BIOSYNTHESIS OF LASALOCID A

METABOLIC INTERRELATIONSHIPS OF CARBOXYLIC ACID PRECURSORS AND POLYETHER ANTIBIOTICS

MARY M. SHERMAN†, STEVE YUE and C. RICHARD HUTCHINSON*

School of Pharmacy, University of Wisconsin,
Madison, WI 53706, U.S.A.

(Received for publication March 19, 1986)

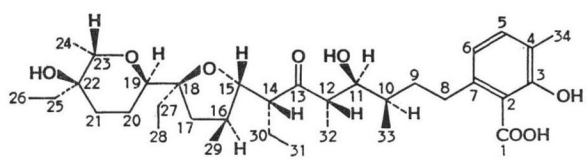
The metabolic interrelationships of isobutyrate, *n*-butyrate, and propionate in *Streptomyces lasaliensis* are established to show how these acids are used as precursors for the biosynthesis of the polyether antibiotic, lasalocid A.

It has been established through feeding experiments with isotopically labeled precursors that polyether antibiotics like lasalocid A (**1**) are built from the simple fatty acids, acetate, butyrate, or propionate, by bacterial secondary metabolism.[1] In two cases, narasin,[2] and lysocellin,[3] the labeling regiochemistry resulting from the incorporation of [1-], [2-], [3-], or [4-^{13}C]-*n*-butyrate, or [1-], [2-], or [3-^{13}C]propionate has been interpreted to be consistent with the metabolic conversion of *n*-butyrate to propionate *via alpha* or *omega* oxidation.[1] POSPÍŠIL *et al.* have shown that [1-^{13}C]isobutyrate labels the polyether antibiotics monensins A and B as if it were converted to [1-^{13}C]-*n*-butyrate before entering the four-carbon unit of these antibiotics.[4] ROBINSON and co-workers recently have elucidated the stereochemistry of the isobutyrate-to-*n*-butyrate rearrangement,[5] and have proved that this is an intramolecular rearrangement in the case of the monensin pathway.[6] Consequently, there are precedents for the metabolic interconversion of three and four carbon carboxylic acids in the biosynthetic pathways of polyether antibiotics.

During our study of lasalocid A biosynthesis,[7] we observed that the labeling of lasalocid A (**1**) by [1-^{13}C]-*n*-butyrate and [3-^{13}C]propionate did not agree with some of the earlier findings for other polyether antibiotics,[2,3] which led us to examine how *n*-butyrate, isobutyrate, and propionate are related metabolically in *Streptomyces lasaliensis*. We present the results of our work in this paper and discuss the pathways through which *n*-butyrate and propionate are interrelated in *S. lasaliensis* when they serve as precursors of **1**.

Results

We determined how variously ^{13}C-labeled forms of *n*-butyrate, isobutyrate, and propionate were



1

† Current address: Department of Chemistry, University of Utah, Salt Lake City, UT 84112, U.S.A.

Table 1. ^{13}C Enrichments[a] of lasalocid A (**1**).

Carbon[g]	[3-^{13}C,2-$^{2}H_2$]-Propionate	[4-^{13}C]-*n*-Butyrate	[1-^{13}C,$^{2}H_2$]-*n*-Butyrate	[1-^{13}C]-Isobutyrate	[1,3,3′-$^{13}C_3$]-Isobutyrate[b]	[2,3,3′-$^{13}C_3$]-Isobutyrate[b]
1	3.6	—	3.9	4.5	—	—
2	4.8	11.7	—	—	—	—
3	1.9	—	12.0	7.2	—	—
4	1.6	3.7	—	—	—	—
5	2.1	—	2.2	2.9	—	—
6	1.3	6.9	—	—	—	—
7	2.8	—	4.6	3.5	—	—
8	3.3	6.2	—	—	—	—
9	1.7	—	12.4	6.1	—	—
10	1.8	—	—	—	—	—
11	1.6	—	10.0	5.1	—	—
12	1.8	4.3	—	—	—	—
13	1.0	—	8.9[e]	11.7	(38.3, d)[e,f]	—
14	2.5	—	—	—	(38.4, d)[e,f]	(37.3, d)[e,f]
15	1.8	—	8.2	5.9	—	—
16	3.3	—	—	—	—	—
17	1.3	—	20.0	13.2	(35.4, d)[e,f]	—
18	2.1	—	—	—	(35.4, d)[e,f]	(41.0, d)[e,f]
19	2.9	1.8	3.3	3.1	—	—
20	3.2	4.5	—	—	—	—
21	1.2	—	17.0	13.7	(37.5, d)[e,f]	—
22	—	—	—	—	(37.5, d)[e,f]	(41.7, d)[e,f]
23	2.2	3.3	3.6	3.1	—	—
24	3.4	13.0	—	—	—	—
25	0.9	—	—	—	—	(~37, dd)[e,f]
26	2.6	28.3	—	—	1.9[d]	6.0[e] (36.3, d)[e]
27	0.9	—	—	—	—	(~37, dd)[e,f]
28	3.5	34.3	—	—	2.5[d]	7.8[e] (35.0, d)[e]
29	20.5	23.3	—	—	—	—
30	1.7	—	—	—	—	(~35, dd)[e,f]
31	—	30.7	—	—	2.7[d]	6.8[e] (35.0, d)[e]
32	16.9	11.3	—	—	—	—
33	17.8	12.0	—	—	—	—
34	16.7	18.9	—	—	—	—

