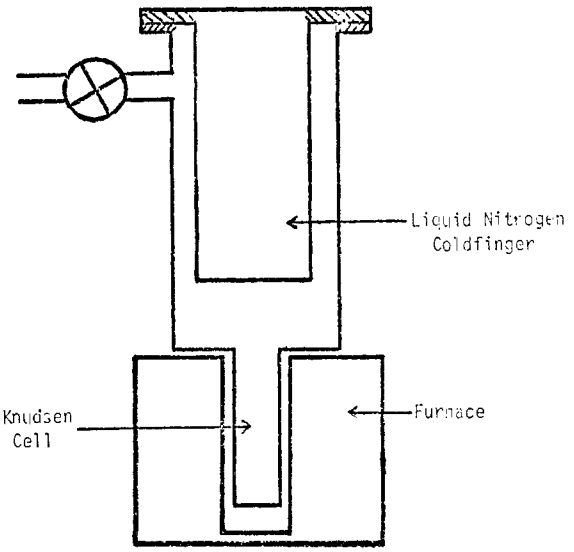


**Figure 1.** Improved reactor.

be made from these experiments is that the species $(C_3Li_4)_n$, which was first prepared by Robert West and co-workers from the reaction of butyllithium and propyne,[7] is an exceedingly stable polylithium compound, with a stability just below that of lithium carbide.

These results illustrate that, although it will be experimentally difficult to do so, there is hope for structurally characterizing gas-phase polylithium organic species; and, perhaps, it may involve techniques such as crossed-beam laser spectroscopy or matrix methods. We have discovered recently that flash vacuum heating of such compounds can result in vapor transport across a 10-cm path with no more than 10% decomposition. For example, if approximately 1 g of dilithiomethane is placed in the lower chamber of a reactor like the one shown in Figure 1 and heated very rapidly (from room temperature to approximately 1500 °C in less than 3 s), dilithiomethane is recovered 90% intact:

$$CH_2Li_2 \xrightarrow[2\ s]{1500\ °C} CH_2Li_2\ (90\%) + C_3Li_4\ (2\%) + C_2Li_2\ (8\%)$$

Such extremely fast flash heating can be effected by heating the Inconel metal Knudsen cell (see Figure 1) with induction coils, using a 30 kW, 300-kHz radio frequency generator. Experiments of this type made it clear that it is possible to obtain short-lived, high-temperature mass spectra, and such experiments are being conducted in our laboratory. These spectra will help elucidate and identify at least the stoichiometries of the gas-phase species actually observed for polylithium compounds. Thus far, the studies have shown that these electron-deficient, three-dimensional polymers produce not only monomers in the gas phase but also small polymeric clusters ranging in size from dimers to octamers; in many cases, the polymeric species are more abundant than the monomeric ones.[13] Specifically, the vapor species observed for $(CH_2Li_2)_n$ include $(CH_2Li_2)_n^+$ ($n$ = 1–6), those for $(CLi_4)_n$ include $(CLi_4)_n^+$ ($n$ = 1–5), and those for $(C_3Li_4)_n$ include $(C_3Li_4)_n^+$ ($n$ = 1–3).

In light of such observations, Schleyer and co-workers have begun focusing more intensive ab initio calculational efforts toward the prediction of the structures and stabilities of dimers and other polymers in the gas phase. Their calculations have indicated that many dimers and trimers are exceedingly stable.[4,14]

**Acknowledgment.** Support of this research by the National Science Foundation and the Robert A. Welch Foundation is gratefully acknowledged.

(13) J. W. Chinn, Jr., J. A. Gurak, and R. J. Lagow, to be published.
(14) P. v. R. Schleyer, Private communication.

