Homonuclear ^{13}C Decoupling in ^{13}C Nuclear Magnetic Resonance Studies of Biosynthesis using Doubly Labelled Precursors. Assembly Pattern of the Acetate Units in Bikaverin

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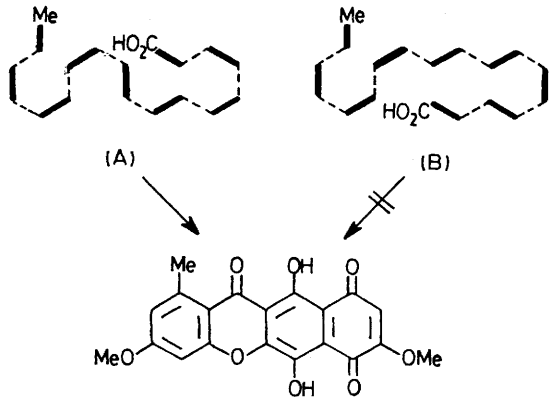
Summary In a ^{13}C n.m.r. study of bikaverin biosynthesis by *Fusarium oxysporum* the use of (1,2-^{13}C)-acetate and homonuclear ^{13}C decoupling overcame the difficulties of low ^{13}C-enrichment, so that the polyacetate origin and the arrangement of precursor units in the metabolite could be established.

BIKAVERIN (I) is a red anti-protozoal mould metabolite[1–3] inducing vacuolation in fungi[4] and is identical to the *Fusarium oxysporum* pigment previously known as lycopersin.[5] The structure established from chemical[2,6] and *X*-ray crystallographic[7] studies, is unique among natural products in containing a benzoxanthone ring system, but no biogenetic information has yet been available. In a preliminary experiment radioactivity from (1-^{14}C)-acetate was incorporated into (I) by *F. oxysporum*, supporting the suggestion[2] that the molecule is derived *via* a polyketide intermediate. However, the efficiency of incorporation was low, and supplementing cultures with (1-^{13}C)- and (2-^{13}C)-acetate failed to enrich (I) sufficiently to establish the alternate labelling of carbon atoms expected from this mode of biogenesis. In such circumstances the use of doubly-labelled precursors is advantageous[8] and we now report an experiment with (1,2-^{13}C)-acetate which confirms that (I) is biosynthesized by condensation of acetate units in pattern (A).

Assignment of peaks was complicated by the absence of hydrogen from all but three of the seventeen ring carbons,

and by the poor solubility of (I) in most solvents. Spectra were recorded with samples dissolved in CF_3CO_2D–$CDCl_3$ (1 : 1), despite difficulty with peak overlap from this solvent, because soluble derivatives could not be prepared in pure form and high yield. Incorporation of intact (1,2-^{13}C)-acetate units was detected by the appearance of satellites due to ^{13}C–^{13}C coupling in the ^{13}C n.m.r. spectrum (Figure) but low enrichment (<0·5% ^{13}C), entailing a low signal-to-noise ratio, and similarities in sp^2 character of the carbons in (I), caused uncertainties in the J_{CC} values which made it difficult to match pairs of labelled carbons from spacings in the spectrum. The usual ^1H decoupling procedures could be used to assign very few of the resonances and provisional assignments from proton-noise decoupled and high resolution pulse Fourier transform spectra were based mainly on comparisons with the model compounds 2,7-dimethoxy-5,8-dihydroxy-1,4-naphthoquinone and 3,7-dimethoxy-1-methyl-9-hydroxyxanthone (lichexanthone). Since these results are prone to errors, single ^{13}C frequency homonuclear decoupling experiments were used to match labelled pairs.



(I)

Proposed assembly of bikaverin from acetate units ($MeCO_2H$).

All resonances, except those of the methoxy carbons, which were labelled by L-(*Me*-^{13}C)-methionine, were accompanied by ^{13}C–^{13}C satellites. This establishes that the

