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ANTIBIOTIC STRUCTURE AND BIOSYNTHESIS[1]

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ABSTRACT.—The first polyether antibiotics were isolated more than thirty years ago, but in the past fifteen years, their numbers have increased from five to nearly eighty. The interest in these naturally occurring acid ionophores has arisen from their coccidiostat activity and their use as growth promoters in ruminants. The polyethers have been classified according to their cation selectivity and chemical structure, and recently a unified stereochemical model has been proposed for their biosynthesis.

Although not identified as polyether antibiotics at the time, the first three members of this class, antibiotics X-206, X-464 (nigericin), and X-537A (lasalocid), were reported in 1951 (1,2). Because of their parenteral toxicity, little interest was shown in these lipophylic, carboxylic acids until sixteen years later, when their ionophorous nature was first described (3) and the first structure, that of monensin (4), was elucidated by X-ray crystallographic analysis. One year later, monensin, nigericin, dianemycin, and antibiotic X-206 were all reported to be potent coccidiostats (5). Coccidia are parasitic protozoa of the subphyllum Sporozoa, which exhibit a particular affinity to the epithelial cells in the digestive tracts of birds and mammals; the genus found most often in poultry is *Eimeria*. The extent of the disease can be estimated by the 1978 world market of $100 million (6). Subsequently, an even more lucrative market was uncovered when it was found that polyether antibiotics improve the efficiency of feed conversion in ruminants (7).

All of these factors caused an exponential growth in the number of novel polyether antibiotics during the next decade to more than seventy (8). In order to keep track of this rapidly expanding group of natural ionophores, it became necessary to classify them according to their cation selectivity and chemical structure (9). Because of the preponderance of polyethers that fell into the monovalent (*1a*) and monovalent glycoside (*1b*) classes, further subdivisions based on the presence and nature of their spiroketal functions have been proposed (10,11) as listed in Table 1 and illustrated in

TABLE 1. Classification of Polyether Antibiotics

Class		Sub-Class	Number Reported	Examples
1a-1	Monovalent	Non-spiroketal	2	alborixin (12), X-206 (13)
-2		Spiroketal	17	lonomycin (14), monensin (4)
-3		Dispiroketal	15	noboritomycin (15), salinomycin (16)
1b-1	Monovalent glycoside .	Spiroketal	17	carriomycin (17), septamycin (18)
-2		Two spiroketals	8	dianemycin (19), lenoremycin (20)
2a	Divalent		8	lasalocid (21), lysocellin (22)
2b	Divalent glycoside . .		2	antibiotic 6016 (23), X-14868B (29)
3	Pyrrole ether		3	calcimycin (25), X-14547A (26)
4	Acyl tetronic acid . . .		2	tetronomycin (27), M-139603 (28)

Figure 1. Until the discovery of antibiotics X-14868A,B,C, and D (24), all of the monovalent glycoside class (*1b*) of polyether antibiotics contained the identical sugar-like moiety, 2,3,5-trideoxy-4-*O*-methyl-D-erythrohexapyranose (4-*O*-methyl-

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FIGURE 1. Monovalent Polyethers Containing no Spiroketals, *1a*-1, a Single Spiroketal, *1a*-2, and a Dispiroketal, *1a*-3