[a] Calcd as described in the Experimental section of ref 16.
[b] ^{13}C Enrichments resulting only from the direct incorporation of this precursor are shown.
[c] $^{1}J_{CC}$, Hz; multiplicity shown in parentheses: d=doublet + singlet; dd=double doublet + singlet.
[d] ^{13}C Enrichment calculated from C-4 of [1,2,4-$^{13}C_3$]-*n*-butyrate derived from the [1,3,3′-$^{13}C_3$]isobutyrate.
[e] ^{13}C Enrichment calculated from C-4 of [2,3,4-$^{13}C_3$]-*n*-butyrate derived from the [2,3,3′-$^{13}C_3$]isobutyrate by dividing total area of satellite peaks by area of center peak.
[f] ^{13}C Enrichment not calculated for this position.
[g] As assigned in ref 16.

incorporated into **1** by ^{13}C NMR analysis. The most important data from an earlier communication[7] and the present work are listed collectively in Table 1 as relative ^{13}C enrichments for the carbons of **1**. The enrichment value is not shown (—) when it was negligible. These data show that the primary ^{13}C enrichments produced by [1-] and [4-^{13}C]-*n*-butyrate and [2-] and [3-^{13}C]propionate are as expected based on the earlier work of WESTLEY *et al.*[8]

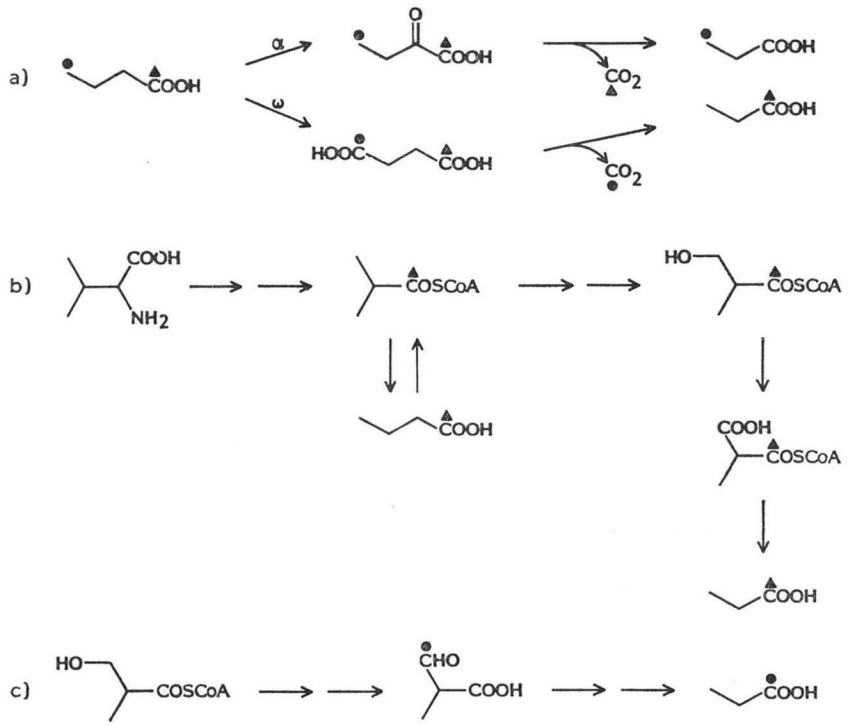
The secondary ^{13}C enrichments are of more interest and there are two metabolic relationships that have a bearing on this. Fig. 1a shows what would happen if [1,4-$^{13}C_2$]-*n*-butyrate were converted to

