated more efficiently than tyrosine. While participation of acetate and methionine in generating the carbon skeleton of (I) is unexceptional, incorporation of phenylalanine as an intact biogenetic unit would clearly require a structural rearrangement at some stage in the pathway. Previous studies have shown the usefulness of ^{13}C-labelling techniques combined with ^{13}C n.m.r. spectroscopy in delineating biosynthetic pathways[2] and in this communication we report the results of such a study on tenellin.

The assigned pulsed Fourier transform (P.F.T.) proton-noise decoupled (p.n.d.) natural abundance ^{13}C n.m.r. spectrum of (I) is shown in Figure 1, and the results of ^{13}C-labelling experiments are summarised in Figure 2. As expected L-[*Me*-^{13}C]methionine labelled the methyl groups on C-10 and C-12, whereas C-2, C-3 and C-7—C-14 were alternately labelled and equally enriched by sodium [1-^{13}C]- and [2-^{13}C]-acetate. Consequently, one step in the biosynthetic pathway involves formation of a ten-carbon polyketide chain methylated at C-6 and C-8 (C-10 and C-12 in tenellin). In the heterocyclic ring, C-4 is labelled by (±)-[1-^{13}C]phenylalanine and C-6 from (±)-[2-^{13}C]phenylalanine. Thus (I) is formed by condensing the methylated polyketide chain with a unit containing all the carbons of phenylalanine, and at some stage in the biosynthetic pathway the carboxy-carbon of phenylalanine migrates to the carbon adjacent to the aromatic ring. Precedents for this type of rearrangement exist in the biosynthesis of tropic[3] and pulvic[4] acids from phenylalanine.

The spectrum of (I) labelled with 90% enriched [1,2-^{13}C]-acetate (81% $^{13}CH_3{}^{13}CO_2Na$; 9% $CH_3{}^{13}CO_2Na$; 9% $^{13}CH_3CO_2Na$; 1% CH_3CO_2Na) is shown in Figure 1b. Incorporation of doubly labelled acetate units is indicated by the presence of satellite resonances, due to ^{13}C–^{13}C spin–spin coupling, symmetrically located about the corresponding singlet resonances arising from isolated ^{13}C nuclei. These satellites, quite apart from providing structural information and a source of spin–spin coupling data, establish in a single experiment that C-2, C-3, and C-7—C-14 derive from the condensation of intact two carbon units.

For various reasons,[5] including the errors in comparing p.n.d. ^{13}C n.m.r. spectral intensities for unenriched and enriched metabolite, it is difficult to detect ^{13}C-enrichments of $<0{\cdot}2\%$ using singly labelled precursors. The satellites

originating from adjacent [^{13}C]-labels, however, should permit smaller enrichments to be observed. The advantages of this approach are analogous to those obtained by the use of ^{13}C-H satellites in ^{1}H n.m.r. spectra for obtaining enrichment data.[6]



(I)

FIGURE 2. Positions in tenellin labelled by various ^{13}C-labelled precursors as determined by comparing the p.n.d. PFT ^{13}C n.m.r. spectra of the labelled and unlabelled compounds. Enrichments due to precursor incorporation varied from 5 to 10%.

As all the relaxation mechanisms for ^{13}C nuclei in naturally occurring (I) are retained in molecules containing doubly labelled two-carbon units, the percentage enrichment at each labelled position in (I), produced from doubly labelled acetate, is equal to $1{\cdot}1\ I_s/(I_c - fI_s)$, where I_s is the sum of the intensities of the two satellite resonaces, I_c the intensity of the central peak, and f is the ratio of the concentrations of appropriate singly and doubly labelled precursors statistically present in the doubly enriched acetate. Enrichment data for (I) calculated in this way (see caption Figure 1) indicate that reasonable estimates are indeed possible. This equation only applies if the contributions to spin–lattice relaxation from bonded ^{13}C nuclei are negligible (as is always the case for carbons bearing hydrogen[7]), and if the probability of doubly labelled units being adjacent is small.

The use of homonuclear and heteronuclear multiply labelled precursors thus promises to become a valuable aid in studying biosynthetic problems, particularly in the case of complex metabolites in which the assignment of quaternary carbon atoms is often ambiguous.

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[1] A. G. McInnes, D. G. Smith, C-K. Wat, L. C. Vining, and J. L. C. Wright, preceding communication.
[2] A. G. McInnes, D. G. Smith, L. C. Vining, and L. Johnson, *Chem. Comm.*, 1971, 325; C-K. Wat, A. G. McInnes, D. G. Smith, and L. C. Vining, *Canad. J. Biochem.*, 1972, **50**, 620.
[3] M. L. Louden and E. Leete, *J. Amer. Chem. Soc.*, 1962, **84**, 4507.
[4] W. S. G. Maass and A. C. Neish, *Canad. J. Bot.*, 1967, **45**, 59.
[5] J. B. Grutzner, *Lloydia*, 1972, **35**, 375.
[6] D. Desaty, A. G. McInnes, D. G. Smith, and L. C. Vining, *Canad. J. Biochem.*, 1968, **46**, 1293; J. Wright, D. G. Smith, A. G. McInnes, L. C. Vining, and D. W. S. Westlake, *ibid.*, 1969, **47**, 945.
[7] K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.*, 1970, **52**, 3439.