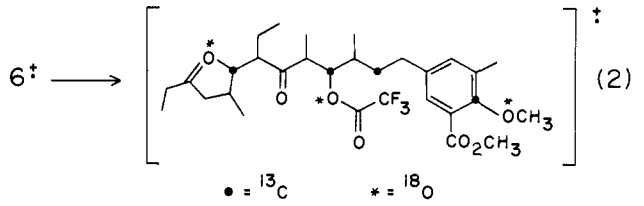

The results of the experiments described above are consistent with the hypothesis shown in Scheme Ib for the biosynthesis of lasalocid A (**2**) but not with that shown in Scheme Ia, provided that in vivo $H_2{}^{18}O$ has a vanishingly small probability of rehydrating the same molecule from which it 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**.

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(17) (a) Gatenbeck, S.; Mosbach, K. *Acta Chem. Scand.* **1959**, *13*, 1561. (b) Scott, A. I.; Wiesner, K. 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.
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Biosynthesis of Macrolides. 6. Mechanism of Stereocontrol during the Formation of Lasalocid A†

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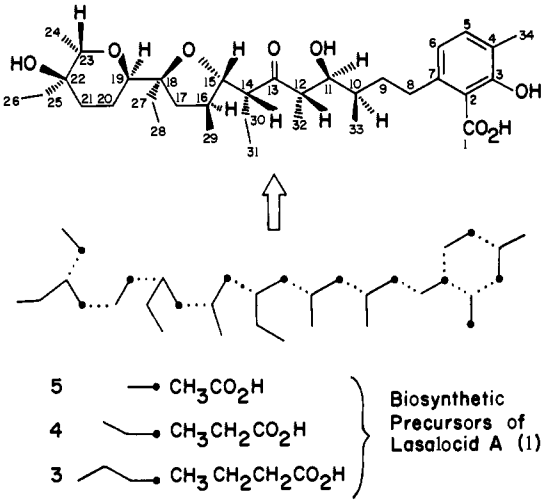
In a previous paper[1] we noted that configurational differences at skeletal carbons bearing identical substituents in macrolide (erythromycin A) and polyether (lasalocid A, **1**) antibiotics appear to be incompatible with a current belief that they are constructed by cycles of stereospecifically identical 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.
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stereochemical course of condensation reactions responsible for chain extension. Tracing the fate of hydrogens at the 2 positions of propionate and butyrate should provide information on the processes involved. To accomplish this it is necessary to isotopically label the precursors with ^2H or ^3H and monitor their presence or absence in **1** by nuclear magnetic resonance and radiotracer techniques.

Epimerization by methylmalonyl-CoA epimerase results in the complete exchange of the methine hydrogens with ^1H from the medium through an enolization process.[3] Thus, the introduction of an intact ^{13}C–^2H unit into **1** from [2-^{13}C,2-$^2H_2$]propionate should unequivocally establish that the enantiomer of methylmalonyl-CoA produced directly from the propionyl-CoA carboxylase reaction was used for chain extension. Chemical studies[4] and a variety of investigations with chirally labeled propionates[5] have shown, at least for those cases examined so far, that the propionyl-CoA carboxylase reaction proceeds with retention of configuration to yield (*S*)-methylmalonyl-CoA. Thus, if this precedent is followed in *S. lasaliensis*, it should be possible for labeling experiments to determine the chirality of the methylmalonyl-CoA precursor and to use the absolute configuration of sites in **1** derived from this precursor to deduce the stereochemical course of the condensation reaction responsible for chain extension.

Similar but less definitive information should also be obtainable from the corresponding labeling experiments with butyrate. Although the active form of the latter is believed to be ethylmalonyl-CoA, its absolute configuration is unknown, and there is no definitive evidence for the occurrence of butyryl-CoA carboxylase or ethylmalonyl-CoA epimerase. Nevertheless, it is known that propionyl-CoA carboxylase converts butyryl-CoA to ethylmalonyl-CoA at a reduced rate;[6] so it seems highly probable that the latter also has an *S* configuration. In spite of these limitations it seemed likely that a comparison of the configurations of sites in **1** derived from methyl- and ethylmalonyl-CoA might shed some light on the configuration of ethylmalonyl-CoA used for chain extension.