propionate by *alpha* or *omega* oxidation. Loss of the ▲ carbon by *alpha* oxidation is assured. It is not clear if the ● or the ▲ carbon would be lost by *omega* oxidation since the involvement of free succinate would scramble the labeling between the C-1 and C-4 positions; thus, loss of the one illustrated is entirely arbitrary. In any event, the primary ^{13}C enrichment of **1** from *n*-butyrate (vertical arrow on the left) should be accompanied by a secondary enrichment from [1-] or [3-^{13}C]propionate (vertical arrow on the right). Fig. 1b shows how *n*-butyrate and propionate would be labeled by [1-^{13}C]isobutyrate based on the proposal of POSPÍŠIL *et al.*[4] and ŌMURA *et al.*[9] explaining their observations about the incorporation of valine and isobutyrate into polyether and macrolide antibiotics, respectively. It is known that valine is degraded to propionate by some microorganisms and mammals, and that β-hydroxyisobutyryl-CoA and 2-methylmalonic semialdehyde are intermediates in this pathway.[10,11] The aldehyde carbon of 2-methylmalonic semialdehyde, which comes from C-3′ of β-hydroxyisobutyrate, becomes the carboxyl of propionate in these systems as shown in Fig. 1c. Evidence presented below shows that C-1 of propionate comes from the carboxyl carbon of isobutyrate in *Streptomyces* species, however, as indicated in Fig. 1b.

The secondary ^{13}C enrichments of **1** show (Table 1) that the enrichment of those positions coming

Fig. 1. Metabolism of (a) *n*-butyrate by *alpha* or *omega* oxidation in streptomycetes; and of valine (b) by a reversible intramolecular rearrangement of isobutyrate and *n*-butyrate in streptomycetes, or (c) by the metabolism of β-hydroxyisobutyrate to propionate in other organisms.

The symbols indicate the predicted relationships between carbons that are retained or lost during the metabolism (a) of *n*-butyrate to propionate, or (b, c) of valine to *n*-butyrate and propionate. In (c) a dot is affixed on the aldehyde carbon of 2-methylmalonic semialdehyde to indicate that this carbon becomes the carboxyl carbon of propionate during the metabolism of valine in bacteria other than the streptomycetes and in mammals.



directly from C-1 of *n*-butyrate (C-13, 17 and 21) was highest in the labeling experiments with [1-^{13}C]-*n*-butyrate and -isobutyrate. Positions labeled by C-1 of propionate (C-3, C-9, C-11 and C-15) had the next highest level of enrichment, and positions labeled by C-1 of acetate (C-1, C-5, C-7, C-19 and C-23) had the lowest level. These data eliminate the occurrence of *alpha* oxidation of *n*-butyrate in the lasalocid A pathway since propionate could not acquire a C-1 label this way, and support the interrelationships of isobutyrate, *n*-butyrate, and propionate shown in Fig. 1b. They also reinforce the knowledge that *n*-butyrate can be degraded to acetate by *beta* oxidation.

[4-^{13}C]-*n*-Butyrate most heavily labeled the positions coming directly from the methyl group of butyrate (C-26, C-28 and C-31) and to a lesser amount the methyl groups of propionate-derived units (C-29, C-32, C-33 and C-34). As expected, [4-^{13}C]-*n*-butyrate also labeled the positions coming from the methyl carbon of acetate directly (C-2, C-6, C-8, C-20 and C-24) and indirectly (C-4 and C-12; C-10 and C-16 were not resolved well enough at 50 MHz to measure) *via* the TCA cycle and the conversion of succinate to 2-methylmalonate. Of the two positions in the propionate-derived units of **1** corresponding to C-2 and C-3 of succinate, which [2-^{13}C]acetate will label, the one corresponding to C-3 of propionate was ^{13}C-enriched much more which suggests that [4-^{13}C]-*n*-butyrate was converted to [3-^{13}C]propionate by more direct route than its degradation to [2-^{13}C]acetate, *etc.* These data are consistent with the *alpha* oxidation route, but as its operation already has been ruled out, this leaves only the *omega* oxidation or isobutyrate routes (Fig. 1) as possible explanations of the labeling data. Yet *omega* oxidation of [4-^{13}C]-*n*-butyrate can give either C-1 labeled or unlabeled propionate but never C-3 labeled propionate; thus only the *n*-butyrate→isobutyrate→propionate pathway explains the observations.

