Table IV
Ionization Potentials of Some Metalloporphyrin Thin Film

	divalent form, kJ/mol	trivalent form, kJ/mol
horse cytochrome *c*	560	589
cytochrome c_3	444	521
zinc tetraphenylporphyrin	482	
protohemin		511

ture was perfectly reproducible either from 243 to 393 K or from 393 to 243 K.

Ionization Potential of Cytochrome c_3

The electrochemical characteristics of cytochrome c_3 described in the preceding sections might be a reflection of the electronic state of the hemes in the cytochrome c_3 molecule that is not shared by other heme groups in single-heme proteins such as eucaryotic cytochrome *c*. Electronic energy states of porphyrins in hemoproteins can be investigated by spectroscopic methods such as optical absorption, emission, magnetic resonance, or Mössbauer effect, but measurement of ionization potentials of the ferri and ferro forms of cytochrome c_3 together with those of monoheme-type cytochrome *c* would provide us with more crucial information for the determination of absolute energy levels. Accordingly they were measured, giving the results summarized in Table IV.[52,66] This table also includes the ionization potentials of zinc tetraphenylporphyrin and protohemin as the standards for divalent and trivalent metal derivatives of porphyrin.

In the case of eucaryotic cytochrome *c*, the difference in ionization potentials between the ferri and ferro forms was 29 kJ/mol, which is in accord with the difference between typical trivalent and divalent metal porphyrins, protohemin and zinc tetraphenylporphyrin. This means that the polarization energy of the metalloporphyrin structure induced by the protein moiety of eucaryotic cytochrome *c* was identical in both redox states. This is in accord with the fact that the conformation change during the reduction of eucaryotic cytochrome *c* is minor, as indicated by X-ray crystallographic measurements.[67]

By contrast, in the case of cytochrome c_3 where the four hemes are unequally oriented to form a heme cluster, the difference of ionization potentials between the ferri and ferro forms was as much as 77 kJ/mol. This remarkable difference may well be ascribed to the change in polarization energy induced by the protein moiety, change in electronic states of heme clusters, or an extraordinarily large intermolecular interaction in cytochrome c_3 film, which can be associated with the unusual electrical properties of cytochrome c_3 as described in the preceding sections. It has to be pointed out that the ionization potential of ferrocytochrome c_3 (444 kJ/mol) is the lowest in several porphyrin derivatives and phthalocyanines (near or over 482 kJ/mol)[68] and even lower than that of graphite (453 kJ/mol).[69,70]

Closing Remarks

In this Account we have presented some characteristics of cytochrome c_3 that make this molecule unique among organometallic compounds from either biological or nonbiological origins. An understanding of the theoretical basis of these characteristics of the tetrahemoprotein system would help us to discover as yet unexpected characteristics from biological materials.

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Biosynthetic Studies of Macrolide and Polyether Antibiotics

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There are two schools of thought concerning the value of heuristic studies of the biosynthesis of natural products. One is that the formation of natural products like alkaloids, antibiotics, and terpenoids must involve complex and, perhaps, unusual biochemical transformations. Investigation of their biosynthesis, consequently, is worthwhile to broaden our insight about the scope of biochemical events. A similar argument can be made for many other scientific endeavors: it is the diversity of scientific achievement, rather than just its singularity, that enriches the basis of our knowledge. The other school is that many of these natural products are useful to mankind as chemotherapeutic, insecticidal, color, and flavor, etc., agents. Information about their biosynthesis, therefore, has practical value to organi-

Charles Richard (Dick) Hutchinson was born in Dayton, Ohio, in 1943 and remained near there until receiving his undergraduate degree at the Ohio State University in 1966. After completing a Ph.D. in organic Chemistry with Professor Edward Leete at the University of Minnesota (1970), he spent a brief period as a postdoctoral fellow with Professor Alan Battersby at Cambridge University (1971). His independent research career began at the University of Connecticut (1971–1974) and has continued since 1974 at the University of Wisconsin, where he is a professor of medicinal chemistry. Major research interests in his laboratory have been the biosynthesis of natural products (alkaloids, antibiotics) and the medicinal chemistry of naturally occurring antitumor agents.

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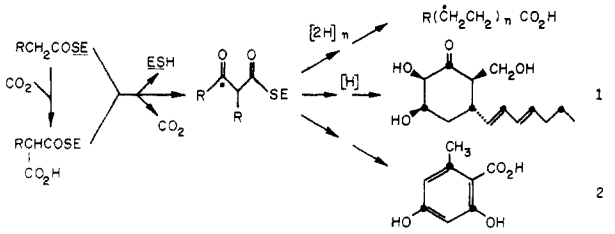

Figure 1. The general feature distinguishing the biosynthesis of fatty acids, in which the dotted carbon is fully reduced, from the biosynthesis of polyketides, in which the dotted carbon is partially (1) or not (2) reduced.

zations producing these compounds commercially. There also is hope that knowledge about the biosynthesis of natural products may reveal why living organisms make such compounds.

The study of antibiotic biosynthesis is particularly interesting due to the frequent occurrence, structural diversity, and useful biological activity of these substances.[1] We have been interested principally in the macrolide[2] and polyether[3] antibiotics. These antibiotics are assembled from simple C_2 to C_4 acids by biochemical pathways that are analogous to the formation of long-chain fatty acids.[4,5] Since the biochemical feature distinguishing the formation of macrolide and polyether antibiotics from saturated fatty acids is the amount of oxygenation (Figure 1), the frequent presence of oxygen-bearing carbons at alternate positions in these two classes of antibiotics places them in a larger class of natural products, the polyketides.[6] It is believed that the biosynthesis of polyketides involves poly-β-ketone intermediates, whose intramolecular cyclization generates aromatic metabolites like orsellinic acid (**2**) or whose partial reduction and cyclization generate metabolites like palitanin (**1**). The poly-β-ketone intermediates have never been found in the growth media of the producing organism; it is assumed, therefore, that they remain enzyme-bound until a certain point in the assembly process. Verification of this concept is one of the most important goals of biosynthetic studies of polyketides.