# Biosynthesis of Macrolides. 5. Regiochemistry of the Labeling of Lasalocid A by $^{13}C$,$^{18}O$-Labeled Precursors

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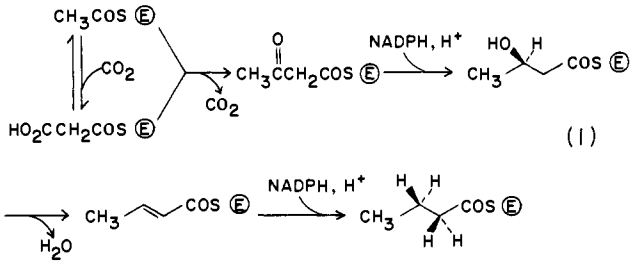
Macrolide and polyether antibiotics as well as fatty acids are believed to be constructed by similar or identical processes in eukaryotic organisms.[1] It is known that fatty acid biosynthesis occurs by the condensation of enzyme-bound acetate and malonate through a repetitive sequence of four stereospecific enzymatically catalyzed reactions (eq 1), within a large, multienzyme complex,




and without the intervention of non-enzyme-bound intermediates.[2] It has therefore been presumed that the archetypal macrolide antibiotic erythromycin A (**1**) and the polyether ionophore antibiotic lasalocid A (**2**) are assembled in the same way from either acetate, propionate, or butyrate via their respective malonate analogues.[3] However, if this were true, corresponding chiral centers in the $C_2$–$C_4$ subunits of **1** and **2** should have the same absolute configurations. The stereochemistry of **1** and **2**, as well as that of their simplest putative precursors, **3**[3] and **4**,[6b] appears to be inconsistent with such a simplistic concept for their biogenesis because the absolute configuration of secondary alcohol and methine carbons derived from separate precursor molecules varies irregularly.[4] Consequently, either the enzymology of macrolide and polyether antibiotic biosynthesis is much more complex than suggested by the analogy to fatty acid biosynthesis or the stereocontrol over the formation of these antibiotics occurs subsequent to the carbon chain elongation process.

†Research Career Awardee of the National Cancer Institute (CA 00253), 1976–1981.

(1) (a) Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 585. (b) Omura, S.; Nakagawa, A. *J. Antibiot.* **1975**, *28*, 401. (c) Queener, S. W.; Sebek, O. K.; Vezina, C. *Annu. Rev. Microbiol.* **1978**, *32*, 593.

(2) (a) Volpe, J. J.; Vagelos, P. R. *Physiol. Rev.* **1976**, *56*, 339. (b) Bloch, K.; Vance, D. *Annu. Rev. Biochem.* **1977**, *46*, 263. (c) Wakil, S. J.; Bressler, R. *J. Biol. Chem.* **1962**, *237*, 687. (d) Sedgwick, B.; Cornforth, J. W.; French, S. J.; Gray, R. T.; Kelstrup, E.; Willadsen, P. *Eur. J. Biochem.* **1977**, *75*, 481. (e) Seyama, Y.; Kasama, T.; Yamakawa, T.; Kawaguchi, A.; Okuda, S. *J. Biochem. (Tokyo)* **1977**, *81*, 1167; **1977**, *82*, 1325. (f) Sedgwick, B.; Morris, C.; French, S. J. *J. Chem. Soc., Chem. Commun.* **1978**, 193. (g) Sedgwick, B.; Morris, C. *Ibid.* **1980**, 96. (h) Kawaguchi, A.; Yoshimura, T.; Saito, K.; Seyama, Y.; Kasama, T.; Yamakawa, T.; Okuda, S. *J. Biochem. (Tokyo)* **1980**, *88*, 1. (i) White, R. H. *Biochemistry* **1980**, *19*, 9.

(3) (a) Hung, P. P.; Marks, C. L.; Tardrew, P. L. *J. Biol. Chem.* **1965**, *240*, 1322. (b) Martin, J. R.; Rosenbrook, W. *Biochemistry* **1967**, *6*, 435.

(4) W. D. Celmer (Celmer, W. D. *J. Am. Chem. Soc.* **1965**, *87*, 1801. Celmer, W. D. *Pure Appl. Chem.* **1971**, *28*, 413) has noted that there is a remarkable stereochemical homogeneity among the different classes of macrolide antibiotics produced by bacteria. This fact suggests the occurrence of evolutionary consistency during the development and maintenance of the pertinent enzymatic systems.