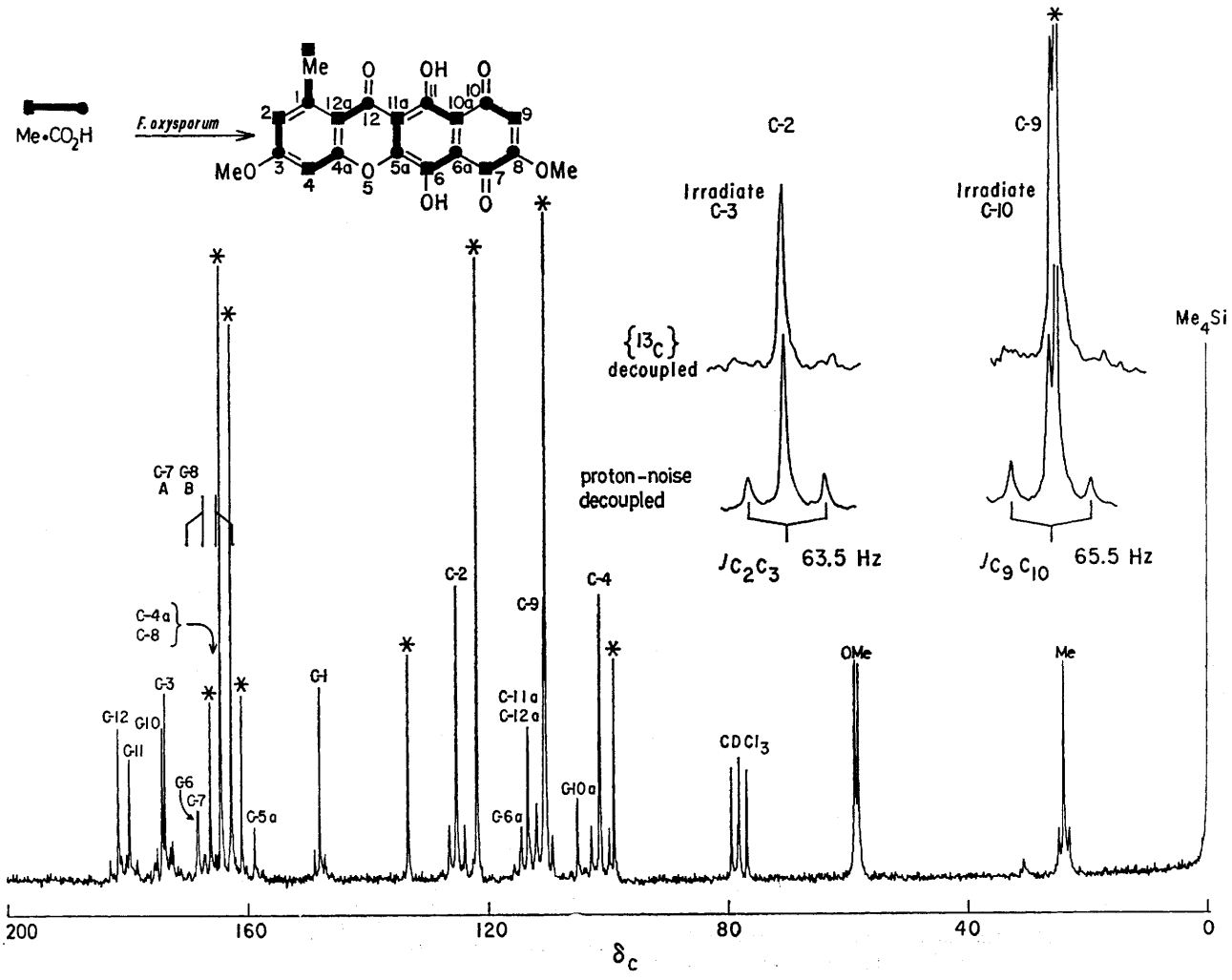


FIGURE. The proton-noise decoupled, pulse Fourier transform ^{13}C n.m.r. spectrum of bikaverin enriched from [1,2-^{13}C]-acetate. Asterisks identify resonances of CF_3CO_2D. The insert shows two examples of ^{13}C–^{13}C homonuclear decoupling ($\gamma H_2/2\pi$ *ca.* 70 Hz) with simultaneous ^1H decoupling.

benzoxanthone ring system is biosynthesized entirely by condensation of intact two-carbon units. Pairs of carbons matched by ^{13}C-decoupling were as follows: $^{1}J_{CC}$ Me, C-1, 41·8; C-2, C-3, 63·4; C-4, 74·2, C-4a, 76 ± 2; C-5a, 68·7, C-11a, 68·0; C-6, 64 ± 2, C-6a, 62 ± 2; C-9, 66·0, C-10, 65·5; C-10a, 65 ± 1, C-11, 66 ± 1; C-12, C-12a, 68·0 Hz; C-7, C-8 were present as a characteristic AB quartet, J_{AB} 65 ± 2 Hz. Chemical shifts, direct and long range coupling to hydrogen, and the pairs of matched carbons all correlated. Thus all spectral peaks could be assigned unequivocally.

The arrangement of ^{13}C–^{13}C pairs in (I) is consonant with a biogenesis *via* the acetate-polymalonate route. ^{13}C Enrichments were 0·40 ± 0·07% above natural abundance for all labelled positions. Values estimated by integrating satellite peaks in the ^{13}C and ^{1}H n.m.r. spectra[8] agreed well. Since *F. oxysporum* cultures were dosed on successive days with the maximum tolerated amount of labelled acetate to improve isotopic incorporation the intracellular pool of labelled precursor would be relatively constant, obscuring differences in enrichment due to starter and extension units, or to assembly from more than one polyketide intermediate. Thus these results do not establish whether (I) is biosynthesized by the folding of a single polyketide chain or by such alternatives as the extension of an orsellinate starter unit[2] or the condensation of preformed orsellinate and naphthoquinone intermediates. However, absence of orsellinic acid from the culture suggests a single polyketide chain as the most likely progenitor, and the labelling pattern obtained with (1,2-^{13}C)-acetate now makes it possible to distinguish between alternative directional modes of cyclization in fused carbocyclic metabolites such as bikaverin.

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