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A ^{13}C NUCLEAR MAGNETIC RESONANCE STUDY OF THE BIOSYNTHESIS OF THE 16-MEMBERED MACROLIDE ANTIBIOTIC TYLOSIN

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Recently we have reported a systematic ^{13}C NMR study on 16-membered macrolide antibiotics [1] and as an application of this investigation a new biosynthetic scheme was established for leucomycin-A_3 [2]. From the point of view of the lactone ring the 16-membered macrolide antibiotics can be classified into two large groups : the magnamycin-leucomycin group and the tylosin-cirramycin group.

In analogy with the origin of the carbon skeleton of the lactone ring of methymycin [3], erythromycin [4] and leucomycin [2] the aglycone of tylosin [5] (Fig. 1) may be derived from two acetates + five propionates + one butyrate. However, the presently unknown origin of carbons-3 and -4 of leucomycin [2],[6],[7] made it advisable to examine whether the corresponding unit of tylosin (C-3 ; C-4 and C-18; Fig. 1) is derived from propionate. In order to elucidate this point and to confirm the origin of the whole lactone ring, a biosynthetic investigation of tylosin was undertaken and in the present paper we would like to report our results.

A tylosin-producing strain, Streptomyces fradiae C-373, was inoculated into a medium containing starch 1.5%, corn steep liquor 1.0%, yeast extract 0.5% and $CaCO_3$ 0.4%, and cultivated at 27°C. A 72-hour culture was transferred into a producing medium containing starch 2.0%, corn steep liquor 1.0%, sugar beat molasses 1.0%, soybean meal 1.5%, soybean oil 1.0% and $CaCO_3$ 0.2% in a reciprocal flask and the initial pH was adjusted to 7.5. After cultivation for 72 hours at 27°C the ^{13}C-labeled precursors ([1-^{13}C]Na-acetate, [1-^{13}C]Na-propionate, [1-^{13}C]Na-butyrate and [4-^{13}C] 2-ethylmalonate) were added at the concentration of 0.1% to the culture and the fermentation was further continued for an additional 96 hours. The culture filtrate was extracted with $CHCl_3$ at pH 8.0 and the extract was concentrated to dryness affording a crude powder. This was chromatographed over silica gel thin layer plate using $CHCl_3$ MeOH (10:1) as a developer to isolate pure ^{13}C-labeled tylosin (70 - 120 mg from 1 liter of culture filtrate).

^{13}C NMR spectra of the cold and the labeled tylosin samples were recorded in chloroform-d solution on a Bruker HX FT NMR spectrometer operating at 22.63 MHz. The incorporation ratio of the ^{13}C-labeled precursors is illustrated in Table 1.

Table 1 Incorporation of ^{13}C-labeled precursors into tylosin

Carbon atoms	Chemical shift δ(ppm) from $(CH_3)_4Si$	Enrichment factor[a]			Relative Intensity[b] (%)
		[1-^{13}C] acetate	[1-^{13}C] Propionate	[1-^{13}C] butyrate	[4-^{13}C]2-ethylmalonate
C-1	173 9	2.8	0 8	1 7	
2	39.4	1 5	1.8	2 0	
3 [c]	68 2	0.1	3 8	9 8	
4	45 1	1.7	1 3	1 1	156
5	81.6	2 2	1 3	22 7	
6	32.2	0.1	1 9	2.5	
7	32 9	2 5	6.1	17.3	
8	40 3	1.3	1.3	0 8	133
9	202.8	3 9	1.6	3.2	
10	118.8	1.0	0 9	0 8	
11	148 0	2.0	6 0	17.0	
12	134 9	1.0	1 0	1 0	100
13	142.2	2 0	5 7	15 6	
14	44 7	1.4	1.5	0.7	233
15	75 3	1.2	4.0	9 7	
16	25 5	1.1	1 1	0.9	200
17	9.0	0 7	1.0	0 8	
18	9.6	1 1	1.0	0 6	
19	43.9	2.8	1.3	1.8	367
20	203.0	1.9	0 5	1.3	
21	17.4	1 1	1 3	1.3	
22	13 0	1.1	1 2	0.8	
23 [c]	69.5	0 8	0.7	2 3	

a) Peak height $\frac{\text{enriched sample}}{\text{natural abundance}}$ from spectra run under essentially identical instrumental conditions.

b) The peak of C-12, as a reference, is taken for 100 % and the height of the other peaks is compared with it.Under the experimental conditions of recording this spectrum the natural abundance peaks were not observed.

c) Biosynthetic evidence indicates that the previously assigned signal[(1)] to C-23 at 68.2 ppm in the spectrum of tylosin represents actually C-3. The C-23 carbon of the antibiotic resonates probably at 69.5 ppm although the assignment of this site is still ambiguous.