The discovery of pikromycin[7] (**3**, Figure 2) was the first milestone in macrolide and polyether antibiotic chemistry. The later discovery of erythromycin A[8] (**4**) sparked intense interest in macrolide antibiotics since **4** has valuable antiinfective activity.[8a] Subsequent

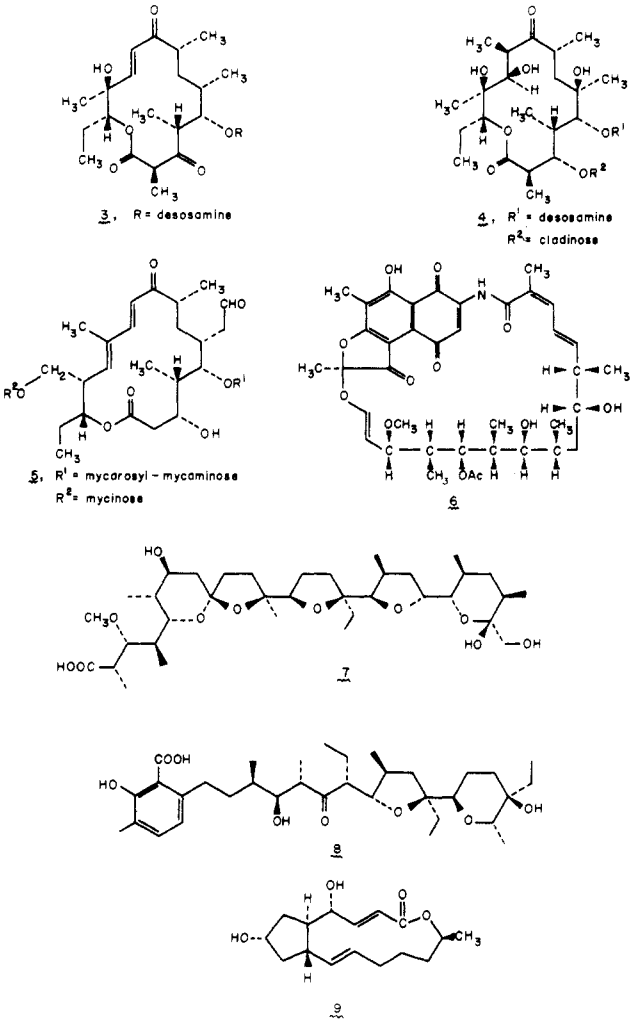

Figure 2. Structures of representative macrolide and polyether antibiotics.

discoveries (tylosin[9] (**5**), carbomycin (magnamycin)[10]) expanded the range of useful macrolide antibiotics, and uncovered the novel ansa macrolides[11] (rifamycins (**6**) and maytansins). Researchers at Eli Lilly and Hoffmann–La Roche in the 1950s found antibiotics that later proved to have useful coccidiostatic properties. Their structural characterization established nigericin,[12] monensin A[13] (**7**), and lasalocid A[12a,14] (**8**) as the first examples of the polyether antibiotic class. Although the former types of antibiotics are produced by bacteria, the fungi also produce macrolide antibiotics,[6c] of which brefeldin A[15] (**9**) is one example.

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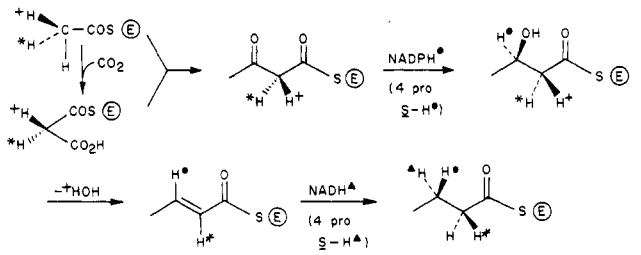

Figure 3. The stereochemical course of fatty acid biosynthesis in living organisms. There are differences in the last step among various genera; the result for *E. coli* is shown.

Researchers involved with the early chemical studies of macrolides speculated that these antibiotics might be formed biologically from fatty acids.[8] Support for this idea first came from the findings of Corcoran,[16] Grisebach,[17] and their co-workers that propionate, 2-methylmalonate, and glucose were the fundamental biochemical precursors of erythromycin A[18]. The lack of intact incorporation of saturated fatty acids into brefeldin A[19] and the absence of unusual polymethyl or cyclic fatty acids in the producing organisms[20,21] were circumstantial evidence against formation of the macrolide antibiotics directly from fatty acids. Nevertheless, even the rapid growth of knowledge about precursor–product relationships among the macrolide and polyether antibiotics during the last decade has not dispelled the notion that the lactone ring is assembled by a fatty acid synthetase.[16] Our understanding of the relationship at the enzyme level between macrolide and polyether antibiotic formation and the biosynthesis of fatty acids[22] thus is still an elusive research goal.