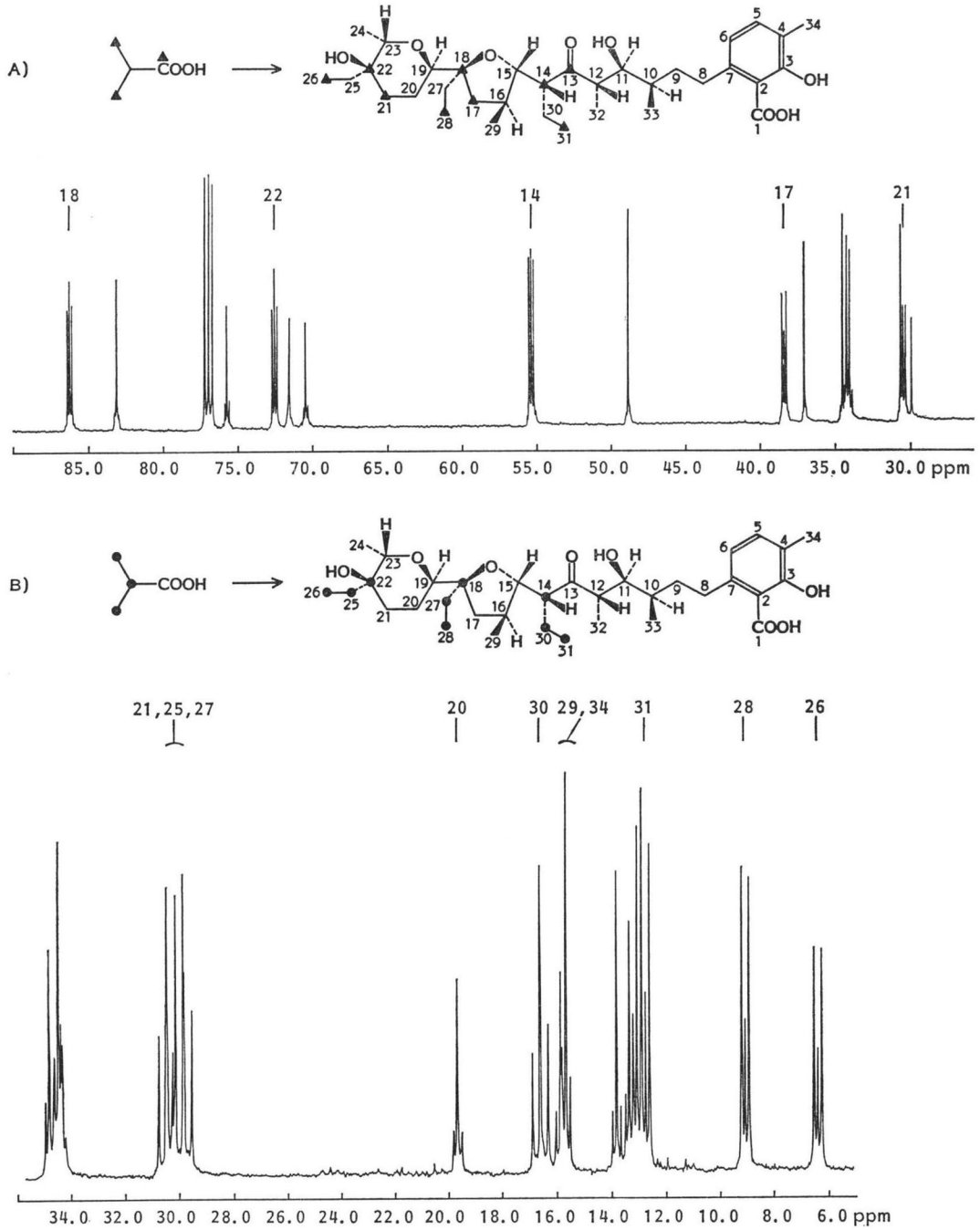
[3-^{13}C]Propionate labeled those positions coming directly from the propionate methyl group (C-29, C-32, C-33 and C-34) much more than any others, such as those coming from C-2 of acetate, which could be labeled *via* the catabolism of propionate to acetyl-CoA as in plants and animals.[10] The data from the [2-^{13}C]propionate experiment confirm that propionate entered **1** directly with a very minor amount of catabolism to acetate.

To solidify the above arguments, we examined the incorporation of multiply ^{13}C-labeled isobutyrate into **1**. Following trial feeding experiments with [*U*-^{14}C]valine and [2,3,3′-^{14}C]isobutyrate to establish the specific incorporation of carbon isotope, samples of [1,3,3′-$^{13}C_3$]isobutyrate and [2,3,3′-$^{13}C_3$]isobutyrate were synthesized and fed to *S. lasaliensis* in separate experiments as described in the Experimental section. Analysis of the 125 MHz $^{13}C\{^1H\}$ NMR spectrum of the resulting samples of ^{13}C-labeled **1** established clearly that the carboxyl carbon of isobutyrate had migrated intramolecularly to C-3/3′ in forming the *n*-butyrate which provides the four-carbon units of lasalocid A. This conclusion rests firmly on the observation of satellite doublets* for the signals of C-13, C-14, C-17, C-18, C-21 and C-22; and strongly enhanced singlets for C-26, C-28 and C-31 in **1** labeled by [1,3,3′-$^{13}C_3$]isobutyrate (Fig. 2A shows representative spectral data). Moreover, the signals shown in Fig. 2B of portions of the three-carbon subsets, C-22, C-25 and C-26; C-18, C-27 and C-28; and C-14, C-30 and C-31, of **1** labeled by [2,3,3′-$^{13}C_3$]isobutyrate exhibited satellites with a doublet-double doublet-doublet

