Studies of Polyketide Chain Assembly Processes: Incorporation of [2-^{13}C]Malonate into Averufin in *Aspergillus parasiticus*

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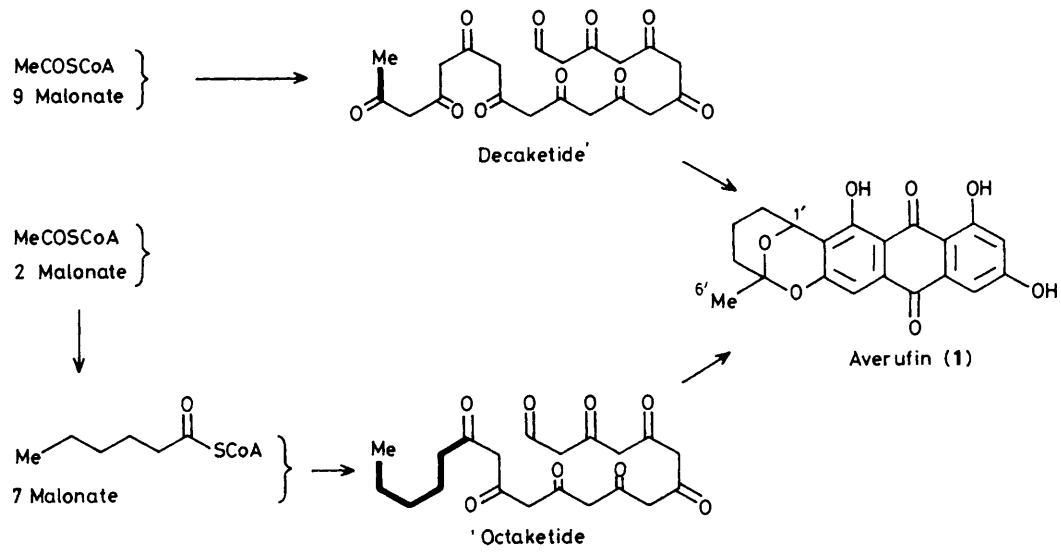
Analysis of the ^{13}C n.m.r. spectrum of averufin (**1**) enriched by feeding diethyl [2-^{13}C]malonate to cultures of *Aspergillus parasiticus* shows high and equal incorporation of ^{13}C-label at *nine* positions to indicate a clear acetate 'starter' effect; thus averufin is a decaketide, not an octaketide derived from a hexanoate starter and seven malonates as suggested by recent studies.

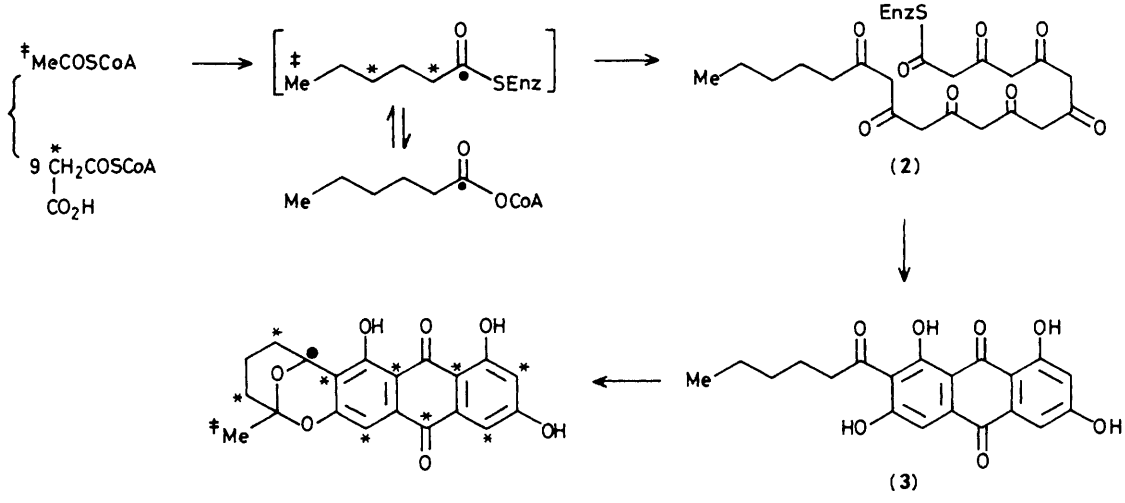
The polyketide pathway[1] is one of the major pathways of secondary metabolism but until recently very little was known of the processes involved in the early stages of polyketide biosynthesis. However, recent studies[2] using precursors labelled with the stable isotopes ^{13}C, ^{2}H, and ^{18}O have provided significant indirect evidence for the nature of the enzyme-bound intermediates produced by the polyketide-synthesising enzymes in different organisms, and for the sequence of events involved in their assembly. These studies indicate that reduction and loss of oxygen in polyketide biosynthesis occurs by mechanisms similar to those involved in fatty acid biosynthesis,[3] during and not after the chain elongation process. Although there has been little success in attempts to obtain direct information on the necessary intermediates which appear to be enzyme-bound throughout the assembly process, the intact incorporation of ^{13}C-labelled hexanoate into averufin was recently reported.[4] Feeding [1-^{13}C]hexanoic acid to cultures of *Aspergillus parasiticus* resulted in high specific incorporation of label at C-1′ of averufin (**1**). This was a most significant observation and led to the proposal that averufin, and therefore the aflatoxins, were not *deca*ketides as previously accepted,[5] but rather that they were *octa*ketides, being formed by elongation of a separate, previously formed hexanoate starter by addition of seven malonates as indicated in Scheme 1. However, a possible alternative interpretation is that a single decaketide synthetase is involved which produces an enzyme-bound C_6 segment which can exchange with free hexanoyl CoA (Scheme 2). In order to test this hypothesis further we have examined the incorporation of ^{13}C-labelled malonate into averufin.