When [1-^{13}C] acetate was added to the culture carbons-1 and -9 were strongly enriched and incorporation could be also detected on the two butyrate-derived carbons C-5 and C-19 as a consequence of the indirect incorporation of acetate at these sites [(2)]. The addition of [1-^{13}C] propionate revealed the enrichment of carbons-3, -7, -11,

-13 and -15. In the experiment with [1-^{13}C]butyrate, in addition to C-5 strong incorporation was unexpectedly also observed on the propionate-carboxyl derived carbons C-3, -7, -11, -13 and -15. When [4-^{13}C] 2-ethylmalonate was added to the culture in addition to C-19 the propionate-methylene derived carbons C-4, -8, -12, -14 and -16 indicated marked signal enhancement.

The incorporation pattern of the used precursors into the aglycone of tylosin is illustrated in Fig. 1.

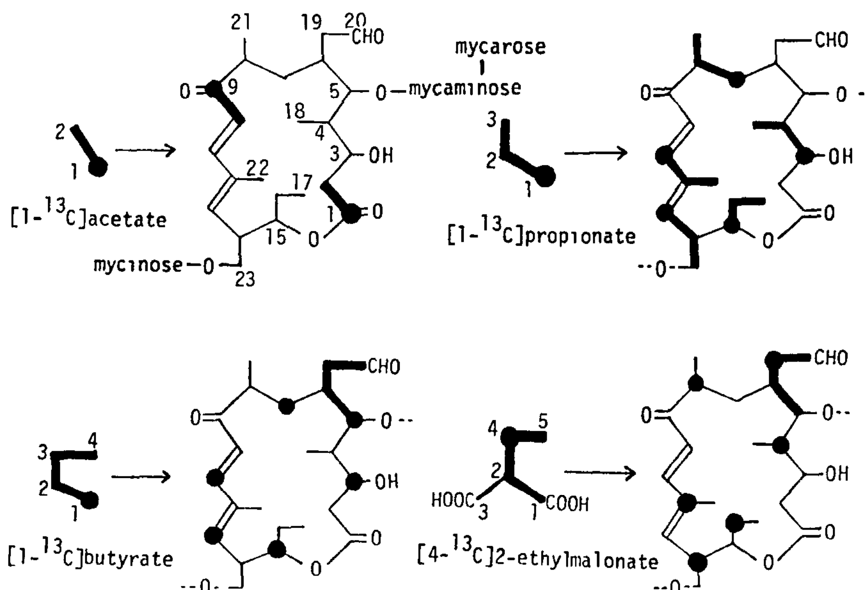

Fig 1 Biosynthesis of Tylosin

On the basis of the results obtained in the experiment with [1-^{13}C]butyrate and [4-^{13}C] 2-ethylmalonate we would like to emphasize two observations :

1. Both precursors indicate evidence for their direct incorporation into carbons-5, -6, -19 and -20 of tylosin.

2. Both precursors can be also metabolized into propionate and then incorporated into the lactone ring.However, their transformation into propionate seems to proceed by two different mechanisms since [1-^{13}C] butyrate and [4-^{13}C] 2-ethylmalonate show enrichment in the carbons originating respectively from C-1 and C-2 of propionate.The incorporation ratio of Table 1 affords evidence that the transformation of these precursors into propionate does not proceed via an acetate.

In conclusion the aglycone of tylosin is derived from two acetates + five propionates + one butyrate as indicated in Fig. 1.

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References

1. S. Omura, A. Nakagawa, A. Neszmelyi, S.D. Gero, A.-M. Sepulchre, F. Piriou and G. Lukacs, J.Amer.Chem.Soc., 97 , 4001 (1975).
2. S. Omura, A. Nakagawa, H. Takeshima, K. Atsumi, J. Miyazawa, F. Piriou and G. Lukacs, ibid., in press.
3. A.J. Birch, C. Djerassi, J.D. Dutcher, J. Majer, D. Perlman, E. Pride, P.W. Rickardo and R.J. Thomson, J.Chem.Soc., 5274 (1964).
4. H. Grisebach, H. Achenbach and M. Grisebach, Naturwiss., 47 , 206 (1960).
5. With respect to our previous paper [1] we have modified the carbon numbering of tylosin.
6. H. Grisebach and C.A. Weber-Schilling, Z. Naturforsch., 23 , 655 (1968).
7. D. Srinivasan and P.R. Srinivasan, Biochem., 6 , 3111 (1967).