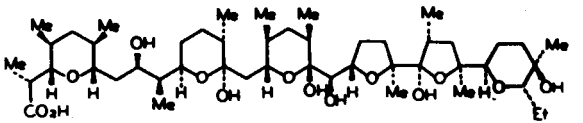
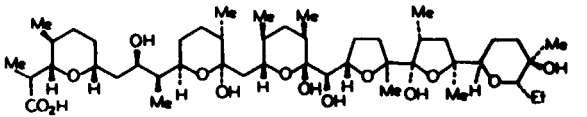
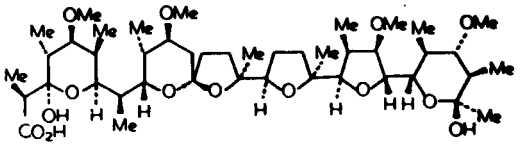
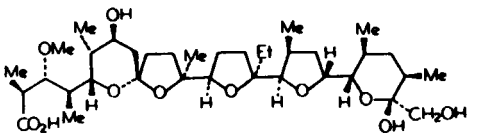
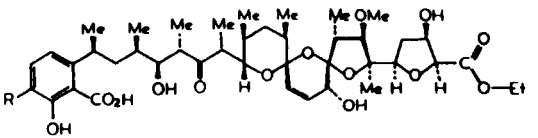
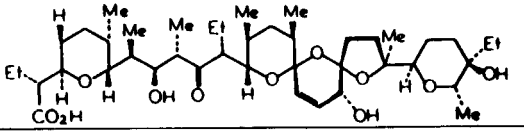
	Name (synonyms) [C. A. Reg. No.]	Formula MW MP (°C)	$[\alpha]_D$ (c, solvent) UV_{max} (ϵ)	Structure	Reference
1a-1	**Alborixin**	$C_{48}H_{84}O_{14}$	-7° (c 4, acetone)		(12)
	(S14750A)	885.2			
	[57760-36-8]	100-105	End abs.		
	X-206	$C_{47}H_{82}O_{14}H_2O$	+15.0 (c 2, MeOH)		(13)
	[36505-48-3]	889.2			
		133-145	End abs.		
1a-2	**Lonomycin A**	$C_{44}H_{76}O_{14}$	+66.6° (c 1, $CHCl_3$)		(14)
	(Emericid, DE-3936, A-218)	829.1			
	[58785-63-0]	109-114	End abs.		
	Monensin A	$C_{36}H_{62}O_{11}H_2O$	+47.7° (c 1, MeOH)		(4)
	(monensic acid, A3823)	688.9			
	[17090-79-8]	103-105	End abs.		
1a-3	Noboritomycin A	$C_{43}H_{63}O_{14}Na$	-28.7° (c 1, $CHCl_3$)		(15)
		827.0			
	[68508-45-2]	235-237	300 nm (3,600), 244 nm (4,100)	NOBORITOMYCIN A, R=Me " B, R=Et	
	Salinomycin	$C_{42}H_{70}O_{11}$	-63° (c 1, EtOH)		(16)
	[53003-10-4]	751.0			
		112.5-113.5	285 nm (108)		

FIGURE 2. Monovalent Glycosides Containing Either One Spiroketal, *1b*-1 or two *1b*-2

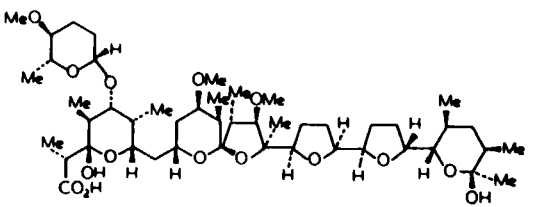
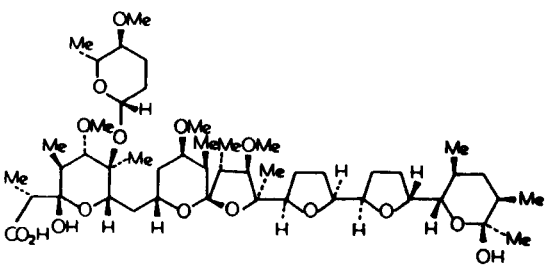
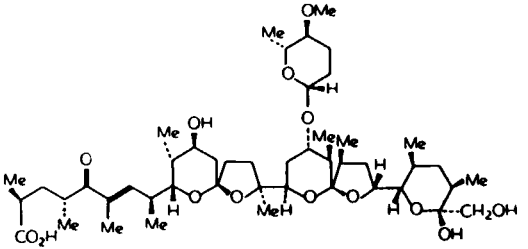
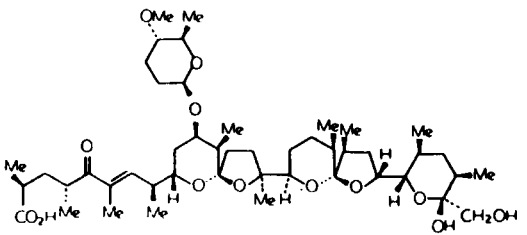
	Name (synonyms) [C. A. Reg. No. 1]	Formula MW MP (°C)	$[\alpha]_D$ (c, solvent) UV_{max} (ϵ)	Structure	Reference
1b-1	**Carriomycin**	$C_{47}H_{80}O_{15}$	-0.5° (c 1, MeOH)		(17)
		885.1			
	[65978-43-01]	120-122	End abs.		
	Septamycin	$C_{48}H_{81}O_{16}Na$	+14.4° (c 1, MeOH)		(18)
	(A28695A)	937.1			
	[54927-63-8]	164-166	End abs.		

