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Biosynthetic Origins Of The Large Macrolide, Oligomycin A

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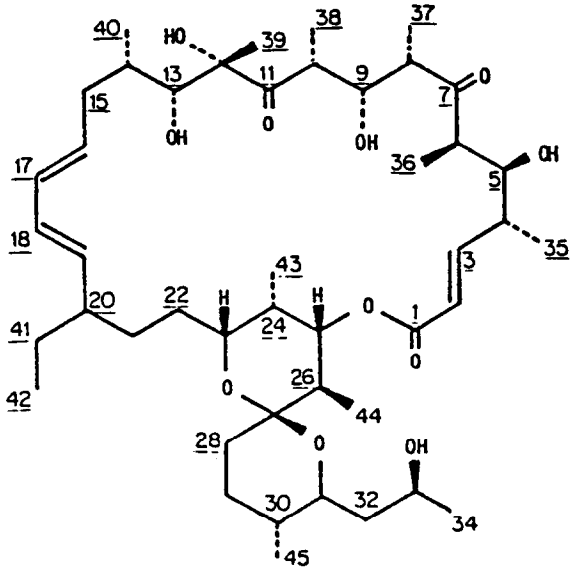
Summary: The overall biosynthesis of the large macrolide oligomycin A from a sequence of acetate, propionate and butyrate units, demonstrated by the specific incorporation of [1-^{13}C]-labelled precursors, is in accord with the known structure, and the indirect incorporations from propionate and butyrate are very low.

The oligomycin complex comprises antifungal macrolide antibiotics of relatively wide distribution amongst Streptomycetes.[1] Their structures were determined by a combination of chemical degradation and mass spectroscopy [2] and confirmed with stereochemical detail by X-ray diffraction.[3] The structures, e.g. oligomycin A (1), are among the more elaborate of macrolides, with a 26-membered ring fused to a spiroketal element. Biosynthetically the macrolides are polyketides and the simple acyl precursors of many have been identified; however the majority of such data relate to assemblies substantially smaller than (1). By inspection, oligomycin A should originate from no less than 17 consecutive acyl (or equivalent malonyl) sub-units, comprising 7 acetate, 9 propionate and 1 butyrate residues. The complexity of this assembly makes experimental verification of this hypothetical biosynthetic origin desirable.



(1)

The availability of a streptomycete producing oligomycin A (1) as a relatively major metabolite, and of full ab initio assignments of the natural-abundance [^{13}C] n.m.r spectum of (1) [4] has allowed us to verify the hypothetical overall biosynthetic origin by the incorporation of [^{13}C]-labelled precursors.

The organism was grown up in shake flask cultures on a glycerol-alanine medium, on which (1), as assayed by h.p.l.c., is produced most rapidly between 3 and 6 days after inoculation. The macrolide accumulates in the cells and reaches a maximum level of up to 100–120 mg/L by day 7. In successive experiments the precursors [1-^{13}C]acetate, [1-^{13}C]propionate and [1-^{13}C]butyrate were added as their Na salts (100 mg of 90% [^{13}C] precursor to each of ten 500 mL shake flasks containing 100 mL of medium) on day 3, and the combined flasks worked up by filtration on day 7. The quantities of precursor to be used per flask were calculated approximately by reference to the assumed turnover rates, comparing the overall rate of cell growth with the measured rate of oligomycin production. It can be calculated that approximately 10% of the total carbon flux in the cultures is by way of the presumed oligomycin precursors, whereas only about 1% of the total carbon supply is actually converted into (1); the amounts of added labelled precursor are thus commensurate with the relevant endogenous carbon flux.

The crude Me_2CO–$CHCl_3$ extracts from the mycelia were dried and purified by (i) flash column chromatography (Kieselgel 60), eluting with 80:20 EtOAc:cyclohexane, followed by (ii) preparative t.l.c., developing twice with 90:10 hexane:Pr^iOH and once with Et_2O, to give 10–20 mg samples of (1) sufficiently pure for spectroscopic measurements of the [^{13}C] enrichments. For the natural-abundance spectra, larger volumes of culture were similarly worked up, with final purification by h.p.l.c. [4]

All spectra were measured at 75MHz in methanol-d_4 (Varian XL-300). The integrated intensities in the measured spectra were first normalized to line intensities of unlabelled carbon atoms, and the enrichment factors for each of the carbon atoms of the three labelled samples were then calculated from the ratios of normalized line intensities in the enriched spectrum to the corresponding signals in the natural-abundance spectrum. Table 1 lists the results for the three labelled precursors in this form; enrichments considered to represent direct incorporation are underlined in bold type.