We have put this enzymological issue aside temporarily to focus on the mechanism of carbon chain assembly in the biosynthesis of macrolide and polyether antibiotics by asking two questions. What process determines (1) the absolute configuration of chiral centers and (2) the sequence of C_2 to C_4-subunit assembly during formation of the carbon chain? The nonregular configurational relationships of asymmetric methine positions and the nonsystematic sequence of acetate- and propionate-derived structural subfragments in the lactone ring or acyclic carbon framework of these antibiotics are intriguing. Celmer's observations about the stereochemical homology among the macrolides,[23] and similar facts for the polyether antibiotics (see below), reveal a strong genetic determinant for stereochemical consistency in the formation of these antibiotics by bacteria but do not provide clues about the stereochemical control of carbon chain assembly for any particular antibiotic. The following Account illustrates some ways we and others are probing this question.

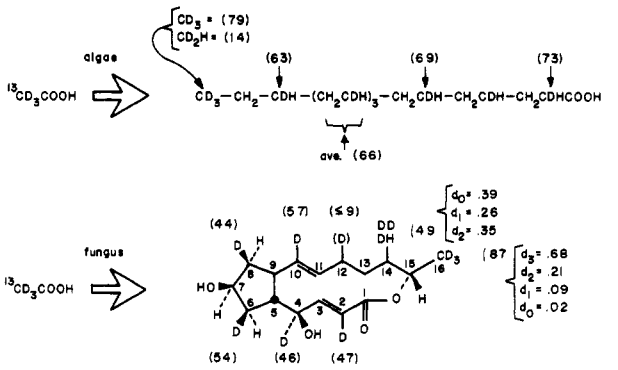

Figure 4. The pattern of ^{13}C,^{2}H labeling of palmitic acid in the algae, *Anacystis nidulans*,[29] and of brefeldin A in *P. brefeldianum* introduced by $^{13}C^{2}H_3CO_2H$. The numbers in parentheses are the relative amounts of ^{2}H at each ^{13}C labeled position as a percentage of that theoretically expected.

Brefeldin A

We are studying the biosynthesis of brefeldin A (**9**) to examine to comparative biochemistry of fatty acid and macrolide formation and to elucidate the mechanism of cyclopentane ring formation. Long-chain fatty acids are formed in all organisms by the four basic transformations shown in Figure 3.[22] Their repetition achieves a two-carbon chain extension for each cycle of acyl-SEnz–malonyl-SEnz condensation, β-keto thioester reduction, dehydration, and enolyl-SEnz reduction with the stereospecificity shown for each step.[24,25] The last step, enolyl-SEnz reduction, has species-specific differences in the stereospecificity of hydride donation from the coenzyme[25,27] and in the hydrogen addition to the heterotopic faces of the α,β-unsaturated thioester.[26] [2,2,2-$^{2}H_3$]Acetate, in traversing this pathway, should retain its three ^{2}H atoms at the chain-starting C_2 unit but lose two of its three ^{2}H atoms at all other C_2 units. The absolute configuration of all the chiral, ^{2}H-labeled positions should be identical and should depend only on the stereospecificity of hydrogen addition during step 4 of the carbon-chain extension cycle. Partial loss of ^{2}H occurs during some steps of this cycle in vivo[26,28,29] and in vitro[24a,b] so that, in fact, less than 1 equiv of ^{2}H per carbon atom resides at each labeled position except for the ω-methyl. This exchange process does not usually affect the configuration of the carbon atoms that are chiral due solely to ^{2}H labeling.[26,27,30] Since **9** had been shown to arise from one acetate and seven malonate molecules,[31] as if it were formed from a C_{16} fatty acid,[19,22] the important question to us was how did its

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^2H labeling stereochemistry from the incorporation of $C^2H_3CO_2H$ compare with that of a saturated fatty acid.

The unique structural features of brefeldin A permit us to view the five types of C–H and C–O stereochemical determinants seen in Figure 3; therefore, it is a good molecule for analysis of the biochemical labeling relationships. We determined the $^{13}C^2H_3CO_2H$-induced[32] and the $C^2H_3CO_2H$-induced[33] ^2H labeling regiochemistry of **9** by $\{^1H,^2H\}^{13}C$ NMR and ^2H NMR spectroscopic analysis. The results show (Figure 4) that ^2H resides at all the even-numbered carbons and that the amount of ^2H loss above theory is similar among the methylene and vinyl carbons, except for C-12.[32] We do not have a satisfactory explanation yet for the large loss of ^2H from C-12, but assume that the ^2H loss from C-16 is due to enolization of the acetyl-SEnz intermediate or to a recycling between the acetyl-SEnz and malonyl-SEnz intermediates. It is still uncertain whether the latter processes, or some other step, causes the exchange of ^2H during fatty acid biosynthesis.[24a,b,27,29,34] We believe the data for **9** indicate that this exchange occurs in the malonyl-SEnz intermediate because the amount of ^2H loss from all the other labeled positions (excluding C-12 and C-16) is not significantly greater than that at C-14. The latter position is not converted to an sp^2 carbon by dehydration[32,35] and thus is identical with C-2 of the malonyl-SEnz intermediate. The ^2H exchange could be spontaneous[34,36] or it could be enzymatically catalyzed by a basic group of the putative "brefeldin synthetase", even if a nonessential part of the carbon-chain assembly process.[24a,b] If the latter occurs, then there should be stereospecific ^2H loss from only one of the two disasterotopic positions at C-14 of **9**.