FIGURE 2. *Continued*

1b-2	**Dianemycin**	$C_{47}H_{78}O_{14}$ H_2O	+39.9° (c 1, MeOH)		(19)
		885.1			
	[35865-33-9]	156-157	232 (15,000)		
	Lenoremycin	$C_{47}H_{77}O_{13}Na$	+95° (c 1, $CHCl_3$)		(20)
	(Ro-21-6150, A-130A)	873.1			
	[51257-82-2]	235	235 nm (14,000)		

amicetose), as indicated in Figure 2. In addition, all of the *1b* antibiotics were β-glycosides with the exception of antibiotic A204A (29) which is, so far, the only 4-*O*-methyl-α-D-amicetoside reported as a polyether. Two examples of monovalent glycosides with a single spiroketal and two with a double spiroketal are shown in Figure 2.

When a polyether antibiotic of the type illustrated in Figures 1 and 2 assumes the characteristic cyclic conformation dictated by a hydrogen bond between the carboxyl and terminal hydroxyl groups, the molecule concentrates all the oxygen functions at the center of the structure where they are available for the complexation of a suitable cation, while the many branched alkyl groups along the carbon backbone are simultaneously spread over the outer surface, rendering the complex lipid soluble. This elegant molecular design gives these antibiotics the ability to conduct monovalent cations across membranes down the concentration gradient by a mechanism known as passive diffusion (3). Lasolocid, monensin, nigericin, salinomycin, dianemycin, and antibiotic X-206 all have been compared by either fluorimetric or two-phase distribution techniques to determine their cation selectivity (30). The results are summarized in Table 2.

TABLE 2. Polyether Antibiotics and Their Cation Selectivities[a] as Determined by Either Fluorimetric or Two-Phase Distribution Studies

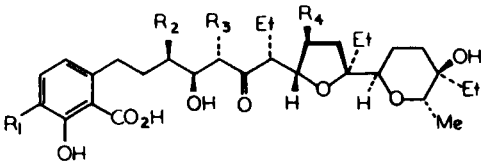
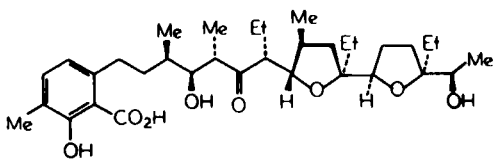
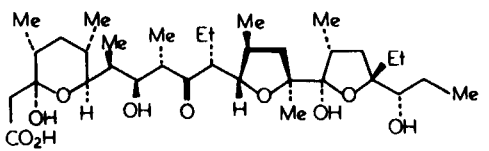
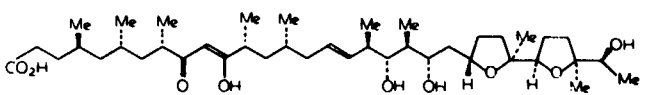
Antibiotic	Molecular weight	Cation selectivity
Lasalocid	590	$Ba^{2+}>>Cs^{+}>Rb^{+}, K^{+}>Na^{+}, Ca^{2+}, Mg^{2+}>Li^{+}$
Monensin	671	$Na^{+}>>K^{+}>Rb^{+}>Li^{+}>Ca^{2+}$
Nigericin	724	$K^{+}>Rb^{+}>Na^{+}>Cs^{+}>Li^{+}$
Salinomycin	750	$K^{+}>Na^{+}>Cs^{+}>>Ca^{2+}$
Dianemycin	866	$Na^{+}, K^{+}>Rb^{+}, Cs^{+}>Li^{+}$
Antibiotic X-206	870	$K^{+}>Rb^{+}>Na^{+}>Cs^{+}>Li^{+}$

[a]Lyotropic series (with ionic radii in nanometers) are: Cs^{+} (0.169)>Rb^{+} (0.148)>K^{+} (0.133)>Na^{+} (0.095)>Li^{+} (0.060) and Ba^{2+} (0.135)>Sr^{2+} (0.113)>Ca^{2+} (0.099)>Mg^{2+} (0.097).