* Since the samples of **1** are an admixture of unenriched (natural abundance) and ^{13}C-enriched (labeled) molecules, each signal consists of a singlet from the natural abundance material and a doublet or double doublet from molecules having two or three adjacent ^{13}C-labeled sites. The spectral resolution of <5 Hz and line widths of the singlet resonances prevented the direct verification that the center carbon of three adjacent ^{13}C enriched sites actually was a double doublet as stated in the text.

Fig. 2. (A) The 30 to 90 ppm portion of the 125 MHz $^{13}C\{^1H\}$ NMR spectrum of lasalocid A (free acid) in $CDCl_3$ labeled by [1,3,3′-$^{13}C_3$]isobutyric acid. (B) The 6 to 36 ppm portion of the 125 MHz $^{13}C\{^1H\}$ NMR spectrum of lasalocid A (free acid) in $CDCl_3$ labeled by [2,3,3′-$^{13}C_3$]isobutyric acid.

The symbols indicate the labeling pattern resulting from the rearrangement of isobutyrate to *n*-butyrate *in vivo*. Secondary labeling due to the catabolism of *n*-butyrate and isobutyrate to acetate and propionate also appear in these spectra; *e.g.*, at C-20, C-29 and C-34 in (B).



pattern*,** (ignoring two-bond carbon-carbon couplings and ^{13}C *alpha* or *beta* isotope shifts) that can only result from intact incorporation of carbons 2,3, and 3′ of isobutyrate into the *n*-butyrate-derived subunits of **1**. These results confirm the earlier observation by REYNOLDS and ROBINSON about the incorporation of [1,3,3′-$^{13}C_3$]isobutyrate into monensin A.[6]

Discussion

Our observations about the ^{13}C-labeling of lasalocid A by its simple fatty acid precursors confirm the results of others,[4~6,9] which have revealed that streptomycetes metabolize isobutyrate and *n*-butyrate through a novel pathway (Fig. 1b). It is reasonable to speculate that valine catabolism may be an important source of these carboxylic acids for the biosynthesis of polyether as well as macrolide antibiotics. This would be consistent with the knowledge that extensive protein breakdown occurs during the most active time of antibiotic formation by streptomycetes,[12] and that *Streptomyces fradiae* mutants deficient in the level of valine dehydrogenase, the first enzyme of the valine catabolic pathway,[13] produce reduced amounts of a macrolide antibiotic precursor whose formation requires the same three carboxylic acids needed for polyether antibiotics.[14] Furthermore, branched-chain amino acids like leucine and valine can be the sole C and N sources for growth and macrolide antibiotic production in defined media.[15]

Experimental

General

The procedures used for culture maintenance and fermentation, standard chemical methods, and isolation and spectral analyses of **1** are described in ref 16.

[2,3,3′-^{14}C]Isobutyrate

Disodium malonate (2.96 g, 20 mmol) and disodium [2-^{14}C]malonate (0.1 mCi, 19.9 mCi/mmol) were admixed, the pH adjusted to 2 with 6 M HCl, the solvent evaporated, and the residue dried *in vacuo*. The resulting [2-^{14}C]malonic acid - NaCl mixture was dissolved in EtOH (15 ml) with heating, HCl gas bubbled in, and the reaction mixture refluxed for 3.5 hours. The reaction was cooled to room temp, the material rinsed out of the flask with 100 ml H_2O, and extracted 3 times with 100 ml Et_2O. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated *in vacuo* at room temp. The recovered material (2.2 g, 13.8 mmol, 68% yield) was checked by ^1H NMR ($CDCl_3$) which showed two resonances corresponding to the ethyl ester at 1.25 ppm and 4.2 ppm, and a resonance at 3.35 ppm corresponding to the methylene protons. Its specific radioactivity was 7.3 μCi/mmol.