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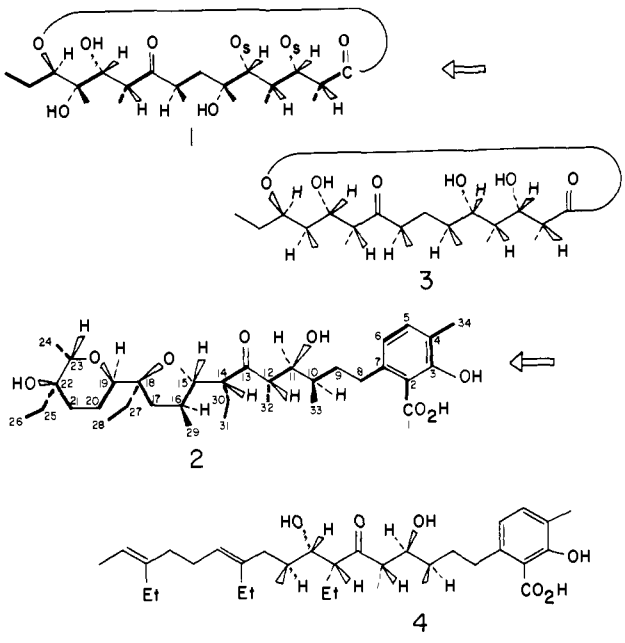
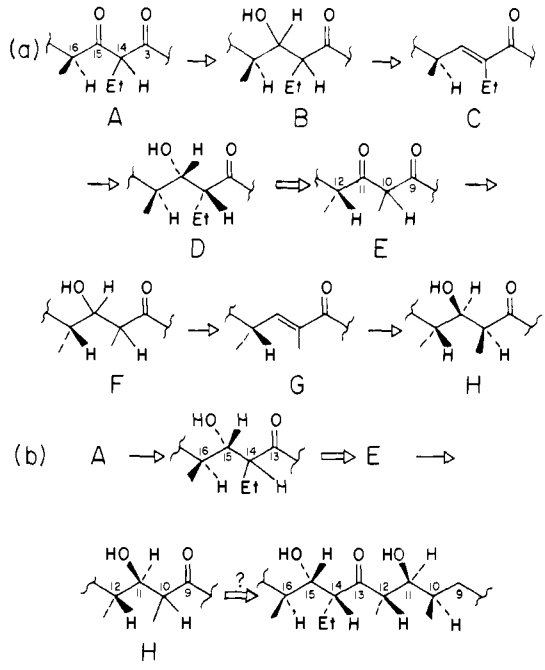


The thickened lines indicate the individual portions of the molecules that are labeled biosynthetically by either acetate, propionate, or butyrate. $O_s$ in I indicates attachment to a sugar molecule.

Scheme I. Two Hypotheses for the Biosynthetic Formation of the C-9–C-16 Fragment of Lasalocid A (2). (a) Dehydration–Enone Rehydration Idea; (b) Stereodivergent Reduction of the β-Ketoacyl Group Idea[a]



[a] The thickened arrows between D and E in (a) and (b) indicate the addition of $C_3$ subunits during carbon chain assembly.

Our curiosity about the biochemical mechanisms underlying the stereocontrol of macrolide[5] and polyether antibiotic biosynthesis led us to examine the biosynthesis of lasalocid A in greater detail than in previous studies.[6] In this study we wish to answer two principal questions: How does Nature determine the absolute stereochemistry and the sequence of precursor assembly during the formation of **2**? These questions are relevant to the biosynthesis of all macrolide and polyether antibiotics. Thus, any insight we obtain from the study of **2** should have general implications.

There are three plausible mechanisms for formation of the hydroxymethylene centers in the C-9–C-16 portion of lasalocid A (**2**): direct reduction of the keto group in the growing β-ketoacyl chain, reduction followed by dehydration and stereospecific rehydration of the resulting enone, or multistep reduction to a deoxygenated chain followed by aerobic oxidation. For example, as a corollary to one of these ideas, one can imagine that the syn relationship of the C-14 ethyl and C-15 oxygen and of the C-10 methyl and C-11 hydroxyl of **2** is not accidental but may mean that some biochemical process simultaneously determines the absolute stereochemistry at C-10/C-11 or at C-14/C-15. An attractive mechanism for such diastereoselection[7] is the hydration of an enone with anti stereoselectivity (Scheme Ia). Hence the hydration of subunit D of **2** would have to have the stereoselectivity shown for C → D; for subunit H, it would be that shown for G → H. The overall biochemical process would be expected to involve carbon chain elongation from the $C_2$ starter unit (C-23, C-24) toward C-16 to give A, followed by reduction to C, and then dehydration to D. The subsequent incorporation of two additional $C_3$ units would give E, and eventually G, which would have to undergo rehydration with a stereoselectivity opposite to that giving D from C. Alternatively (Scheme Ib), biochemical reduction of C-15 in A, or C-11 in E, could establish the absolute stereochemistry at these carbons. However, there is no obvious mechanism by which the stereocontrol can be exercised simultaneously at C-14 and C-15 as well as at C-10 and C-11.[8]

