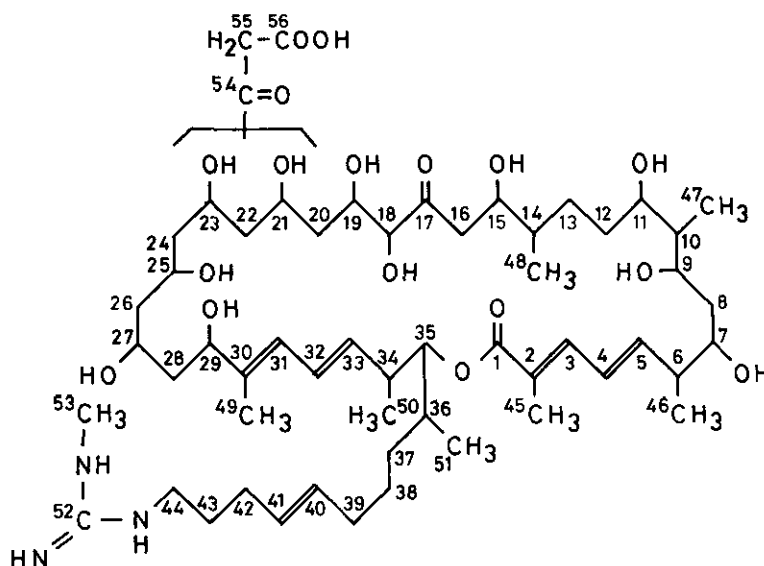


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Abstract --- Incorporation of [1-¹³C]acetate, [1-¹³C]propionate and [3-¹³C]propionate into azalomycin F_{4a} produced by cultured Streptomyces hygroscopicus var. azalomyceticus was studied. Fourteen molecules of acetate and 7 molecules of propionate were incorporated in the azalomycin F_{4a} molecule.

The antibiotic azalomycin F complex, produced by Streptomyces hygroscopicus var. azalomyceticus,¹⁾ shows broad anti-microbial spectrum against gram positive bacteria, yeast, fungi and protozoa.²⁾ Its clinical utility against vaginal trichomoniasis and candidiasis has also been demonstrated.^{3,4)}

Fig.1 The Structure of Azalomycin F_{4a}



The skeletal structure of azalomycin F_{4a} , $C_{56}H_{95}N_3O_{17}$, a main component of the F complex, was determined by summarizing the analytical and spectroscopic data of the compound and its degradation products, as reported in the preceding papers.⁵⁾ The structure is composed of a 36-membered $\Delta^{2,4,30,32}$ -lactone ring bearing 6 methyls, one ketone (at C-17, forming a hemiketal) and 11 hydroxy groups, and of a side chain attached to C-35 bearing a methyl, an olefinic and a terminal N-methylguanidine group (Fig. 1). Among 11 hydroxy groups, one forms a hemiketal ring with the 17-keto group and another forms a hemiester with a malonic acid at either the 21 or 23 position.

This structure suggests that the compound is biosynthetically related to the polyene macrolide antibiotics which are derived via so called polyketide biosynthetic pathway. The biosynthesis of 14- and 16-membered macrolide antibiotics has been studied for many years, using $C_2 \sim C_4$ carbon precursors labelled with ^{14}C or ^{13}C .⁶⁾ However biosynthetic studies of such polyene macrolides have so far been reported for only a limited number of examples. Incorporation of some ^{14}C -labelled precursors, including acetate and propionate, into nystatin,⁷⁾ mycoticin,⁸⁾ lucensomycin⁹⁾ and candidicin¹⁰⁾ has been proved, but the accurate positions of their incorporations were not determined in detail.

Here we describe the experiments using $[1-^{13}C]$ acetate, $[1-^{13}C]$ propionate and $[3-^{13}C]$ propionate, which enable us to determine accurately the incorporation positions of these precursors by ^{13}C -NMR spectroscopy. This also helps us to confirm the previously elucidated structure of azalomycin F_{4a} .⁵⁾

Two-day-old culture of *Streptomyces hygroscopicus* var. *azalomyceticus* were refloated on to fresh baker's yeast-medium¹¹⁾ (100 ml per flask), and cultivated for 48 hr at 27°. Then 100 mg of $[1-^{13}C]$ sodium acetate, $[1-^{13}C]$ sodium propionate or $[3-^{13}C]$ sodium propionate was added separately to respective culture (2 x 100 ml each) and they were shaken for 5 more days at 27°. The culture medium incubated with the respective precursors were worked up separately by the previously described procedure¹¹⁾ for obtaining the F complex. This was followed by silica gel column chromatography eluted with sec-butanol-water (9 : 1), and recrystallization of crude F_{4a} from methanol-water to give ca 20 mg of the respective specimens of pure ^{13}C -labelled azalomycin F_{4a} .

