Biosynthesis of the Antibiotic Thermozymocidin. Incorporation of [1-^{13}C]Acetate: ^{13}C Nuclear Magnetic Resonance Study

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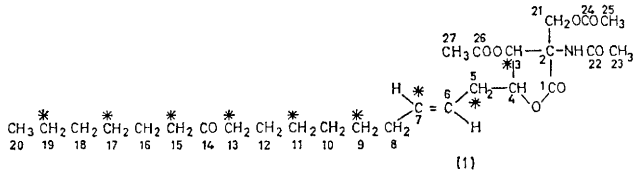
Summary A ^{13}C n.m.r. analysis of [1-^{13}C]acetate-derived thermozymocidin confirms the mixed biosynthesis of this antibiotic from serine and an acetogenine.

^{13}C NUCLEAR MAGNETIC RESONANCE spectroscopy of natural compounds makes an important contribution to the study of the biosynthetic pathways involved.[1]



(1)

Incorporation experiments[2] with [^{14}C]-labelled substrates revealed that the carbon skeleton of the microbial metabolite thermozymocidin is assembled from acetate units and L-serine.

The non-degradative procedure of ^{13}C n.m.r. can be used to examine whether or not acetate participates in the biosynthesis of serine in thermophilic fungi. Furthermore, the distribution of the label gives indications about the possible intervention of propionate as a starting unit in the biosynthesis of thermozymocidin, followed by loss of one carbon atom after coupling with serine.

The incorporation of [1-^{13}C]acetate (90%) was achieved on its addition (0·08 mg/ml) to a 1 day culture broth; the addition was repeated every 8 h. 1 Day after the first addition, isotope enriched thermozymocidin was isolated as previously described[3] and converted into the soluble triacetyl-γ-lactone (**1**).

The ^{13}C n.m.r. chemical shifts in the natural abundance of most of the 27 carbon atoms of (**1**) are: 172·9, 170·5, 170·2, 169·5 (C-1, -22, -24, -26); 62·6 (C-2); 72·6 (C-3); 81·6 (C-4); 32·3 (C-5); 123·7 (C-6); 135·2 (C-7); 31·7 (C-8); 29 (C-9, -10, -11, -17); 23·9 (C-12, -16); 42·9 (C-13, -15); 212·2 (C-14); 32·5 (C-18); 22·6 (C-19, -23); 14 (C-20); 63·4 (C-21); 20·4, 20·6 (C-25, -27). Assignments were made with the aid of (a) magnitudes and multiplicities of the residual splittings in the off-resonance CW decoupled

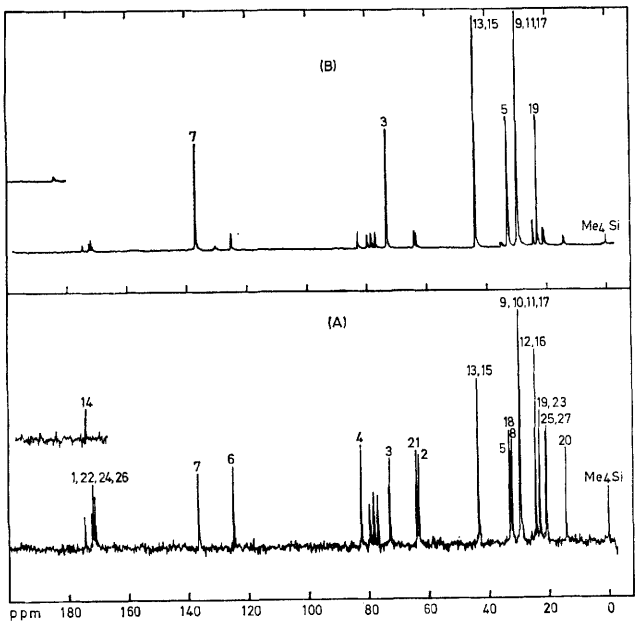


FIGURE. (A) Natural abundance ^{13}C n.m.r. spectrum of thermozymodicin triacetyl-γ-lactone (**1**); (B) corresponding spectrum of the [1-^{13}C]acetate-derived thermozymocidin triacetyl-γ-lactone.

The proton noise-decoupled spectra were recorded under identical experimental conditions at 25·2 MHz on a Varian XL-100 spectrometer using $CDCl_3$ as solvent and for field/frequency lock and Me_4Si as an internal reference.

spectrum,[4] and (b) chemical shift comparison with straight-chain hydrocarbons.[4]

The ^{13}C selectively enriched spectrum of (**1**) (Figure) shows that alternate C-3—C-20 carbon atoms have been labelled. No significant incorporation at C-1, C-2, and C-21 can be observed.

The mass spectrometric analysis demonstrated a difference in the ratios $M+1/M$ for the labelled and unlabelled thermozymocidin triacetyl-γ-lactone of 0·27. This corresponds to an average of 3% enrichment at each of nine positions or a total of 4·1% ^{13}C (with natural abundance of 1·1% ^{13}C included) at each of the labelled carbons.

The non-homogeneous distribution of the label could be observed by inspection of the isotope content of the fragment *m/e* 382 ($M - CH_3[CH_2]_5COCH_2$): the label on C-3, C-5, C-7, C-9, and C-11 shows an average of 4·3% ^{13}C enrichment at each of the five positions. Therefore the label on C-13, C-15, C-17, and C-19 is lower than the average value of 3%.

The ^{13}C n.m.r. spectrum of enriched thermozymocidin demonstrates that acetate does not participate appreciably, under the experimental conditions used, in the biosynthesis of serine and that the acetogeninic precursor is a polyketide of the type nC_2, thus ruling out the propionate–acetate route.

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[1] J. B. Stothers, 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York and London, 1972, p. 487 and refs. therein; G. Lukacs, *Bull. Soc. chim. France*, 1972, 351.

[2] F. Aragozzini, P. L. Manachini, R. Craveri, M. G. Beretta, B. Rindone, and C. Scolastico, *Ann. Microbiol. Enzym.*, 1972, in the press.

[3] F. Aragozzini, P. L. Manachini, R. Craveri, B. Rindone, and C. Scolastico, *Tetrahedron*, 1972, **28**, 5493.

[4] J. B. Stothers, 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York and London, 1972, p. 146 (ref. 9), p. 56 (ref. 1), and p. 81 (refs. 4 and 18).