These two hypotheses can be distinguished by determining the biochemical origin of the oxygen atoms at C-11, C-13, and C-15 and the hydrogen atoms at C-9, C-12, and C-14 in **2**. If precursors carrying isotopes of these atoms label appropriate "early" intermediates of the biosynthetic pathway, the absence of these isotopes in **2** would be consistent with Scheme Ia, whereas their presence in **2** would be consistent with Scheme Ib. In this paper and the following one we report that the results of biosynthetic feeding experiments are consistent only with the latter.

Since Westley and co-workers[6a] had established the simple precursor–product relationships for **2**, we could direct our attention to determining whether incorporation of [1-$^{13}$C,1-$^{18}$O]propionate and [1-$^{13}$C,1-$^{18}$O]butyrate labels **2** with $^{13}$C and $^{18}$O at C-3/O-3, C-11/O-11, C-13/O-13, and C-15/O-15. The presence of intact $^{13}$C–$^{18}$O units was established by observing $^{18}$O-induced isotope shifts of carbon resonances[9] in the high-field $^{13}$C NMR spectrum of **2** and confirmed by high-resolution electron-ionization mass spectrometry of **2**.

Precursor feeding experiments with sodium [1-$^{13}$C,1-$^{14}$C,1-$^{18}$O]propionate and sodium [1-$^{13}$C,1-$^{14}$C,1-$^{18}$O]butyrate carried out separately in shaking-flask fermentations of *Streptomyces lasaliensis* (NRRL 3382R)[10] gave samples of **2** containing suf-

(5) (a) Hutchinson, C. R.; Kurobane, I.; Mabuni, C. T.; Kumola, R. W.; McInnes, A. G.; Walter, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 2474. (b) Hutchinson, C. R.; Kurobane, I.; Cane, D. E.; Hasler, H.; McInnes, A. G. *J. Am. Chem. Soc.* **1981**, *103*, 2477.

(6) (a) Westley, J. W.; Evans, R. H.; Harvey, G.; Pitcher, R. G.; Pruess, D. L.; Stempel, A.; Berger, J. *J. Antibiot.* **1974**, *27*, 288. (b) Westley, J. W.; Blount, J. F.; Evans, R. H.; Stempel, A.; Berger, J. *Ibid.* **1974**, *27*, 597. (c) Westley, J. W.; Benz, W.; Donahue, J.; Evans, R. H.; Scott, C. G.; Stempel, A.; Berger, J. *Ibid.* **1974**, *27*, 744.

(7) (a) Heathcock, C. H.; White, C. T. *J. Am. Chem. Soc.* **1979**, *101*, 7076. (b) Bartlett, P. A. *Tetrahedron* **1980**, *36*, 2.

(8) An attractive mechanism would be one that is akin to the least hindered approach rationalization for nucleophilic addition to carbonyls (Cram's Rules: Cram, D. J.; Abd Elhafez, F. A. *J. Am. Chem. Soc.* **1952**, *74*, 5828, 5851. Cram, D. J.; Kopecky, K. R. *Ibid.* **1959**, *81*, 2737) if we were to consider the biochemical reductant as the nucleophile.

(9) (a) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252. (b) Vederas, J. C. *Ibid.* **1980**, *102*, 374. (c) Diakur, J.; Nakashima, T. T.; Vederas, J. C. *Can. J. Chem.* **1980**, *58*, 1311. (d) Risley, J. M.; Van Etten, J. M. *J. Am. Chem. Soc.* **1980**, *102*, 4609, 6699.