It could be anticipated that variable amounts of isotopic hydrogen labels at activated methylene and methine groups would be lost by exchange with ^1H from the medium during precursor processing in the biosynthetic pathway leading to **1**. Such losses have been observed previously in studies on the incorporation of [2-^{13}C,2-$^2H_3$]acetate into fatty acid,[7] hydrocarbon,[8] elsinochrome D,[9] cytochalasin B,[10] and the macrolide antibiotic brefeldin A.[11] However, although this complication can reduce the quantity of information obtained from the labeling experiments through complete loss of label at some sites, it does not affect conclusions based on isotopically enriched sites.

Isotopically labeled precursors were administered in aliquots (5 mM) over a 4-day period to shaken-flask cultures of *S. lasaliensis* in the manner described by Westley et al.[2] This procedure maximizes isotopic enrichment of **1** but can lead also to scrambling of labels by a number of metabolic processes prior to incorporation into the secondary metabolite. The ^{14}C radioactivity of **1** enriched from each precursor listed in Table I showed that

Table I. Carbon-13 Enrichments (% ^{13}C from Labeled Precursor[c]) for Labeling of Lasalocid A (1) with Precursors Shown[a]

	precursors[b]	
	expt 2	expt 3
carbon	$^{13}C^2H_3CO_2H$[d]	$CH_3{}^{13}C^2H_2CO_2H$[d]
1		1.3
2	10.1	1.0
3	0.9	
4	3.2	10.8
6	10.5	1.0
8	12.8	
9	0.3	
10	3.7	12.3
11	0.2	
12	4.3	10.6[h]
14	6.0	
15	1.1	
16	2.6	12.3
18	5.6	
19		1.2
20	13.0	1.5
21		0.7
22	6.6	
23		0.8
24	11.7	1.7
25		0.7
26	6.3[e]	0.7
27		0.6
28	5.7[e]	0.6
29	4.7[e,f]	0.8[f]
31	7.0[e,g]	0.8
32	5.6[e,g]	1.2
33	5.3[e,g]	1.3
34	4.7[e,f]	0.8[f]

[a] Apparatus, experimental conditions, definitions of parameters, and method of calculation are the same as those for ref 8 with the following additional conditions. Experiment 2: 53 mg of 1 in 0.4 mL of 4:1 C^2CHl_3/C_6F_6 (also 2:1 $C^2H_2Cl_2/C_6F_6$), with 8 mg/mL Cr(acac)$_3$ added, SW 5500 Hz, AT 1.48 s, FA 40°, PD 1.5–5.0 s, {^1H,^2H}-broadband decoupling, {^1H} decoupling off during -PD to suppress residual nuclear Overhauser enhancement, internal lock to ^{19}F. Experiment 3: 40 mg of 1 in 0.45 mL of 4:1 C^2HCl_3/C_6F_6 with 8 mg/mL of Cr(acac)$_3$ added; conditions the same as for experiment 2 with PD 3.2 s. Three spectra of **1** at natural isotopic abundance (58 mg in 0.45 mL of solvent) were obtained under same conditions as experiment 3 and used to standardize peak intensities in experiments 2 and 3. Isotopically shifted peaks were determined from spectra of the labeled materials with {^1H} decoupling only. ^{13}C spectra at 100.6 MHz were recorded with a Bruker WH-400 spectrometer under the following conditions. Experiment 2: 53 mg of 1 in 0.5 mL of C^2HCl_3, SW 250 ppm, AT 0.65 s, FA 20°, {^1H}-broadband decoupling. Experiment 3: 40 mg of 1 in 0.5 mL of C^2HCl_3, and all other conditions are the same as for experiment 2. ^2H spectra at 30.7 MHz were obtained with a Bruker WH-200 spectrometer under the following conditions. Experiment 1: 50 mg of 1 in 0.5 mL of C_6H_6, SW 10 ppm, AT 6.8 s, FA 60°, {^1H}-broadband decoupling. [b] The precursors contained 90–91% ^{13}C and/or ≥95% ^2H. For experiment 1, precursor was $C^2H_3CO_2H$. In all cases, the precursors shown were mixed with a small amount of the same compound containing ^{14}C at position 1. [c] Calculated from integrals of three ^{13}C spectra at 25.2 MHz of each labeled compound. [d] Average from three spectra. Error for comparison of enrichments: ± 0.4% for % ^{13}C < 3; ± 0.7% for 3 < % ^{13}C < 7; ± 1.4% for % ^{13}C > 10. Error in absolute % ^{13}C = above comparitive error + % ^{13}C × 0.13. [e] ^2H retained. Distributions of labeled species (error ± 0.03) and isotope chemical shifts in ppm (brackets, error ± 0.04 ppm) are as in Table II. [f] Resonances superposed in C^2HCl_3/C_6F_6. [g] $^{13}C^1H^2H_2$ component of C-33 resonance overlaps $^{13}C^1H_2{}^2H$ of C-32 and $^{13}C^1H_3$ of C-31; $^{13}C^1H_2{}^2H$ of C-33 overlaps $^{13}C^1H_3$ of C-32. Proportions calculated by assuming intensity of C-32 and C-33 $^{13}C^1H_2{}^2H$ components equals average intensity of $^{13}C^1H_2{}^2H$ for C-29 and C-34. [h] ^2H retained. Distribution of labeled species: $^{13}C^1H$ 0.90 ± 0.02, $^{13}C^2H$ 0.10 ± 0.02.