Clean sodium (700 mg, 30.4 mmol) was placed in a dry, N_2 flushed 25 ml flask fitted with reflux condenser attached to an oil bubbler. EtOH (3 ml, dried over Mg) was added dropwise to the Na with stirring at room temp. The reaction was heated until all the Na had dissolved; additional EtOH (4.5 ml) was then added dropwise and the solution refluxed for 1 hour more. The reaction was cooled until reflux had subsided, then all of the diethyl [2-^{14}C]malonate prepared above was added dropwise. The reaction mixture was stirred vigorously with mild heating until all of the precipitate had dissolved and then maintained at this temp for an additional 1.5 hours. [^{14}C]Methyl iodide (58 mCi/mmol, diluted with CH_3I to 8.5 μCi/mmol, 15.4 mmol) was added slowly to the reaction mixture without heating. After the addition was complete, the reaction was refluxed for 5 hours, cooled to room temp, and the mixture was washed with H_2O, evaporated partially to remove excess EtOH, then extracted several times with Et_2O (50 ml). The combined Et_2O layers were dried over anhydrous Na_2SO_4 and evaporated to give 1.0 g (5.32 mmol, 39% yield) of [3,3′-^{14}C]dimethylmalonic acid diethyl ester.

---

* See footnote on p. 1138, *.

** The $^1J_{CC}$ couplings among the various subsets of ^{13}C-labeled carbons of **1** were confirmed by one-dimensional Inadequate spectra in this experiment.

A cold solution of 5% aq NaOH (2.2 mol per mol) was added to the crude diethyl ester and the reaction stirred at room temp for 20 hours. After cooling, the reaction mixture was acidified to pH 1 with 6 M HCl and extracted 3 times with Et_2O. The Et_2O extracts were combined and evaporated *in vacuo* to give 690 mg (5.23 mmol, 98% yield) of orange crystals of 2,2-dimethylmalonic acid which were recrystallized once from hexane.

The crystals were placed in a dry 25 ml flask fitted with a reflux condenser attached to an oil bubbler and a stir bar. The temperature of the bath surrounding the flask was raised slowly to 170°C and then held at 150°C for 1 hour. The flask was cooled to room temp to give a residue weighing 307 mg (3.53 mmol, 67% yield, 4.2 μCi/mmol). ^{1}H NMR analysis ($CDCl_3$) of this material showed resonances at 1.22 ppm (d, J=7.5 Hz) and 2.58 ppm (heptet, J=7.5 Hz) for the protons at C-3,3′ and C-2, respectively, of isobutyric acid.

Sodium [1,3,3′-$^{13}C_3$]Isobutyrate

[*cyano*-^{13}C]Ethylcyanoacetate was prepared from bromoacetic acid (1.04 g, 7.5 mmol) and [^{13}C]-KCN (0.5 g, 7.6 mmol, 90 mol % ^{13}C) according to the literature.[17] Following the method of PHILLIPS,[18] the crude product (0.65 g, 5.7 mmol) and [^{13}C]methyl iodide (2 g, 14 mmol, 99 mol % ^{13}C) were dissolved in EtOH (20 ml) containing 2 drops of phenolphthalein, then a solution of NaOEt (2.4 mol per mol) in EtOH (5 ml) was added dropwise to the magnetically stirred mixture at such a rate that the color was not intensely purple. When the color had been sustained for about 20 minutes, the addition of base was stopped, the majority of the solvent evaporated, and the resulting residue partitioned between H_2O and Et_2O. Evaporation of the ether layer and combined ether extracts gave crude ^{13}C-labeled 2,2-dimethyl ethylcyanoacetate (0.53 g).

To prepare the free acid, crude 2,2-dimethyl ethylcyanoacetate (1.10 g) was stirred with 4 M NaOH (1.2 equiv, 2.5 ml) in MeOH (8 ml) overnight at room temp, then the solution was acidified with dilute acid to pH ~ 1 and extracted repeatedly with CH_2Cl_2. After evaporation of the organic solvent extract, the crude acid was heated to 140°C and distilled (Kugelrohr) to effect decarboxylation. Upon repeating this distillation twice, it was found that some [3,3′-$^{13}C_2$]isobutyric acid also had been formed from loss of the cyano group, rather than CO_2, and had co-distilled with the desired nitrile (ratio of nitrile to acid=5:1 by ^{1}H NMR analysis). The crude yield of the combined products was 0.34 g. This mixture was dissolved in H_2O (*ca.* 5 ml), titrated to a phenolphthalein endpoint, and the aqueous layer and ^{13}C-labeled isobutyronitrile recovered by distillation (Kugelrohr).

The crude isobutyronitrile was dissolved in H_2O (12 ml), solid KOH (5 g) was added to make a 30% solution followed by a 30% solution of H_2O_2 (2 ml), and the mixture was stirred at 40°C for 1 hour in a flask fitted with a condensor. A stream of nitrogen was slowly bubbled through the reac-

Table 2. Precursor feeding experiments data.