(10) The fermentations were carried out according to ref 6a in which we added the precursors once daily at concentrations of 5 mM in the fermentation medium on days 6–9 of the 10-day fermentations.

Table I. $^{13}C,^{18}O$ Content of Lasalocid A and Its Derivatives As Determined by $^{13}C$ NMR Spectroscopic Analysis[a]

| compd | carbon[b] | $\delta_c$,[b] ppm | $\Delta\delta_c$,[c] Hz | peak heights, cm | | isotopic labeling ratios,[e] % | retention of $^{18}O$,[f] % |
|---|---|---|---|---|---|---|---|
| | | | | $^{13}C$-$^{16}O$[d] | $^{13}C$-$^{18}O$ | | |
| **2**[g] | 15 | 83.2 | 2.0 | 9.5 | 21.4 | 69 | 96 |
| **5**[g] | 3 | 155.4[i] | 1.6 | 11.4 | 24.1 | 68 | 95 |
| | 15 | 85.1 | 2.2 | 12.8 | 25.5 | 67 | 92 |
| **5**[h] | 13 | 215.6 | 5.3 | 13.6 | 26.2 | 66 | 95 |
| | 1 | 169.1[i] | 1.5 ($COCH_3$) | 23.8 | 14.1 | 16[j] | 52[j] |
| | | | 3.7 (C=O) | | 17.3 | 20[j] | |
| **6**[g] | 3 | 155.6[i] | 1.5 | 11.5 | 22.9 | 67 | 92 |
| | 15 | 86.4 | 2.0 | 9.7 | 23.1 | 70 | 98 |
| | 11 | 80.6[k] | 4.2 | 14.2 | 24.0 | 63 | 87 |

[a] The $^{13}C$ NMR spectra were determined on a Bruker WH 400 spectrometer at 100.6 MHz. Signals for carbons bearing oxygens were obtained by using a 32 K data block/1000-2000 Hz, 60–400 scans, 8–16 s acquisition time and 45° pulse angle. [b] The $\delta_c$ are from ca. 0.1 M solutions of labeled compound in $CDCl_3$ and are relative to $Me_4Si$ as internal standard. Assignments of $\delta_c$ are as reported by; Seto, H.; Westley, J. W.; Pitcher, R. G. *J. Antibiot.* 1978, *31*, 289. [c] Measured from center of signal at half-height. [d] Corrected for the contribution due to natural abundance $^{13}C$–$^{16}O$. [e] Calculated by dividing the height of $^{13}C$–$^{18}O$ signal by the total height of the two signals and multiplying by 100. [f] Calculated by dividing the isotopic ratio percentage of the $^{13}C,^{18}O$-labeled site in lasalocid A or its derivative by the [$^{13}C,^{18}O$]-labeling probability percentage of the appropriate 1-$^{13}C$,1-$^{18}O$ precursor. [g] Labeled by the incorporation of [1-$^{13}C$,1-$^{18}O$]propionate ($^{13}C$–$^{18}O$ content = 72% as shown by EIMS analysis of its *p*-phenylphenacyl derivative). [h] Labeled by the incorporation of [1-$^{13}C$,1-$^{18}O$]butyrate ($^{13}C$–$^{18}O$ content = 70% as determined in *g*). [i] $\delta_c$ assigned by characteristic upfield shift on *o*-methylation relative to value for these signals in **2**. [j] See ref 15. [k] $\delta_c$ 73.4 in **2**.