The results from the experiment with $C^2H_3CO_2H$ reveal the stereochemistry of ^2H incorporation at C-4, C-6, and C-8 of **9** but not at C-14 for the d_1 species due to inadequate spectroscopic resolution.[33] We have proposed that stereospecific reduction of RCH=CHCOSEnz intermediates produces the ^2H labeling stereochemistry at C-6 and C-8. The absolute configuration of these positions is identical with the corresponding asymmetric centers in C_{16}–C_{18} fatty acids made from $C^2H_3CO_2H$ by algae[33] and *Escherichia coli*[26,27] but not by yeast.[25d] On the other hand, the relative configuration of the C-4 ^2H of **9** is opposite to the C-6 and C-8 positions. We have suggested that this fact supports creation of the C-4 ^2H labeling stereochemistry by a stereospecific oxidation of a C-4–C-5 double bond, perhaps to an epoxide that may be a prerequisite of cyclopentane ring formation[35] rather than by hydroxylation of a C-4 sp^3 carbon. Biochemical hydroxylations of sp^3 carbons usually involve stereospecific insertion of an oxygen atom into a C–H bond with retention of its configuration.[37] Had this happened at the C-4 methylene of some postcyclization intermediate of brefeldin A biosynthesis, it is reasonable to believe that this C^2H^1H center would have had the L configuration like C-6 and C-8 and, therefore, would have lost its ^2H upon hydroxylation.

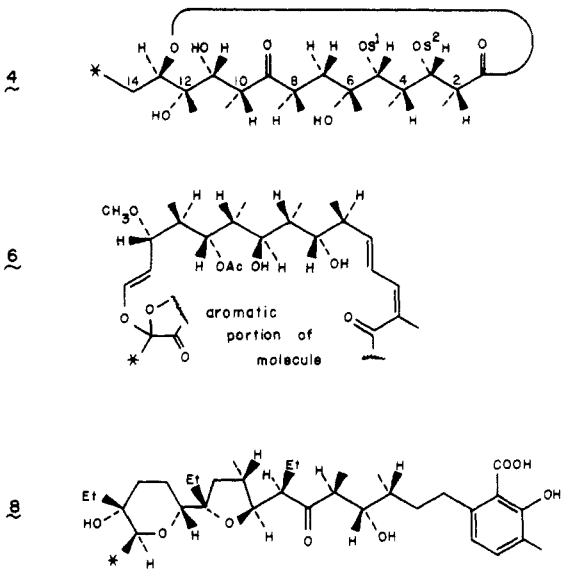

Figure 5. The structures of three antibiotics whose polyketide portion is known to be made from simple C_2–C_4 fatty acids starting at the starred carbon and proceeding to the right. In **4**, S_1 = desosamine and S_2 = cladinose.

The stereochemistry of the incorporation of [^2H]-acetate into brefeldin A is identical with the ^2H labeling pattern of C_{16}–C_{18} fatty acids in three other organisms.[38] Consequently, the mechanism of the formation of the carbon chain of **9** parallels the mechanism of fatty acid formation (Figure 3), although further work on the biosynthesis of **9** must be done to validate this statement thoroughly. The most important conclusion to reach at this time is that the implicit primary (fatty acid) and secondary (macrolide) biochemical processes have essential mechanisitic similarities from a stereochemical viewpoint. There are, however, characteristic species-specific differences, some of which result in the uniqueness of the antibiotic structures and in all of which lie the novelty of the secondary metabolic pathways.

Lasalocid A

The mechanism of carbon chain assembly for the biosynthesis of the bacterially produced macrolide and polyether antibiotics is more complex than fungal macrolide antibiotic biosynthesis. Erythromycin A (**4**), rifamycin S (**6**), and lasalocid A (**8**) are typical examples of the former type of antibiotics. These antibiotics are made from acetate, butyrate, and propionate plus other primary metabolites[5,11,16] in a manner superficially like fatty acid biosynthesis. Since most of the oxygen atoms found in the polyketide-derived portion of these antibiotics originate directly from the C_2–C_4 precursors (vide infra), there clearly are fundamental differences in the mechanism of carbon chain assembly between the two biochemical systems.

I can illustrate these differences best by drawing the polyketide-derived portion of **4**, **6**, and **8** as in Figure 5, from which **4** serves as a useful model to discuss a hypothesis for the mechanism of carbon chain assembly.

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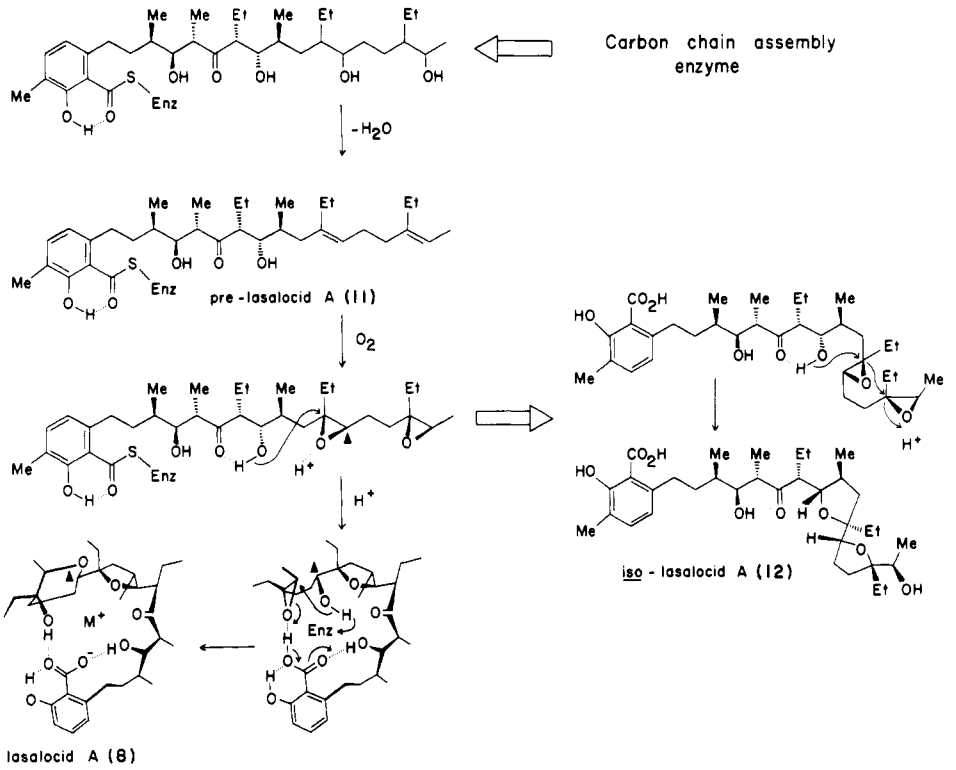