(3) (a) Overath, P.; Kellerman, G. M.; Lynen, F. *Biochem. Z.* **1962**, *335*, 500–518. (b) Mazumder, R.; Sasakawa, T.; Kaziro, Y.; Ochoa, S. *J. Biol. Chem.* **1962**, *237*, 3065–3068.
(4) Sprecher, M.; Clark, M. J.; Sprinson, D. B. *J. Biol. Chem.* **1966**, *241*, 872–877.
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(7) McInnes, A. G.; Walter, J. A.; Wright, J. L. C. *Tetrahedron Lett.* **1979**, 3245–3248.
(8) McInnes, A. G.; Walter, J. A.; Wright, J. L. C. *Lipids* **1980**, *15*, 609–615.
(9) Kurobane, I.; Vining, L. C.; McInnes, A. G.; Smith, D. G.; Walter, J. A. *Can. J. Chem.* **1981**, *59*, 422–430.
(10) Wyss, R.; Tamm, C.; Vederas, J. C. *Helv. Chim. Acta* **1980**, *63*, 1538–1541.
(11) Hutchinson, C. R.; Kurobane, I.; Mabuni, C. T.; Kumola, R. W.; McInnes, A. G.; Walter, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 2474.

Table II. Distribution of Labeled Species and Isotope Chemical Shifts for the Methyl Groups of **1**

	labeled species (chemical shift, ppm)			
carbon	$^{13}C^{1}H_3$	$^{13}C^{1}H_2{}^{2}H$	$^{13}C^{1}H^{2}H_2$	$^{13}C^{2}H_3$
C-26	0.13		0.15 (0.58)	0.72 (0.81)
C-28	0.14		0.17 (0.57)	0.69 (0.84)
C-29	0.33	0.18 (0.28)	0.49 (0.57)	
C-31	0.10		0.28 (0.60)	0.62 (0.89)
C-32	0.37	0.15 (0.30)	0.48 (0.60)	
C-33	0.39	0.17 (0.32)	0.45 (0.59)	
C-34	0.33	0.18 (0.28)	0.49 (0.57)	

a high isotopic enrichment had been obtained. The regiochemical distribution of ^{2}H labels in **1** from experiments 2 and 3 was monitored by ^{13}C NMR spectroscopy, but isotopic enrichments were measured from the intensities of isotopic chemically shifted ^{13}C resonances in the $\{^{1}H,^{2}H\}$ broad-band-decoupled Fourier transform ^{13}C spectrum. Removal of the ^{2}H decoupling in another experiment identified the resonances for carbons bonded to deuterium. A relaxation reagent [$Cr(acac)_3$] was used to equalize ^{13}C relaxation times and eliminate nuclear Overhauser enhancements. The ^{13}C spectrum of **1** has been assigned previously.[2,12]