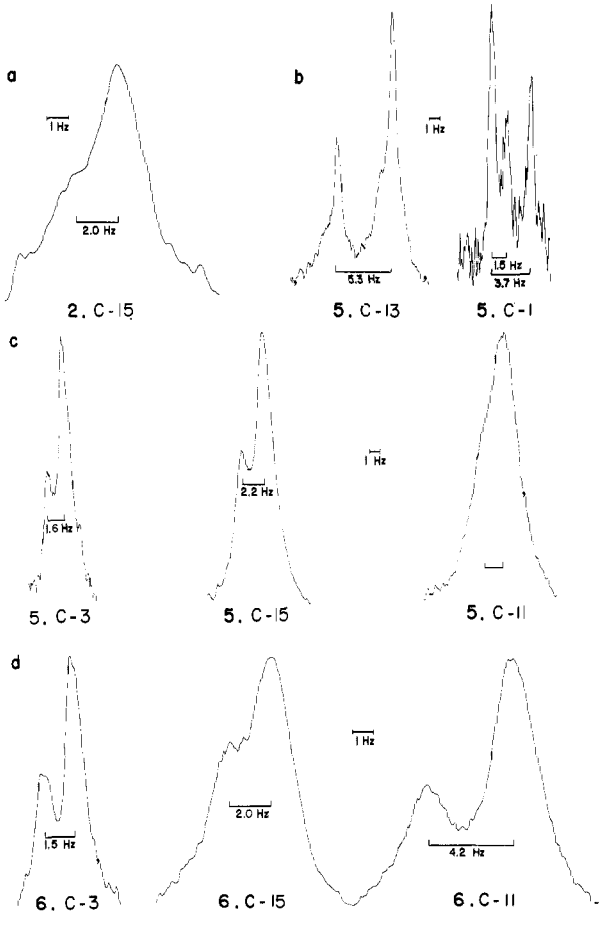



**Figure 1.** Signals for the $^{13}C$–$^{18}O$-labeled carbons of lasalocid A (**2**) and its two derivatives, **5** and **6**, as they appear in the 100.6-MHz $^{13}C\{^1H\}$ NMR spectra. The resonances due to $^{13}C$–$^{16}O$-labeled carbons are on the left-hand side (lower field) and those due to $^{13}C$–$^{18}O$-labeled carbons on the right-hand side of each set of signals. A line-broadening factor of 0.1–0.5 Hz was applied to each spectrum before Fourier transformation of the FID. (a), (c), and (d) are from $^{13}C$ NMR spectra of **2** that was labeled by the biosynthetic incorporation of [1-$^{13}C$,1-$^{18}O$]propionate; (b) is from $^{13}C$ NMR spectra of **2** that was labeled by the [1-$^{13}C$,1-$^{18}O$]butyrate.

ficient isotopic enrichment[11] for NMR analysis. $^{13}C$ NMR analysis at 100.6 MHz of [$^{13}C,^{18}O$]-**2** enriched from the propionate resolved (Figure 1) only one isotopically shifted resonance, C-15/O-15, of 1,3-bis(*O*-methyl)-[$^{13}C,^{18}O$]-**2**(**5**)[12] resolved two isotopically shifted resonances, C-3/O-3 and C-15/O-15, and of 11,22-bis(*O*-trifluoroacetyl)-**5**(**6**)[13] resolved three isotopically shifted resonances, C-3/O-3, C-11/O-11, and C-15/O-15.[14] Isotopic ratios [$I_{^{13}C-^{18}O}/(I_{^{13}C-^{18}O} + I_{^{13}C-^{16}O})$], determined from the intensities ($I$) of $^{13}C$ resonances arising from enrichment for each site in **2**, **5**, and **6**, when compared with the corresponding ratio for [1-$^{13}C$,1-$^{18}O$]propionate established that 94 ± 7% of the $^{18}O$ in the $^{13}C$–$^{18}O$ units had been retained (Table I). An identical analysis of **5**, which had been prepared from **2** enriched from butyrate, revealed isotopically shifted resonances at C-1 and C-13 only. The isotopic ratio for C-13 was ca. 95% of that of the precursor, as was that for C-1, which must have been labeled by incorporation of the [1-$^{13}C$,1-$^{18}O$]acetate derived from C-1 and C-2 of the butyrate by $\beta$ oxidation.[15]

The isotopic distribution $^{13}C,^{18}O$-labeled ions resulting from fragmentation of the $M^{+\cdot}$ in the EIMS of **5** and **6** was consistent with the labeling regiochemistry revealed by $^{13}C$ NMR analysis. For example, the base peak at $m/z$ 571.292 in the EIMS of **6**, which had been prepared from **2** labeled by the propionate, exhibited isotopically labeled ions at $m/z$ 581.319 whose intensity were 7.5 mol % of all of the ionic species at 571. These data are consistent with fragmentation of **6** to produce ions containing $^{12}C_{25}{}^{13}C_4H_{42}{}^{16}O_4{}^{18}O_3CF_3$ (eq 2).