The direct incorporations unambiguously confirm the expected precursor sequence in (1), starting from the presumed "starter" residue (at C-34) as:

$$A_2\text{-}P\text{-}A\text{-}P_2\text{-}A\text{-}B\text{-}A_2\text{-}P_6\text{-}A \qquad (A = \text{acetate};\ P = \text{propionate};\ B = \text{butyrate}).$$

This 'heptadecaketide' is the largest macrolide sequence to have been established in this way, and the results are gratifyingly free from the ambiguities that have sometimes been encountered in studies of this kind.

From [1-^{13}C]acetate the enrichment factors at directly-labelled carbons vary between ca 2.5 and ca 5.6; the corresponding figures for [1-^{13}C]propionate are 9 to 16 and for [1-^{13}C]butyrate the enrichment is 32. These figures reflect the expected relative turnover rates for the corresponding acyl-coenzyme A species. The turnover of acetyl-CoA will be especially rapid since the cultures were provided with glycerol as the main carbon source and this is metabolized extensively through pyruvate and acetyl-CoA, so that a relatively low enrichment factor from [1-^{13}C]acetate was expected.

Incorporation of [^{13}C]-labelled precursors into oligomycin A (1).

Carbon atom no.[a]	Enrichment factor (see text) from incorporation of: [1-^{13}C]acetate	[1-^{13}C]propionate	[1-^{13}C]butyrate
1	**4.96**[b]	1.55	1.85
2	0.91	0.93	1.10
3	1.49	**10.60**	1.08
4	1.10	1.09	1.30
5	2.00	**15.80**	1.30
6	1.10	0.94	0.77
7	1.85	**9.20**	1.89
8	(obscured by solvent impurity)		
9	2.00	**14.30**	1.30
10	0.97	1.00	1.26
11	2.62	**9.13**	1.10
12	1.88	1.14	1.37
13	2.00	**14.90**	1.30
14	1.10	0.99	0.87
15	**4.02**	1.20	1.10
16	0.84	1.02	0.90
17	**3.56**	1.05	0.91
18	0.94	1.06	0.80
19	**2.49**	1.10	**32.00**
20	0.84	1.05	0.60
21	**4.00**	0.99	1.40
22	1.49	0.97	1.36
23	1.65	**12.80**	1.10
24	1.10	0.93	1.30
25	1.90	**13.73**	1.60
26	1.26	1.09	1.40
27	**4.47**	0.80	1.90
28	1.07	1.06	2.20
29	2.79	**14.93**	3.50
30	1.46	0.97	3.30
31	**5.57**	1.07	2.90
32	1.07	1.16	2.03
33	**5.57**	1.10	2.50
34	2.04	1.22	3.10
35	1.30	0.97	3.10
36	1.72	1.14	1.80
37	1.88	1.05	0.91
38	1.17	1.10	1.05
39	1.52	1.10	1.16
40	1.36	1.10	0.88
41	**3.58**	1.10	1.10
42	2.00	1.14	1.80
43	1.80	1.07	1.30
44	2.90	1.05	3.26
45	2.70	1.11	3.10

[a] numbering as (1) : signals assigned from natural-abundance spectra.[4]

[b] **bold type** : enrichments suggesting specific incorporation

Though the individual enrichments of the directly-labelled positions for both acetate and propionate vary over a range of ca 170% much of this variation is almost certainly due to the relative inaccuracy with which the intensities of the ^{13}C signals can be measured; beyond this, it appears that no detailed explanations are called for. There is no systematic trend in the figures, such as would, for example, tend to confirm suppositions of distinct biosynthetic intermediates for any particular section of the assembly (such as the starter sequence, the spiroketal system, or the six adjacent propionate units), even though comparisons of the structure of (1) with related macrolides might make such hypotheses attractive.

The indirect labelling from [1-^{13}C]acetate is expectedly high, reflecting the formation of other precursors from this intermediate, particularly from the glycerol carbon source. It is clear from Table 1 that the propionate units in (1) are then labelled predominantly at C-1. This label in the propionate (methylmalonyl) units arises from the expected route to methylmalonyl-CoA from acetyl-CoA, by way of the tricarboxylic acid cycle and the isomerisation of succinyl-CoA. The incorporation from [1^{13}C]acetate into the butyrate unit is into C-1 and C-3 of this unit, as expected.

In contrast to the situation with [1^{13}C]acetate, there is remarkably little indirect incorporation from either [1-^{13}C]propionate or [1-^{13}C]butyrate. The low incorporation from butyrate into the propionate units into the butyrate unit, and vice versa, is particularly noteworthy since considerable indirect interconversions of these precursors has been remarked in some other macrolides, e.g. narasin. [5] While such interconversions in themselves may generate considerable biochemical information they undoubtedly complicate the direct interpretation of biosynthetic data; our results suggest that they can be minimised by rational selection of the correct precursor levels and times of addition.

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