Lasalocid can be distinguished from the other five polyether antibiotics listed in Table 2 by the ability to transport *divalent* cations in addition to the effect on alkaline metals exhibited by all the polyether antibiotics. This is the basis of classification for the divalent antibiotics illustrated in Figure 3. The last two classes of polyether antibiotics are structurally distinct from all the other polyethers. They are the pyrrole ethers (Figure 4), which all contain a pyrrole-2-carbonyl function, and the most recently discovered, class 4 polyethers, also known as the acyl tetronic acids (Table 1). These latter antibiotics differ from all other polyethers in that they lack a carboxlic acid moiety. As illustrated in the structure of tetronomycin (27), the carboxlic group is replaced by an acylidene tetronic acid, and another distinguishing characteristic is the presence of a trisubstituted cyclohexane ring representing the first example of a homocyclic aliphatic ring in a polyether antibiotic (Figure 5). The complex structures of these different classes of polyether antibiotics has presented a considerable challenge to several chemists involved in the total synthesis of these unique natural products (33).

The structure of lasalocid was published in 1970 (21), and in that same year, the first report on the biosynthesis of that versatile divalent polyether antibiotic also appeared (34). Since that time, many studies on polyether biosynthesis have been reported, including a number of reviews (35). From these reports, a universal biosynthetic scheme has emerged. The major building blocks for the skeletons of all the polyethers are acetate, propionate, and butyrate, and, in the case of polyethers contain-

FIGURE 3. Divalent Polyethers *2a* and the Divalent Glycoside *2b*, Antibiotic A6016

Name (synonyms) [C. A. Reg. No.]	Formula MW MP (°C)	$[\alpha]_D$ (c, solvent) UV_{max} (ϵ)	Structure	Reference
Lasalocid A (X-537-A) [25999-31-9] [11054-70-9]	$C_{34}H_{54}O_8 \cdot C_2H_5OH$ 636.9 100-109	-39.8° (c 1, $CHCl_3$) 318 nm (4300), 248 nm (6750)	 LASALOCID A, $R_1=R_2=R_3=R_4=Me$ LASALOCID B, $R_1=Et$, $R_2=R_3=R_4=Me$ LASALOCID C, $R_2=Et$, $R_1=R_3=R_4=Me$ LASALOCID D, $R_3=Et$, $R_1=R_2=R_4=Me$ LASALOCID E, $R_4=Et$, $R_1=R_2=R_3=Me$	(21)
Iso-lasalocid [54156-67-1]	$C_{34}H_{54}O_8$ 590.8 203	-39.2° (c 1, $CHCl_3$)		(31)
Lysocellin (K-5610) [55898-33-4]	$C_{34}H_{59}O_{10}Na \cdot \frac{1}{2}H_2O$ 659.8 158-160	+11.5 (c 1, MeOH) 292 nm (45)		(22)
Ionomycin	$C_{41}H_{70}O_9Ca$	+33° (c 0.4, MeOH)		
[56092-81-0]	746.5 205-206	300 nm (21,600), 200 nm (17,700)		(6)

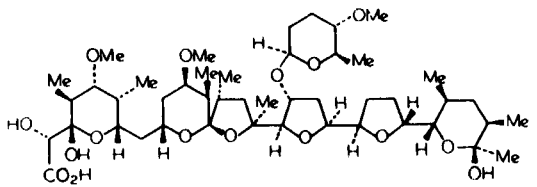
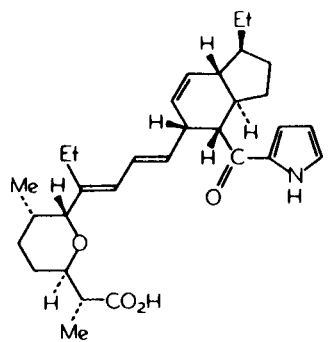
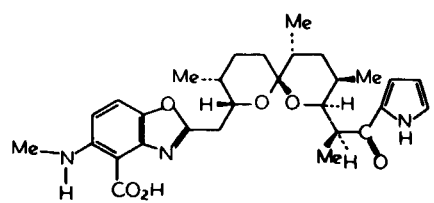
A6016	$C_{46}H_{77}O_{16}Na$	-42.5° (c 1, MeOH)		(32)
	909.1			
[69421-39-23]	192-195	End abs.		