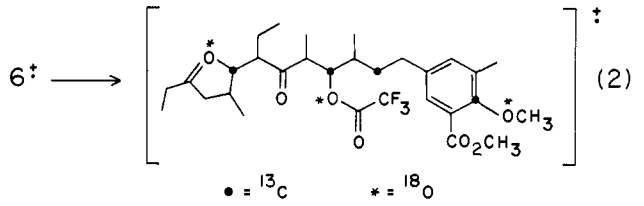
(11) The $^{14}C$ radioactivity of **2** indicated the following specific incorporations of C-14 labeled precursors: propionate, 96%; butyrate, 150%. If the $^{14}C$ were evenly distributed among the sites in **2** labeled by these two precursors,[6a] then the specific $^{14}C$ incorporation per $C_3$ or $C_4$ subunit would be 24% and 50%, respectively.

(12) Westley, J. W.; Oliveto, E. P.; Berger, J.; Evans, R. H.; Glass, R.; Stempel, A.; Toome, V.; Williams, T. *J. Med. Chem.* **1973**, *16*, 397.

(13) This derivative gave spectral data fully consistent with the assigned structure.

(14) Although the dependency of $\Delta\delta_C$ and signal resolution on structure is not well understood yet,[9] the present data are consistent with it being dependent in part on intramolecular hydrogen bonding. Thus as the opportunity for the latter process decreases (**2** → **5** → **6**), the resolution of the $^{13}C$–$^{16}O$ and $^{13}C$–$^{18}O$ signals increases. The electronegativity of substituents attached to the C and the O also affect $\Delta\delta_C$ (e.g., **5** vs. **6** at C-11).[9]

(15) The maximum $^{13}C,^{18}O$-labeling probability of C-1 of **2** should be one-half that of the butyrate-derived [1-$^{13}C$,1-$^{18}O$]acetate due to the introduction of $H_2{}^{16}O$ at C-1 upon release of the antibiotic by hydrolysis of its C-1 thioester bond at some stage of the biosynthetic pathway.[16]




The results of the experiments described above are consistent with the hypothesis shown in Scheme Ib for the biosynthesis of lasalocid A (**2**) but not with that shown in Scheme Ia, provided that in vivo $H_2{}^{18}O$ has a vanishingly small probability of rehydrating the same molecule from which it was eliminated, nor with a mechanism involving aerobic oxidation at C-1, C-3, C-11, C-13, or C-15 of **2**. [Westley has postulated that aerobic oxidation introduces the C-19 and C-22 oxygen atoms,[6b] which is consistent with our results.] These data also show that [1-$^{13}$C,1-$^{18}$O]-propionate and -butyrate lose very little $^{18}$O from their carboxyl groups between the time they are added to the fermentation medium and they are incorporated into **2**. This observation contrasts with the loss of 46–64% of the $^{18}$O content of acetate during the biosynthesis of the macrolide antibiotic brefeldin A[16] and several aromatic polyketide metabolites.[17] We cannot reconcile this difference with the information available to us. We note, however, that the most likely biochemical processes for converting exogenous fatty acids into their enzyme-bound thioesters[18] should not result in a significant loss of $^{18}$O. This belief is underscored by the observation that the acetate responsible for labeling C-1 of **2** must have contained ≥95% of the theoretical maximum $^{13}$C–$^{18}$O content based on the butyrate from which it was derived.[15]

We have not obtained conclusive answers to the questions raised in the introduction from the outcome of the present experiments. However, we have gained some insight into the general problem of stereocontrol during macrolide and polyether antibiotic biosynthesis. The accompanying papers by Cane and co-workers concerning the biosynthesis of erythromycin A and monensin provide additional important information,[19] which together with our results give us valuable direction for future experimentation. We shall pursue the problem further with the aim to resolve the following issue: Is the stereocontrol over these antibiotics' biosynthesis effected by a one enzyme–one reaction relationship, which would require many enzymes, or by a more intricate relationship involving fewer enzymes but ones that are apparently stereodivergent?