Figure 6. The proposed biosynthetic pathway to lasalocid A (8) according to Westley et al.[5] based on the results of precursor incorporation studies and the isolation of isolasalocid A (12).

Erythromycin A (4) is assembled from two hexoses and a 14-membered lactone, called erythronolide B, which results from the hydroxylation of 6-deoxyerythronolide B (10), itself the immediate product of the carbon chain assembly enzymes.[16] The structure of 10 thus is derived from 4 (Figure 5) by replacing the S^1 and S^2 at positions 3 and 5 and the hydroxyls at positions 6 and 12 with hydrogens.

It is believed that 10 grows from left to right on the enzyme surface by successive additions of 2-methylmalonyl-SEnz to the $*CH_3CH_2COSEnz$ starter unit (C-15 to C-13).[16] Each unit of 2-methylmalonate may undergo inversion of its C-2 configuration during C–C bond formation with the preceding $R^1CH(R^2)COSEnz$ unit, as occurs for malonate in palmitic acid biosynthesis.[24b] Yet the configuration of carbons 2, 4, and 10 (D) in 10 is opposite to that of carbons 6, 8, and 12 (L). Hence, either these two groups of C_3 subunits of 10 come from different enantiomers of 2-methylmalonate[47] or an epimerization event subsequent to bond formation between C_3 subunits determines the absolute configuration of some—but not all—asymmetric $R^1CH(CH_3)R^3$ centers in 10 and thus 4. A second mechanistic question is represented by the stereochemistry of carbons 3, 5, and 11 vs. carbon 13 of 10. Since these oxygen-bearing methine carbons originate from the carbonyl group of $R^1COCH(R^2)COSEnz$ intermediates, their configuration could be set by single-step biochemical reduction with hydride donors (Figure 3) or by a lengthier biochemical sequence of reduction, dehydration, and rehydration of the resulting $R^1CH{=}C(R^2)COSEnz$ intermediate in specified instances. The latter would be analogous to the mechanism of fatty acid β oxidation.[22c] The first idea requires reduction of the carbonyl group by two enzymes with differing substrate stereospecificities or by one and the same enzyme acting upon opposite faces of the carbonyl group, perhaps as directed by alternative orientations of the substrate's carbonyl group in the catalytic site. The second idea requires that some carbonyl groups be reduced to a unique configuration, with the heterotopic secondary alcohol configuration resulting from the sterospecificity of H_2O addition to the $R^1CH{=}C(R^2)COSEnz$ and the possibly synchronous correlation of the resulting α- and β-carbon configurations during rehydration.

Lasalocid A (8) is a good model for examination of the above hypotheses because of the extensive knowledge of its chemistry[3] and biosynthesis[5] from the pioneering studies of Westley and co-workers. These researchers had shown 8 to arise from three butyrate, four propionate, and five acetate molecules and had proposed the acyclic diene 11 to be the keystone for formation of 8 or isolasalocid A (12), as shown in Figure 6. Therefore, to test our ideas, we designed experiments that involved examination of the fate of C-1 ^{18}O and C-2 ^{2}H labels in the three precursors during their incorporation into 8. The anticipated outcome of these experiments was the retention or loss of the stable isotopes at the positions of 8 known to be labeled by direct precursor incorporation.[5] A probable complication was the partial exchange of ^{18}O with ^{16}O, or ^{2}H with ^{1}H, as had been found in other studies.[29,39,41]

The results of several experiments (Figure 7) have given us some insight about the various mechanistic possibilities. There is an insignificant loss beyond the theoretically expected amount of the ^{18}O introduced at carbons 1, 3, 11, 13, and 15 of 8 during incorporation of $R^{13}C^{18}O_2H$ precursors.[40] This result contrasts several

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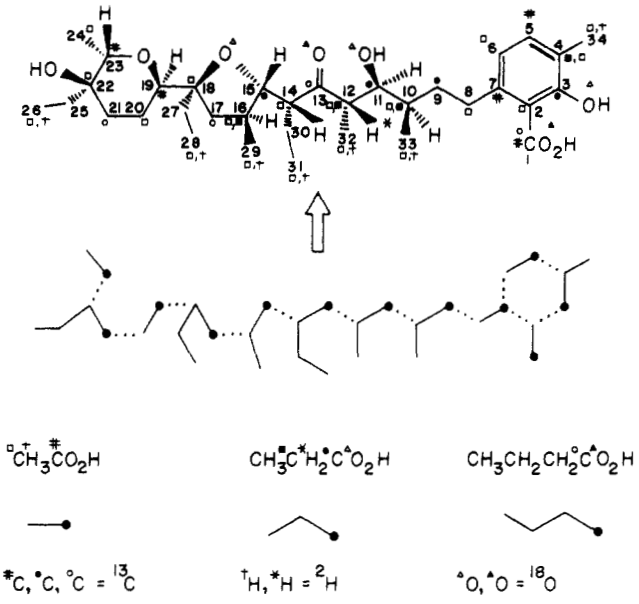