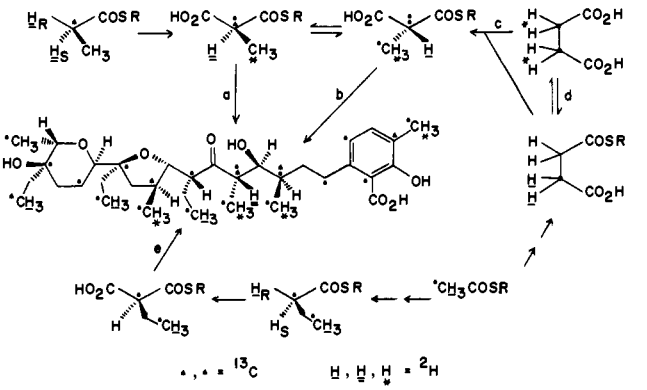
The 30.7-MHz ^{2}H NMR spectrum of **1** enriched from [2-$^{2}H_3$]acetate (experiment 1) indicated that only methyl groups were ^{2}H labeled.[13] In particular, a separate resonance was observed for ^{2}H-34 although the C-34 methyl group is derived from propionate.[2] Overlapping of the remaining ^{2}H resonances prevented enrichments at other methyl groups from being assigned.

Intensities enhanced over those expected for natural ^{13}C abundance and the presence of isotopically chemically shifted $^{13}C-^{2}H$ coupled resonances in the 100.6-MHz ^{13}C spectrum of **1** enriched from [2-^{13}C,2-$^{2}H_3$]acetate were used to locate labeled positions, but the enrichment data in Table I was measured from corresponding signals in $\{^{1}H,^{2}H\}$ broad-band-decoupled 25.2-MHz ^{13}C spectra. Carbons derived directly from C-2 of acetate (C-2, C-6, C-8, C-20, and C-24) were ^{13}C enriched to a significantly greater extent than those originating from acetate via butyrate (C-14, C-18, C-22, C-26, C-28, and C-31) and propionate[4] (C-4, C-10, C-12, C-16, C-29, C-32, C-33, and C-34). The carbons derived from C-2 and C-4 of butyrate units were about equally labeled, whereas those from C-2 and C-3 of propionate were enriched to a greater extent than those from C-1. It is also noteworthy that only the methyl groups of **1** were labeled with ^{2}H. Methyl carbons originating from butyrate bore three, two, or one ^{2}H in contrast to those from propionate which had only two or one ^{2}H attached. The apparent absence of ^{2}H at the C-24 methyl group was unexpected and has important implications with regard to the assignment of acetate as the starter group[2] in lasalocid A biosynthesis.

The corresponding 100.6- and 25.2-MHz ^{13}C spectra of **1** enriched with [2-^{13}C,2-$^{2}H_2$]propionate indicated that C-4, C-10, C-12 and C-16 were ^{13}C enriched by about an order of magnitude more than any other site (experiment 2, Table I). The only site in the molecule labeled with ^{2}H, C-12, was enriched 10.6% ± 2.8%.

Incorporation of an intact $^{13}C-^{2}H_1$ unit at C-12 of **1** enriched from [2-^{13}C,2-$^{2}H_2$]propionate, as pointed out above, implies chain extension with (*S*)-[2-^{13}C,2-$^{2}H_1$]methylmalonyl-CoA, the enantiomer found to be formed directly from the propionyl-CoA carboxylase reaction in all previous studies[5] (Scheme Ia). In polyketide biosynthesis this extension process is known to produce intermediates of the type $RCOC(R_1R_2)COSEnz$ via the biochemical equivalent of the Claisen reaction.[14] As it is known that the 12 position of **1** has an *S* configuration,[15] it follows that the

Scheme I



corresponding site in the $RCOCH(CH_3)COSEnz$ intermediate has an *R* configuration. Thus, chain extension must take place with inversion of configuration at C-2 of (*S*)-[2-^{13}C,2-$^{2}H_1$]-methylmalonyl-CoA. This property is shared by analogous condensation reactions in fatty acid biosynthesis[16] and may therefore be a general feature of polyketide biosynthesis also. Conversely, in the unlikely event that the propionyl-CoA carboxylase reaction produces (*R*)- instead of (*S*)-methylmalonyl-CoA the condensation reaction would have to proceed with retention of configuration.