The results obtained by the proton noise decoupled ^{13}C -NMR spectra of these labelled

Chem.shift (ppm)	Multi- plicity	Assign- ment	¹³ C-enriched	Chem.shift (ppm)	Multi- plicity	Assign- ment	¹³ C-enriched
10.52	q	47	▲	65.53	d		●
12.88	q	45	▲	65.62	d		●
13.33	q	49	▲	66.32	d	27	●
14.37	q	51	▲	69.70	d		●
14.87	q	48	▲	70.69	d	bearing hemi- ester (21 or 23)	●
17.00	q	46	▲	72.34	d		●
17.63	q	50	▲	72.40	d		●
27.89	t			74.24	d	29	■
28.36	q	53		75.10	d	9	■
29.84	t			75.78	d	7	●
30.57	t + t	13*	■*	77.27	d	18	
33.57	t + t		●	80.77	d	35	■
34.47	t		●	99.78	s	17 (hemiketal)	●
35.16	d			125.12	d	31	●
39.24	t			126.74	s	2	
40.73	d			127.57	d	4	
40.90	d			128.52	d	32	
41.19	t			130.19	d	41	
41.85	t			132.51	d	40	
41.98	t + t			136.14	d	33	■
44.04	t			140.09	s	30	
44.40	d			140.17	d	3	●
44.49	d			146.07	d	5	■
44.58	t			158.27	s	52	
46.10	t	55		170.05	s	1	■
46.37	t			171.60	s	54	●
				174.06	s	56	

¹³C-NMR spectra were measured on JNM FX-400 (¹H:400.5 MHz, ¹³C:100.7 MHz).

Signal assignments were made from their chemical shifts and multiplicities as well as by ¹H-¹³C selective decoupling experiments.

● Incorporation of 1-¹³C acetate. ■ Incorporation of 1-¹³C propionate.

▲ Incorporation of 3-¹³C propionate.

* One of these two triplets.

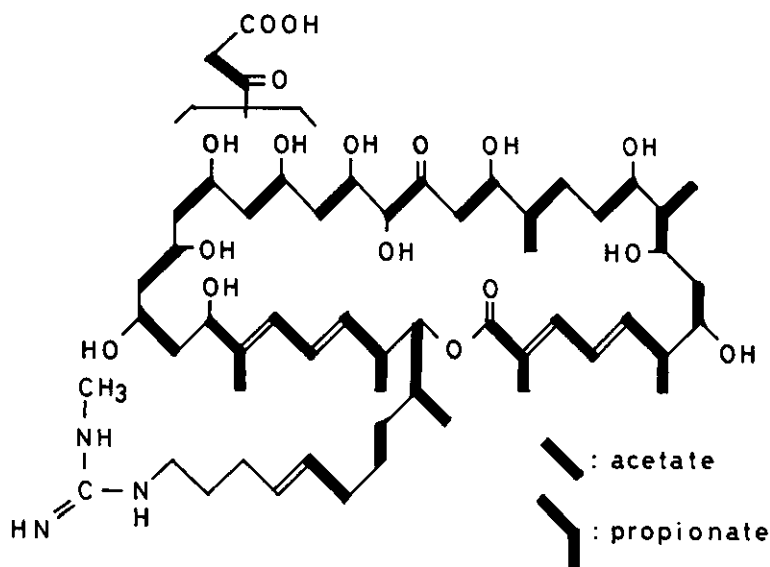
compounds are shown in the Table. The signal assignments in the Table are taken from the preceding paper^{5c}). These results demonstrated that :

1. Fourteen molecules of acetate and 7 molecules of propionate were incorporated in the F_{4a} molecule.
2. All C-methyl groups originated from C-3 of propionate.
3. Except C-18 and C-56, all of the carbons bearing oxygen function originated from C-1 of either acetate or propionate.
4. Olefinic carbons of $\Delta^{2,4,30,32}$ in the lactone ring should be derived from either acetate or propionate.

The carbon signals enriched by [1-¹³C]acetate, appearing at δ 33.57 and 34.47 have not been assigned yet. These signals, however, may be due to C-37 and C-39 since the terminal carbon chain, C-41 ~ C-44, probably is derived biogenetically from arginine, although no experiment using isotope labelled arginine has been carried out yet.

Consequently, incorporation of acetate and propionate into the azalomycin F_{4a} molecule could be indicated as depicted in Fig. 2.

Fig.2 Incorporation of Acetate and Propionate



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Received, 2nd September, 1981