Incorporation of [1-^{13}C]Butyrate into Antibiotic X-537A: ^{13}C Nuclear Magnetic Resonance Study

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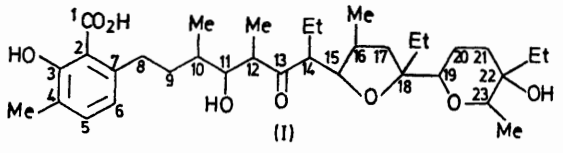
Summary The ^{13}C n.m.r. analysis of [1-^{13}C]butyrate derived antibiotic X-537A has clearly established butyric acid as the biosynthetic precursor of *all three* *C*-ethyl groups in the molecule.

INCORPORATION experiments[1] with ^{14}C labelled substrates revealed that the carbon skeleton of antibiotic X-537A (I) is assembled from acetate, propionate, and butyrate units. The four *C*-methyl groups at C-4, 10, 12, and 16 are propionate derived, whereas the *C*-methyl group at C-23 is formed from the initial acetate unit. Although it was established that butyrate incorporation was involved solely with the production of ethyl groups, it could not be determined directly whether one, two, or all three ethyls were butyrate derived.

The use of sodium [1-^{13}C]butyrate at 0·5 g l^{-1} in X-537A fermentations, followed by ^{13}C n.m.r. spectroscopy[2] of the isolated antibiotic has now clearly demonstrated that *all three* ethyl groups are derived from butyric acid (Table). In addition, incorporation experiments with [1-^{13}C]propionate have confirmed our earlier conclusions[1] concerning the origin of the methyl groups in antibiotic X-537A. The proton-decoupled natural abundance ^{13}C spectrum of the



(I)

antibiotic in chloroform exhibited thirty-three singlets (Figure) of which twenty have been assigned by either comparison with model compounds and degradation products or from biosynthetic considerations. The signals not included in the Table were at 34·5, 34·0, 33·5, 31·2, 19·7

Incorporation of [1-^{13}C]*butyrate and* [1-^{13}C]*propionate into antibiotic* X-537A *as determined by* ^{13}C *n.m.r.*

^{13}C Shift δ_C (p.p.m.)[a]	Assignments in(I)	function	% Abundance[b] of ^{13}C in X-537A produced from: $CH_3CH_2CH_2{}^{13}CO_2H$	$CH_3CH_2{}^{13}CO_2H$
	C=O			
218·4	C-13	Ketone	4	1
177·0	C-1	CO_2H	1	1
	Aromatic			
166·0	C-3	C–OH	1	4·5
143·3	C-7	C–CH_2	1	1
131·3	C-5	CH	1·5	1
123·2	C-4	C–Me	1	1
119·8	C-6	CH	1	1
118·7	C-2	C–CO_2H	1	1
	CO			
87·7	C-18	C(Et)–O	1	1
83·4	C-15	CH–O	1·5	4
77·3	C-23	CH(Me)–O	1·5	1
71·3	C-22	C(Et)–OH	1	1
70·7	C-11	CH–OH	1·5	4
68·7	C-19	CH–O	1	1
	CH, CH_2			
56·0	C-12	CH	1	1
49·0	C-8	CH_2	1	1
38·5	C-21	CH_2	5	1
37·8	C-9	CH_2	1	4
29·5	C-17	CH_2	4	1

[a] δ_C p.p.m. downfield from internal Me_4Si. [b] Corrected to nearest 0·5%.

(aromatic methyl), 16·2 (possibly 2 carbons), 15·8, 15·5, 13·6, 13·3, 12·7, 12·1, 9·4, and 7·0 p.p.m.

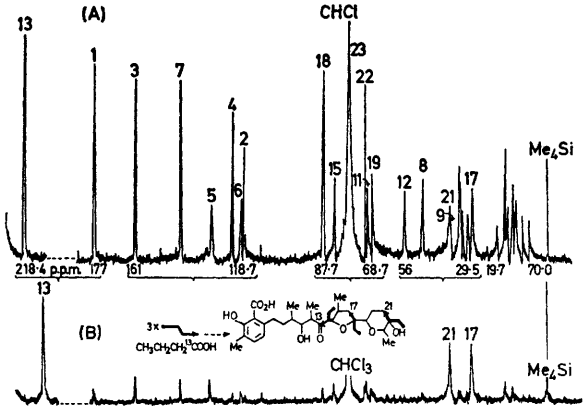


FIGURE (A) *Natural abundance* ^{13}C *n.m.r. of antibiotic* X-537A. *Concentration* 0·8 M, *analysis time* 32 *min*; (B) ^{13}C *n.m.r. of* [1-^{13}C]*butyrate derived antibiotic* X-537A. *The* ^{1}H *decoupled* ^{13}C *n.m.r. Fourier Transform spectra*[3] were recorded in 10 *mm spinning sample tubes on a Bruker* HFX-90/6 *spectrometer at* 22·63 MHz, *using an internal* ^{19}F *lock of* C_6F_6 *at* 84·66 MHz. *A Fabritek* FT-1083 *Computer was used for accumulation of free induction decays and Fourier Transformation.*

The ratio of peak heights in the spectra of the antibiotic produced on ^{13}C substrates to the natural abundance peak heights were calculated. These ratios are only an indication of the extent to which each carbon was enriched in ^{13}C content, as the correspondence between peak height (or area) and ^{13}C abundance is not a direct one.[2]

The enrichments considered most significant are those which resulted in at least a doubling of the natural abundance peak height. Using this criterion, the carbons at C-13, 17, and 21 were enriched in ^{13}C when [1-^{13}C]butyrate was added to the fermentation, whereas C-3, 9, 11, and 15 were enriched when [1-^{13}C]propionate was the labelled substrate.

The results using [1-^{14}C]acetate as substrate which were discussed in our earlier communication[1] probably involved partial conversion of acetate into butyrate prior to incorporation into the antibiotic. The reverse process, β-oxidation of butyrate may account for the apparent ^{13}C enrichment at C-5 and C-23 in the [1-^{13}C]butyrate incorporation results presented here.

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[2] A. G. McInnes, D. G. Smith, L. C. Vining, and L. F. Johnson, *Chem. Comm.*, 1971, 325, and references therein.
[3] T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR", Academic Press, New York, 1971.