Absence of ^{2}H at other chiral centers of **1** ^{13}C enriched from [2-^{13}C,2-$^{2}H_2$]propionate can be rationalized as follows. ^{2}H which may have been attached to the methine carbon of the intermediate giving rise to C-16 would be entirely lost by the sequence of reactions $RCOC^{2}H(CH_3)COSEnz \rightarrow RCHOHC^{2}H(CH_3)COSEnz \rightarrow RCH{=}C(CH_3)COSEnz \rightarrow RCH_2CH(CH_3)COSEnz$. The explanation for the lack of ^{2}H at C-10 is more complicated. Maximum loss of isotopic hydrogen labels through enolization and exchange with ^{1}H from the medium would be expected to have occurred at C-12 and C-14 because they are adjacent to a carbonyl in **1** and must have been adjacent to this group for a considerable part of the biosynthetic process. Retention of isotopic hydrogen labels at one of these carbons in experiment 3, therefore, make it unlikely that enolization during chain extension is responsible for the absence of ^{2}H at C-10 of **1**. Conversion of (*S*)-[2-^{13}C,2-$^{2}H_1$]methylmalonyl-CoA to the (*R*)-[2-^{13}C] epimer by methylmalonyl-CoA epimerase[3] prior to incorporation into the growing chain is a more likely explanation (Scheme Ib). This implies that the condensation reaction responsible for the incorporation of C-10 has proceeded with inversion of configuration, as in the case of C-12, and that enzymes with different stereospecificities are available for the addition of enantiomeric methylmalonate units. Epimerization with loss of ^{2}H by an enzyme subsequent to chain extension with (*S*)-[2-^{13}C,2-^{2}H]methylmalonate, however, is not excluded by the evidence. Furthermore, the data in Table I for experiment 3 establishes that significant scrambling of labels did not occur as a consequence of propionyl-CoA metabolism via the methylmalonate pathway and citric acid cycle.

The regiochemical distribution of labels in **1** obtained from [2-^{13}C,2-$^{2}H_3$]acetate (experiment 2, Table I) indicates that the precursor has labeled sites originating from propionate and butyrate as well as acetate. All centers (C-2, C-6, C-8, C-20 and C-24) labeled directly from the methyl group of the doubly labeled acetate were not ^{2}H labeled although highly ^{13}C enriched. The absence of ^{2}H at C-24 was particularly noteworthy as it suggests that C-23 and C-24 of **1** are not derived from a normal acetate starter group,[2] since in all cases examined to date the acetate starter group has retained most of the ^{2}H content of the [2-^{13}C,2-$^{2}H_3$]acetate precursor.[7-11] It is clear that the precursor for these carbons in **1** has either lost the ^{2}H labels during its formation from acetate or has a structure that facilitates facile exchange of ^{2}H with ^{1}H from the medium. Total loss of ^{2}H from sites

(12) Seto, H.; Westley, J. W.; Pitcher, R. G. *J. Antibiot.* **1978**, *31*, 289–293.

(13) Direct incorporation of acetate could have [^{2}H]-labeled positions 5, 8, 20, and 24, whereas indirect introduction via propionate and butyrate could have labeled positions 10, 12, 29, 32, 33, 34 and 14, 25, 27, 30, respectively.

(14) Money, T. *Biosynthesis* **1973**, *2*, 184–186.

(15) Westley, J. W.; Evans, R. H.; Williams, T.; Stempel, A. *Chem. Commun.* **1970**, 71–72.