Precursor	Amount fed (mM, μCi, ^{3}H/^{14}C ratio)	Lasalocid A isolated	
		Amount (mg)	Incorporation[b] (%)
[2,3,3′-^{14}C]Isobutyrate[a]	1	6	31
[2,3,3′-^{14}C]Isobutyrate[a]	2.5	9	89
[2,3,3′-^{14}C]Isobutyrate[a]	5	5	141
[*U*-^{14}C]Valine	1	nd	17
[*U*-^{14}C]Valine	5	nd	78
[*U*-^{14}C]Valine	10	nd	116
[1-^{13}C]Isobutyrate	5	37	—
[1,3,3′-$^{13}C_3$]Isobutyrate[a]	*	50	—
[2,3,3′-$^{13}C_3$]Isobutyrate[a]	*	55	—

[a] Prepared as described in Experimental section.

[b] Calcd as described in ref 16.

* 1 mmol ^{13}C-labeled plus 1 mmol unlabeled sodium salt of the acid was fed to 200 ml of culture.

nd: Not determined.

tion mixture as its temperature was raised to 95°C. After 3 hours at this temp, the reaction mixture was cooled to room temp, saturated aq $FeSO_4$ (1 ml) was added, and the mixture stirred for 2 more hours. The reaction mixture was acidified to pH ~1 with concd sulfuric acid and the product acid azeotropically distilled with H_2O until the pH of the distillate was *ca.* 5. The distillate was titrated with 1 M NaOH to a phenolphthalein endpoint and evaporated to give sodium [1,3,3'-$^{13}C_3$]isobutyrate (0.23 g, 27% overall yield): ^{1}H NMR (90 MHz, D_2O-DSS) δ 2.36 (1H, m), 1.09 (6H, dm, J=126 Hz); ^{13}C NMR (22.5 MHz, D_2O-DSS) δ 176.5, 9.3; MS *m/z* (% relative intensity) 91 (11, M^+ for $^{13}C_3$), 75 (43) 45 (100).

Sodium [2,3,3'-$^{13}C_3$]Isobutyrate

Following the method of SINGH,[19] a mixture of diethyl [2-^{13}C]malonate (1.0 g, 6.24 mmol, 99 mol % ^{13}C) and [^{13}C]methyl iodide (0.97 ml, 15.6 mmol, 99 mol % ^{13}C) was added dropwise to a magnetically stirred slurry of benzyltriethylammonium chloride (1.42 g) in 14 ml of 12.5 M NaOH at room temp. The resulting mixture was stirred for another 4 hours at the same temp, diluted with H_2O and extracted with Et_2O several times. The aqueous layer was acidified to pH ~1 and re-extracted with Et_2O. The combined ether extracts were evaporated to a residue of crude, ^{13}C-labeled diethyl 2,2-dimethylmalonate (1.06 g, 90% yield) which was hydrolyzed to the acid by stirring with 3.2 ml (2.3 equiv) of 4 M NaOH in MeOH (10 ml) at room temp overnight. The reaction mixture was acidified to pH ~1 and extracted repeatedly with Et_2O, then the combined ether extracts dried ($MgSO_4$) and evaporated to give crude ^{13}C-labeled 2,2-dimethylmalonic acid. This material was decarboxylated and simultaneously distilled (Kugelrohr) at 150°C, and the distillate was titrated with 1 M NaOH to a phenolphthalein endpoint then evaporated to give sodium [2,3,3'-$^{13}C_3$]isobutyrate (0.11 g).* ^{1}H NMR (90 MHz, D_2O-DSS) δ 2.37 (1H, dm, J=130 Hz), 1.09 (6H, dm, J=126.4 Hz); ^{13}C NMR (22.5 MHz, D_2O-DSS) δ 27.5 (t, J=31.7 Hz), 10.1 (d, J=31.7 Hz); MS *m/z* (% relative intensity) 91 (9.5, M^+ for $^{13}C_3$), 75 (45), 46 (100).

Precursor Feedings

The conditions used are described in refs 7 and 16, and the results are listed in Tables 1 and 2.

Acknowledgments

This research was supported by a grant from the National Institutes of Health (GM 25799). We thank Dr. ED MOOBERRY, Department of Biochemistry, and especially Dr. BRUCE R. ADAMS, Department of Chemistry, UW Madison, for assistance with NMR spectral determinations. One of us (S.Y.) also thanks Dr. JANICE DUNCAN, School of Pharmacy, for assistance with the fermentation of *S. lasaliensis*.