Figure 7. The isotopic labeling pattern of lasalocid A (8) resulting from the incorporation of ^{13}C,^{18}O- and ^{13}C,^{2}H-labeled precursors. The labeling regiochemistry was deduced by observation of the isotope-induced shifts of the ^{13}C resonances at ^{13}C enriched positions due to the simultaneous presence of ^{2}H or ^{18}O at these positions. The symbols on the atoms of the precursors and 8 indicate the precursor–product labeling relationships.

examples of ca. 50% ^{18}O exchange in the biosynthesis of fungal polyketides from $CH_3C^{18}O_2H$.[41] Cane and co-workers have reported similar results for erythromycin A[42] and monensin A.[43] The lack of ^{18}O at carbons 19 and 22 of 8 from $R^{13}C^{18}O_2H$ supports Westley's proposal for the role of 11 in lasalocid A formation, since the oxygens at these two positions should come from O_2. Cane and Liang have proven such a labeling relationship for monensin A directly using $^{18}O_2$,[44] which supports their suggestion for the involvement of a triene analogous to 11 in its biosynthesis.[43]

In contrast, there is a large loss of ^{2}H from $R^{13}C^{2}H_2CO_2H$ precursors on their incorporation into 8 at all positions other than the methyl groups.[45] Retention of ^{2}H only in these methyl carbons agrees with several other reports describing $^{13}C^{2}H_3CO_2H$-induced labeling patterns of polyketides,[39] but the absence of ^{2}H in the starter C_2 group methyl (C-24 of 8) does not. Thus, either carbons 23 and 24 in the form of $CH_3COSEnz$ are not the starting point of carbon chain assembly or, more likely, the C_2 starter unit experiences complete ^{2}H exchange before addition of the C_4 chain-extending unit due to some unique characteristic of "lasalocid synthetase". The loss of ^{2}H from most of the methylene and methine carbons is not surprising in view of the discussion in the previous section. Fortuitously, C-12 of 8 retained a significant amount of ^{2}H (10%) from the $CH_3{}^{13}C^{2}H_2CO_2H$ precursor.[45] We have proposed that this labeling result is due to conversion

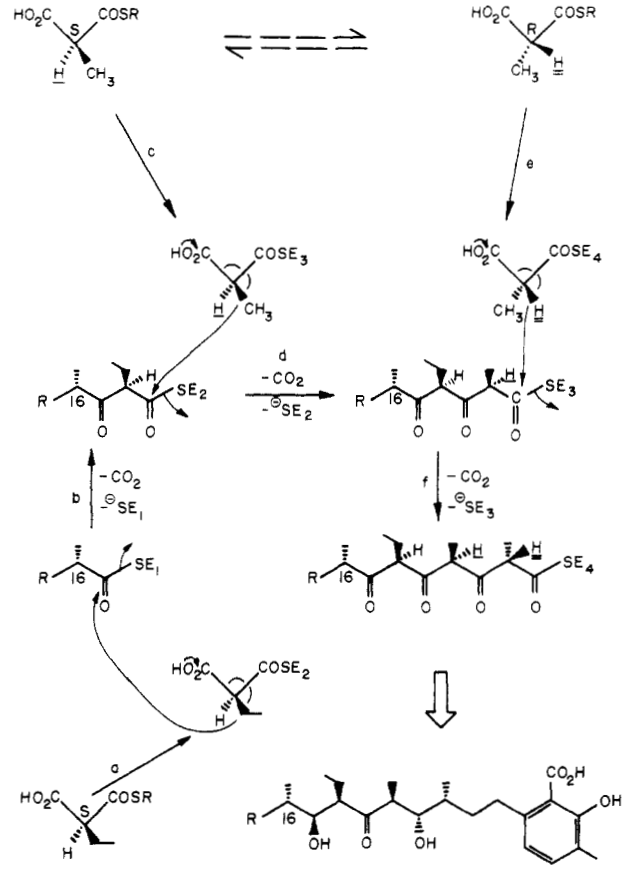

Figure 8. A scheme for the proposed formation of the C_1 to C_{17} portion of lasalocid A (8) via the corresponding linear portion of 11, its putative C_{34} diene precursor. The subscripts on the enzyme symbol, E, indicate the different hypothetical catalytic sites (or enzymes) governing carbon chain assembly. The letters a–f indicate the sequence of events in the assembly of precursors onto the growing carbon chain (R). The timing of the reduction of the C-11 and C-15 carbonyls is not specified in this figure, for it represents only the processes we believe are controlling the stereochemistry at C-10, C-12, and C-14.

of the propionate to ((2*S*)-[2-^{2}H,2-^{13}C]-2-methylmalonyl)-SEnz and then direct incorporation of it into C-11, C-12, and C-32 of 8 with inversion of its C-2 configuration.[45] We believe the lack of ^{2}H but presence of ^{13}C at C-10 of 8 from this precursor is due to biochemical epimerization of (2*S*)- to (2*R*)-2-methylmalonate. Since epimerization of (2*S*)- to (2*R*)-2-methylmalonate by the known epimerase[36,46] would result in loss of the C-2 ^{2}H label from the propionate precursor, then only the ^{13}C reference atom should label C-10 of 8 as well as carbons 4, 12, and 16. The latter carbon should lose its ^{2}H label via a $RC_{17}{=}C_{16}(C_{29}H_3)C_{15}OSEnz$ intermediate. It then is reasonable to propose that the asymmetric methine carbons of 8 with the D configuration are derived from (2*S*)-2-methyl(ethyl)malonate and those with an L configuration from its 2*R* enantiomer, as shown in Figure 8.