**Acknowledgment.** This research was supported in part by grants to C.R.H. from the National Institutes of Health (GM 25799) and to J.C.V. from the Natural Sciences and Engineering Research Council of Canada (NSERC). The Stable Isotopes Resource of Los Alamos National Laboratory provided generous gifts of $H_2{}^{18}O$ for the preparation of $^{18}$O-labeled precursors. We are indebted to Mel Micke, Biochemistry Department, University of Wisconsin, for the high-resolution EIMS analyses, Professor A. G. McInnes for critical reading of the manuscript, and Dr. John Westley, Hoffmann-La Roche, Nutley, NJ, for a sample of **4**.

(16) Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1979**, *101*, 707.
(17) (a) Gatenbeck, S.; Mosbach, K. *Acta Chem. Scand.* **1959**, *13*, 1561. (b) Scott, A. I.; Wiesner, K. J. *J. Chem. Soc., Chem. Commun.* **1972**, 1075. (c) Vederas, J. C.; Nakashima, T. T. *Ibid.* **1980**, 183.
(18) Conversion to the mixed anhydride with ATP and reaction of the latter compound with CoASH or EnzSH. See: Raczynska-Bojanowska, K.; Ruczaj, Z.; Sawnor-Korszynska, D.; Rafalski, A. *Antimicrob. Agents Chemother.* **1973**, *3*, 162.
(19) Cane, D. E.; Hasler, H.; Liang, T. C. *J. Am. Chem. Soc.*, third paper in this series. Cane, D. E.; Liang, T. C.; Hasler, H. *Ibid.*, fourth paper in this series.

# Biosynthesis of Macrolides. 6. Mechanism of Stereocontrol during the Formation of Lasalocid A†

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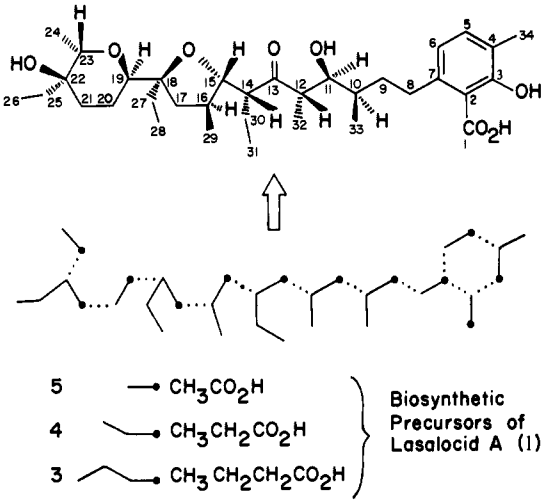
*Received June 1, 1981*

In a previous paper[1] we noted that configurational differences at skeletal carbons bearing identical substituents in macrolide (erythromycin A) and polyether (lasalocid A, **1**) antibiotics appear to be incompatible with a current belief that they are constructed by cycles of stereospecifically identical reactions, as in fatty acid biosynthesis. Studies were, therefore, initiated to determine how stereochemical control is exercised during the construction of such antibiotics in the hope that comparisons between fatty acid and polyketide biosynthesis can be placed on a firmer foundation.




The origin of carbons in lasalocid A biosynthesis has been established in a previous study,[2] and we have also shown[1] that intact carbon–oxygen fragments from 1-$^{13}$C,1-$^{18}$O-labeled propionate were incorporated by *Streptomyces lasaliensis* into hydroxymethylene groups with different configurations at the 11 and 15 positions of **1**. Stereochemical control during reduction of carbonyl groups in β-ketoacyl thioester type intermediates seemed to be the most likely explanation for this result. This requires a single enzyme capable of promoting stereochemically divergent reductions or the presence of two reductases with different stereospecificities.

How stereochemical control is exercised during chain extension is another aspect of lasalocid A biosynthesis that requires study. This might be expected to depend on the absolute configurations of the methyl- and ethylmalonyl-CoA precursors as well as the

† NRCC No. 19537.
(1) Hutchinson, C. R.; Sherman, M. M.; Vederas, J. C.; Nakashima, T. T. *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) Westley, J. W.; Evans, R. H.; Harvey, G.; Pitcher, R. G.; Pruess, D. L.; Stempel, A.; Berger, J. *J. Antibiot.* **1974**, *27*, 288–297.

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