Diethyl [2-^{13}C]malonate was prepared from sodium [2-^{13}C]-acetate[6,7] and fed to static cultures of *A. parasiticus*. ^{13}C N.m.r. analysis† of the enriched averufin revealed that high

† Averufin was converted into the triacetate for n.m.r. analysis. 50 MHz ^{13}C n.m.r. spectra were determined in $CDCl_3$ using inverse gated decoupling conditions to suppress nuclear Overhauser enhancement (n.O.e.) effects[8] in the presence of the paramagnetic relaxation agent trisacetylacetonatochromium.[9] Percentage enrichments[10] were obtained by comparing the intensities of resonances in both the natural abundance and enriched spectra after normalisation.[11]



Scheme 1. Alternative octaketide and decaketide routes to averufin (**1**).



Scheme 2. Proposed pathway for incorporation of malonate into averufin. [2-^{13}C]malonate enrichment: 1.1 ± 0.1% (*), 0.4 ± 0.2% (‡).

and essentially equal incorporation of ^{13}C-label from malonate had occurred at *nine* of the possible positions as indicated in Scheme 2, with only C-6′ showing a lower level of incorporation. This clearly demonstrates that an acetate starter effect is operating. Thus it appears that averufin is indeed a decaketide being formed from an acetate starter unit by addition of two successive malonates with a full reduction–elimination–reduction sequence, *cf.* fatty acid synthetase, following each condensation step to produce enzyme-bound hexanoate. Assembly then continues by successive condensation of seven further malonates with no further reduction steps to give the requisite precursor (**2**) for direct cyclisation to produce norsolorinic acid (**3**)[5] as the first enzyme-free intermediate and subsequently averufin.

The significance of the previous observations,[4] therefore, is that exogenous hexanoate can equilibrate with the enzyme-bound intermediate and so be incorporated intact without prior degradation. This suggests that if intermediates with the correct oxidation level are fed under the right conditions then success can be achieved in the direct study of polyketide chain assembly processes.

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References

1 A. J. Birch and F. W. Donovan, *Aust. J. Chem.*, 1953, **6**, 360.
2 C. R. Hutchinson, I. Kurobane, C. T. Mabuni, R. W. Kumola, A. G. McInnes, and J. A. Walter, *J. Am. Chem. Soc.*, 1981, **103**, 2474; C. Abell, D. M. Doddrell, M. J. Garson, E. D. Laue, and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1983, 694; F. E. Scott, T. J. Simpson, L. A. Trimble, and J. C. Vederas, *ibid.*, 1984, 756; T. J. Simpson and G. I. Stevenson, *ibid.*, 1985, 1822; A. K. Demetriadou, E. D. Laue, and J. Staunton, *ibid.*, 1985, 1125.
3 B. Sedgwick and C. Morris, *J. Chem. Soc., Chem. Commun.*, 1980, 96; F. Lynen, *Eur. J. Biochem.*, 1980, **112**, 431.
4 C. A. Townsend and S. B. Christensen, *Tetrahedron*, 1983, **39**, 3575; C. A. Townsend, S. B. Christensen, and K. Trautwein, *J. Am. Chem. Soc.*, 1984, **106**, 3868.
5 P. S. Steyn, R. Vleggaar, and P. L. Wessels in 'The Biosynthesis of Mycotoxins,' ed. P. S. Steyn, Academic Press, New York, 1980, p. 105.
6 G. A. Ropp, *J. Am. Chem. Soc.*, 1950, **72**, 2299.
7 M. E. Mueller and E. Leete, *J. Org. Chem.*, 1981, **46**, 31511.
8 L. Cattel, J. F. Grove, and D. Shaw, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2626.
9 R. Freeman, K. G. R. Pachler, and G. N. La Mar, *J. Chem. Phys.*, 1971, **55**, 4586.
10 T. J. Simpson, *Chem. Soc. Rev.*, 1975, **4**, 497.
11 J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2135.