We had planned that the known formation of ((2*R*)-2-methylmalonyl)-CoA from succinyl-CoA,[47] which $^{13}C^{2}H_3CO_2H$ labels at C-2 and C-3 by one turn only of the Krebs cycle, would result in ^{13}C,^{2}H labeling at C-10 of 8 via ((2*R*)-[2,4-$^{13}C_2$, 2,4-$^{2}H_2$]-2-methyl-

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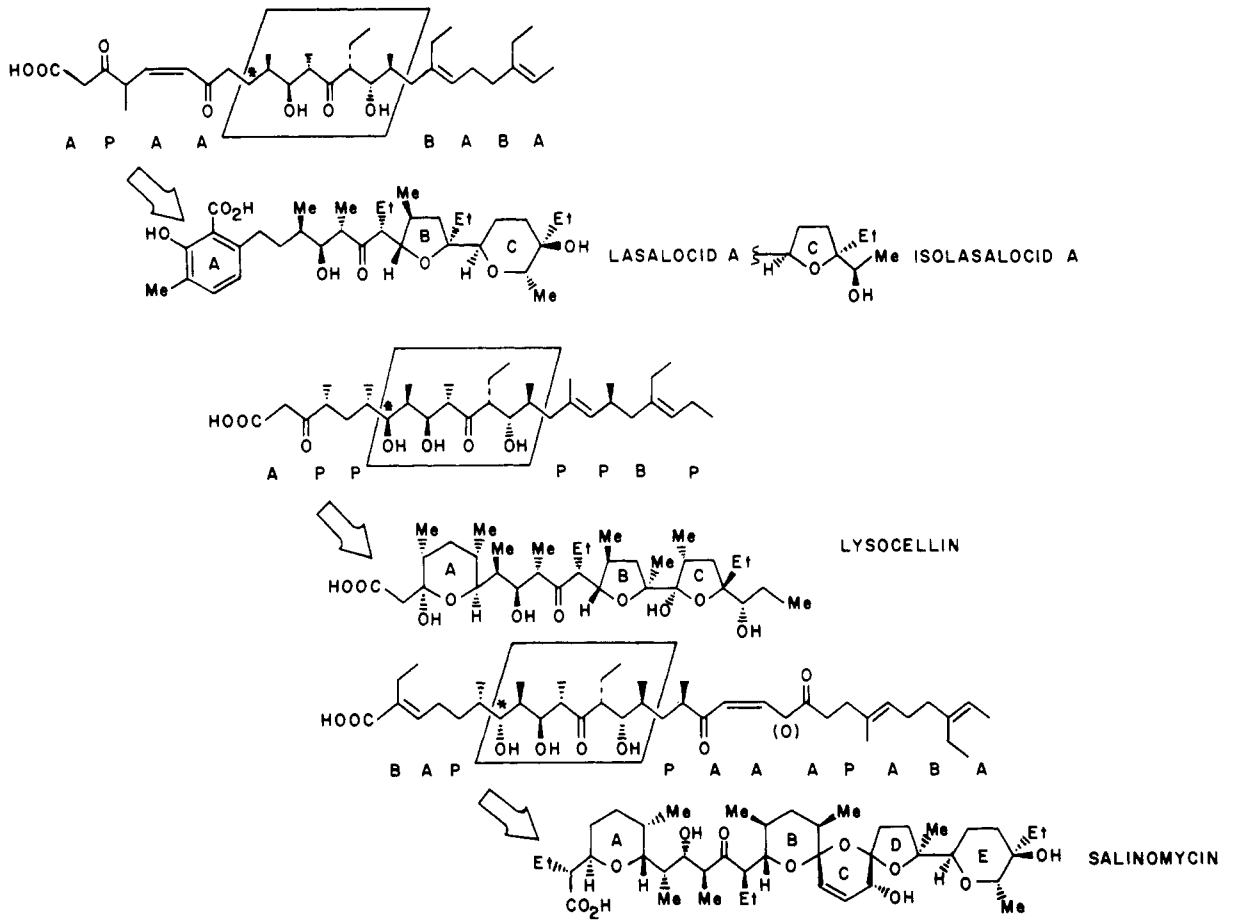

Figure 9. Structural and stereochemical homology among three polyether antibiotics. Letters below each linear polyene structure indicate the C_2–C_4 acid precursor: A, acetate; B, butyrate; C, propionate. The absolute stereochemistry of the portions of the putative acyclic biosynthetic precursors of these antibiotics enclosed in boxes is identical, and the structural subfragments differ only in oxidation state at the starred carbons. These facts and structural homologies among other polyether antibiotics (D. E. Cane, W. D. Celmer, and J. A. Westley, submitted for publication) strongly suggest that the information for polyether antibiotic biosynthesis is arranged by combining several genes, which each encode the formation of subfragments of the carbon chain, into the particular array that results in the antibiotics characteristic of a given species.