(16) Sedgwick, B.; Morris, C.; French, S. J. *J. Chem. Soc., Chem. Commun.* **1978**, 193–194.

derived from malonate (C-2, C-6, C-8, C-20) has also been observed in other studies of polyketide biosynthesis.[9,10] However, although enolization processes were clearly responsible for the loss of label, the actual stage(s) at which this occurred could not be identified, and this is also true for the present study.

It can be assumed that a portion of the [3-^{13}C,3-^{2}H$_2$]-succinyl-CoA produced from [2-^{13}C,2-^{2}H$_3$]acetyl-CoA in the citric acid cycle would be isomerized to (*R*)-[2-^{13}C,2-^{2}H$_1$,*methyl*-^{2}H$_1$]methylmalonyl-CoA by methylmalonyl-CoA mutase[17] (Scheme Ic). Two-thirds of the ^{2}H would be lost during ^{2}H transfer from C-3 of succinyl-CoA to the methyl group of methylmalonyl-CoA because of coenzyme B_{12} participation,[18] and most of the ^{2}H incorporated at this group would be bonded to ^{12}C. Thus, although ^{13}C enrichments at C-4, C-10, C-12, and C-16 of **1** arise from (*R*)-[2-^{13}C,2-^{2}H$_1$,*methyl*-^{2}H$_1$]methylmalonyl-CoA or its epimer, neither of them could be responsible for the presence of the intact ^{13}C^{2}H$_2$ and ^{13}C^{2}H$_1$^{1}H$_1$ units at the C-29, C-32, C-33, and C-34 methyl groups (Table I, footnote e). Incorporation of these units requires the structurally symmetric but cryptically unsymmetric intermediate [2-^{13}C,2-^{2}H$_2$]succinic acid which would yield [2-^{13}C,2-^{2}H$_2$] in addition to [3-^{13}C,3-^{2}H$_2$]succinyl-CoA when reesterified by succinyl-CoA synthetase (Scheme Id). Isomerization of [2-^{13}C,2-^{2}H$_2$]succinyl-CoA would give (*R*)-[*methyl*-^{13}C^{2}H$_2$]methylmalonyl-CoA which can be converted to the *S* epimer by methylmalonyl-CoA epimerase. These two isotopic species of methylmalonyl-CoA can account for the presence of the ^{13}C^{2}H$_2$ units at C-29, C-32, C-33, and C-34, the ^{13}C^{2}H$_1$^{1}H$_1$ residues arising from partial loss of ^{2}H during their formation.

The absence of ^{13}C^{2}H$_1$ units at position 10 of **1** was unexpected as this site was probably derived from (*R*)-[2-^{13}C,2-^{2}H$_1$,-*methyl*-^{2}H$_1$]methylmalonyl-CoA. However, it can be calculated[19] that ~59% of the ^{13}C–^{2}H$_1$ bonds of [2-^{13}C,2-^{2}H$_3$]acetate survived at the methine group of the above precursor, and that ~90% of the ^{2}H remaining was lost subsequently during interconversion of epimers with methylmalonyl-CoA epimerase. As C-10 was 3.7% ^{13}C enriched (see Table I), it follows that 0.10 × 0.59 × 3.7 = 0.2% ^{13}C would be the amount bearing ^{2}H. This low concentration of ^{13}C^{2}H$_1$ units would not be detected by our NMR procedure because overlap of signals precludes the observation of a small isotopically shifted component at the C-10 resonance. On the other hand, the lack of ^{2}H at C-12 is explained by ^{2}H loss during conversion of (*R*)-[2-^{13}C,2-^{2}H$_1$,*methyl*-^{2}H$_1$]- to (*S*)-[2-^{13}C,*methyl*-^{2}H$_1$]methylmalonyl-CoA, the precursor for position 12.

Carbons of **1** derived from the methyl and methine groups of this isotopically labeled mixture of methylmalonates are expected, and found, to be about equally ^{13}C enriched. Much smaller ^{13}C enrichments at C-3, C-9, C-11, and C-15 result from processing [2-^{13}C,2-^{2}H$_3$]acetate through more than one rotation of the citric acid cycle to give succinic acid and therefore methylmalonyl-CoA, ^{13}C labeled at the carboxyl groups.