References

1) WESTLEY, J. W.: Polyether antibiotics—Biosynthesis. *In* Antibiotics. IV. Biosynthesis. *Ed.*, J. W. CORCORAN, pp. 47~73, Academic Press, New York, 1981
2) DORMAN, D. E.; J. W. PASCHAL, W. M. NAKATSUKASA, L. L. HUCKSTEP & N. NEUSS: The use of C-13 NMR spectroscopy in biosynthetic studies. II. Biosynthesis of narasin, a new polyether ionophore from fermentation of *Streptomyces aureofaciens*. Helv. Chim. Acta 59: 2625~2634, 1976
3) ŌTAKE, N.; H. SETO & M. KOENUMA: The assignment of the C-13 NMR spectrum of lysocellin and its biosynthesis. Agric. Biol. Chem. 42: 1879~1886, 1978
4) POSPÍŠIL, S.; P. SEDMERA, M. HAVRÁNEK, V. KRUMPHANZL & Z. VANĚK: Biosynthesis of monensins A and B. J. Antibiotics 36: 617~619, 1983
5) GANI, D.; D. O'HAGAN, K. REYNOLDS & J. A. ROBINSON: Biosynthesis of the polyether antibiotic monensin A: Stereochemical aspects of the incorporation and metabolism of isobutyrate. J. Chem. Soc. Chem. Commun. 1985: 1002~1004, 1985
6) REYNOLDS, K. & J. A. ROBINSON: Biosynthesis of monensin. The intramolecular rearrangement of isobutyryl-CoA to *n*-butyryl-CoA. J. Chem. Soc. Chem. Commun. 1985: 1831~1832, 1985

* The yield was low in this particular preparation due to an abnormal loss of product during the work-up; normally the yield was *ca.* 75%.

7) HUTCHINSON, C. R.; M. M. SHERMAN, J. C. VEDERAS & T. T. NAKASHIMA: Biosynthesis of macrolides. 6. Mechanism of stereocontrol during the formation of lasalocid A. J. Am. Chem. Soc. 103: 5953~5959, 1981
8) WESTLEY, J. W.; R. H. EVANS, Jr., G. HARVEY, R. G. PITCHER, D. L. PRUESS, A. STEMPEL & J. BERGER: Biosynthesis of lasalocid. I. Incorporation of ^{13}C and ^{14}C labeled substrates into lasalocid A. J. Antibiotics 27: 288~297, 1974
9) ŌMURA, S.; K. TSUZUKI, Y. TANAKA, H. SAKAKIBARA, M. AIZAWA & G. LUKACS: Valine as a precursor of *n*-butyrate unit in the biosynthesis of macrolide aglycone. J. Antibiotics 36: 614~616, 1983
10) KAZIRO, Y. & S. OCHOA: The metabolism of propionic acid. Adv. Enzymol. 26: 283~374, 1964
11) TANAKA, K.; D. A. ABERHART, J. I. AMSTER, E. E. JENKINS & C.-T. HSU: Metabolism of valine chirally labeled with carbon 13 and deuterium in vitamin B_{12}-folate-deficient rats *in vivo*. J. Biol. Chem. 255: 7763~7768, 1980
12) CHATER, K. F.: Morphological and physiological differentiation in *Streptomyces*. *In* Microbial Development. *Eds.*, R. LOSICK & L. SHAPIRO, pp. 89~115, Cold Spring Harbor Press, New York, 1984
13) MASSEY, L. K.; J. R. SOKATCH & R. S. CONRAD: Branched-chain amino acid catabolism in bacteria. Bacteriol. Revs. 40: 42~54, 1976
14) ŌMURA, S.; Y. TANAKA, H. MAMADA & R. MASUMA: Ammonium ion suppresses the biosynthesis of tylosin aglycone by interference with valine catabolism in *Streptomyces fradiae*. J. Antibiotics 36: 1792~1794, 1983
15) STARK, W. M.: The erythromycin fermentation. Progr. Industr. Microbiol. 3: 213~230, 1961
16) SHERMAN, M. M.: The biosynthesis and production of the polyether antibiotic lasalocid A. Ph. D. Thesis, Univ. Wisconsin, 1985
17) INGLIS, J. K. H.: Ethyl cyanoacetate. Org. Synth. 8: 74~76, 1924
18) PHILLIPS, M. A.: Malonic and cyanoacetic esters—Decomposition by sodium ethoxide. Ind. Chemist 21: 678~680, 1945
19) SINGH, R. K.: A convenient synthesis of diethyl dialkyl- and dibenzylmalonates via extractive alkylation. Synthesis 1985: 54~55, 1985