malonyl)-SEnz. This would have confirmed our concept for the stereochemistry of carbon chain assembly. The amount of ^{13}C enrichment at C-10 from $^{13}C^2H_3CO_2H$ was too low, however, to detect the presence of ^2H. An identical explanation may be why this acetate also did not appear to label C-14 of 8 with ^2H via [2,4-$^{13}C_2$,2,4-$^2H_2$]butyrate. But there is an alternative explanation of the latter result—loss of the ^2H from C-2 of this butyrate upon α carboxylation before its entry into carbons 13, 14, 30 and 31 of 8—as we noted.[45]

The data from the biosynthetic investigations of erythromycin A, lasalocid A, and monensin A enable some discrimination among the hypotheses I have presented for the mechanism of carbon chain assembly during the formation of these and possibly all other macrolide and polyether antibiotics. They invalidate the dehydration–enone rehydration idea[49] as long as $H_2{}^{18}O$ does not reenter the same molecule from which it was removed. (The latter possibility seems unlikely, as precedented by the mechanistic studies of aconitase.[48]) Hence, biochemical processes more direct than this one must establish the absolute configuration of the α-alkyl and β-oxygen bearing carbons in each C_3 or C_4 subunit of the carbon chain. I believe that this stereochemical control occurs by stereospecific selection of chiral 2-substituted malonate analogues, as proposed above, and by stereodivergent reduction of β-keto thioesters during the chain growth. Perhaps the frequent occurrence in the antibiotic structures of vicinal alkyl groups and oxygen atoms having the threo relative stereochemistry across the new C–C bond that is formed upon addition of a C_3 or C_4 chain-extending unit (Figure 5) indicates that the α-carbon configuration can control the stereospecificty of β-keto thioester reduction. Yet this idea cannot be applied universally since the macrolides made from acetate only (e.g., polyenes) contain –CH(OH)– sites having an irregular pattern of D and L configurations. Nevertheless, it is clear that the overall assembly of the carbon chain of these antibiotics is very complex becasue the configurational features evident in their structures do not follow a regular pattern as in fatty acid biosynthesis.

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Prognosis for the Future

Detailed insights into the mechanism of macrolide and polyether antibiotic biosynthesis, especially knowledge about the control over the sequence of C_2–C_4 subunit assembly, will have to come from studies of the molecular biology and enzymology of their formation. We can assume by analogy to yeast fatty acid synthetase[22b,50] and oligopeptide antibiotic synthases[51] that the

sequence of subunit assembly is a function of the spatial arrangement of catalytic (ligase) sites on the enzyme(s), which in turn is encoded in the mRNA,[52] hence DNA that ultimately determines the bacterial genotype of antibiotic formation. Some things we need to know more about for these levels of bacterial cell biology are the following: (1) whether an assembly of single enzymes or one multi-catalytic-site polypeptide govern formation of the earliest macrolactone or acyclic polyketide intermediate; (2) the mechanism regulating the amount of antibiotic made; (3) the cellular location of the genes coding for the structural and regulatory factors of antibiotic production; and (4) the methods of maintenance and transfer of these genes among the producing organisms. It is, therefore, fortunate that the new methods of recombinant DNA research and the advances in actinomycetes genetics discovered in the past 10 years[53] now have provided the ways to learn new information about these topics, especially for the streptomycetes, which make about 75% of the antibiotics produced commercially.[54]

Summary

The study of macrolide and polyether antibiotic formation in microorganisms during the past 20 years has resulted in a wealth of information concerning the identity of the primary metabolites that are the biochemical precursors of these antibiotics, the probable biochemcial mechanisms by which the precursors unite to form the structures of these antibiotics, and the sequence of biochemical transformations for the assembly of the two macrolides, erythromycin A and tylosin. The study of the enzymology of these antibiotics' biosynthesis is in its infancy,[16] and the investigation of the molecular biology of antibiotic formation is just beginning.[55] For the next decade and beyond the emphasis of most biosynthetic studies of these antibiotics should be on these latter two research topics rather than on the continued analysis of precursor–product relationships. These areas of research offer challenging and important problems in cell biology whose study will provide much new knowledge with both esthetic and practical appeal to a broad group of scientists.

We and others have raised questions about the stereochemical control of macrolide and polyether antibiotic formation, which are pretinent to the mechanism of carbon chain assembly, and have provided some insight into how this comes about. Of more importance, our research and other work have raised several questions concerning the molecular biology of this process. What determines an organism's ability to make a particular macrolide or polyether antibiotic? How has this genetic determinant resulted in the interspecies structural relationships among the macrolides observed by Celmer years ago, which also can be extended to the polyether antibiotics as demonstrated in Figure 9 for lasalocid A, lysocellin, and salinomycin? What regulates the amount of antibiotic produced by a microorganism such that *Streptomyces albus* 80614, e.g., can make 60 g/L of salinomycin in batch fermentation by converting 38% of the media's soybean oil into this antibiotic![56] It surely will be interesting to see how the application of new research techniques in molecular biology (gene cloning in *Streptomyces*)[53] and in spectroscopy (dynamic NMR observation of whole cells)[57] as well as continued exploition of the methodology discussed herein, results in a better understanding of the fascinating biosynthesis of macrolide and polyether antibiotics.

It has been a pleasure to have had the company and seen the enthusiasm and capability of the several co-workers and scientific collaborators whose names appear on the articles cited in this Account of research done at Wisconsin. We also enjoyed the enthusiastic help of senior research collaborators at Providence (D. E. Cane), Halifax (A. G. McInnes and J. A. Walters), and Alberta (J. C. Vederas). The National Institutes of Health (GM 25799) and the University of Wisconsin Graduate School have supported this research financially since 1975.

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