The labeling results for sites enriched from [2-^{13}C,2-^{2}H$_3$]acetate via butyrate were also instructive. Observation of ^{13}C^{2}H$_3$ units at C-26, C-28, and C-31 identified the starter acetate groups of butyric acid biosynthesis, the ^{13}C^{2}H$_2$^{1}H$_1$ and ^{13}C^{1}H$_3$ residues arising from loss of ^{2}H as a result of acetate–malonate interconversion by acetyl-CoA carboxylase.[20]

The reason for the absence of ^{13}C^{2}H$_1$ units at position 14, which is probably derived from (*S*)-ethylmalonyl-CoA (Scheme Ie), is more subtle. Sites originating from the methyl group of [2-^{13}C,2-^{2}H$_3$]acetate during fatty acid biosynthesis possess ^{13}C^{2}H$_1$ units in which 40–75% of the original ^{13}C–^{2}H$_1$ bonds have been retained[7,21] and which have absolute configurations that depend on the organism producing the fatty acids.[21,22] Thus, *S. lasaliensis* can be expected to have a pool of chiral [2-^{13}C,2-^{2}H$_1$]butyryl-CoA, with a significant concentration of ^{13}C^{2}H$_1$ residues, available for the biosynthesis of **1**. In addition propionyl-CoA carboxylase substitutes a carboxyl group for the pro-*R* hydrogen of propionyl-CoA to yield (*S*)-methylmalonyl-CoA.[5] If the enzyme responsible for the conversion of butyryl- to ethylmalonyl-CoA has the same stereospecificity, then chiral [2-^{13}C,2-^{2}H$_1$]butyryl-CoA will yield (*S*)-[2-^{13}C]ethylmalonyl-CoA if the ^{2}H has a pro-*R* and (*S*)-[2-^{13}C,2-^{2}H$_1$]ethylmalonyl-CoA if it has a pro-*S* configuration. Thus the lack of ^{2}H at position 14 is explained if the site was derived from (*S*)-[2-^{13}C]ethylmalonyl-CoA. If position 14 originated from (*S*)-[2-^{13}C,2-^{2}H$_1$]ethylmalonyl-CoA, the situation would be very different as this potential precursor should have retained 40–75% of the original ^{13}C–^{2}H$_1$ bonds (see above) and would be responsible for the 6% ^{13}C enrichment observed at this site. The ^{2}H/^{1}H exchange processes which now would have to be invoked to rationalize the ^{2}H loss seem to be incompatible with available evidence. From a previous study on methylmalonyl-CoA[3a] it can be deduced that the spontaneous rate of exchange of ^{2}H from (*S*)-[2-^{13}C,2-^{2}H$_1$]ethylmalonyl-CoA with ^{1}H from the medium at physiological pH would be very slow. Butyryl-CoA epimerase or other enzymes which could accelerate this exchange have not been identified. Nonetheless, we cannot exclude the possibility that significant isotope loss may occur after addition of ethylmalonate to the growing polyketide chain. We do not have sufficient data at this time to permit a distinction among these possibilities, but plan to answer this question by experiments in progress.

It should be noted that the participation of 2(*R*)- or 2(*S*)-[2-^{13}C,2-^{2}H$_1$]butyryl-CoA is open to verification. This could be accomplished by determining the absolute configuration at C-2 of fatty acids from *S. lasaliensis* enriched from [2-^{2}H$_3$]butyrate by established procedures.[23] In this regard it is interesting to observe that the fatty acid synthetase of baker's yeast ^{3}H labels the pro-*R* hydrogens of alternate methylene groups from [2-^{2}H,^{3}H]acetate[22a] whereas the corresponding enzymes from *E. coli*,[22b] *A. nidulans*, and *C. pyrenoidosa*[21] label the pro-*S* hydrogens.

Our investigations on lasalocid A biosynthesis strongly suggest that reductions of carbonyl groups and the introduction of methylmalonate units proceed by stereochemically divergent processes and thus provide direction for further study of the biochemistry of macrolide and polyether antibiotics.

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