FIGURE 4. Pyrrole Ether (Class 3) Antibiotics

Name (synonyms) [C. A. Reg. No.]	Formula MW MP (°C)	$[\alpha]_D$ (c, solvent) UV_{max} (ϵ)	Structure	Reference
X-14547A	$C_{31}H_{43}NO_4$	-328° (c 1, $CHCl_3$)		(26)
	493.7			
[66513-28-8]	138-141	291 nm (16,100), 244 nm (32,000)		
A23187	$C_{29}H_{37}N_3O_6$	-56° (c 1, $CHCl_3$)		(25)
(Calcimycin)	523.6	378 nm (8200),		
[52665-69-71]	181-182	278 nm (18,200)		

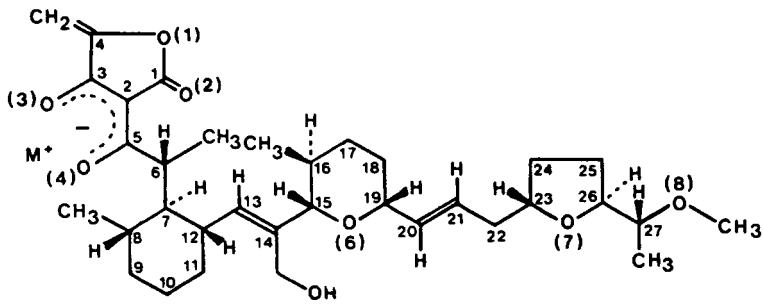


FIGURE 5. Tetronomycin (27), one of the two known acyltetronic acid class polyether antibiotics (class 4).

ing methoxyl groups, these C_1 additions are made via methionine. Taking lasalocid as an example, the starter molecule is acetyl CoA, and the antbiotic appears to be assembled by an enzyme complex. In a sequence of transformations analagous to, but certainly not identical with, classical saturated fatty acid biosnythesis, four malonates, four 2-methlymalonates, and three 2-ethylmalonates are added to the initial acetyl CoA in the correct sequence to form a polyketide (36) that, after suitable reductions, is converted to a linear precursor equivalent to **1** (Figure 6.) In seeking intermediates in the biosynthetic route between **1** and lasalocid A, the mother liquor from extracts of fermented cultures of *Streptomyces lasaliensis* was investigated in some detail. As a result, four homologs, lasalocid B, C, D, and E, were isolated, separated, and fully characterized. As illustrated in Figure 3, in these four homologs, the four methyl groups denoted by R_1 through R_4 were replaced in turn by *C*-ethyl groups. The level of the four homologs in lasalocid fermentation varied from 4% up to 20% with higher levels occurring in fermentations that were oxygen deficient. Our conclusion was that, in cases where there were high levels of butyrate in the fermentation medium resulting from only partial β-oxidation of long-chain fatty acids such as oleate and stearate, propionate was replaced by butyrate in one of the four possible positions in the polyketide chain with the resultant production of each of the four homologs. This occurred between one and five times per hundred molecules of lasalocid A produced by *S. lasaliensis* and gave four isomeric compounds, each containing four *C*-ethyl groups, a unique combination amongst known natural products. The counter current separation and structural elucidation of the four homologs by gc/ms presented quite a challenge (37).

A more instructive cometabolite of lasalocid A was discovered at a much lower concentration (one part in five thousand) in the *S. lasaliensis* fermentation. This particular compound was an isomer of lasalocid A with virtually identical uv and ir spectra. The structure of iso-lasalocid A was determined by X-ray crystallography (31) and is illustrated in Figure 3. The only difference between lasalocid A and this naturally occurring isomer resides in the terminal ring, which is a tetrahydrofuran in iso-lasalocid A in contrast to the tetrahydropyran present in the major antibiotic and its homologs. In addition, the analysis showed that the absolute configuration of iso-lasalocid A is identical at eight asymmetric centers but is reversed in the two terminal centers at C-22(*S*) and C-23(*R*). These differences in ring size and configuration suggest that iso-lasalocid A is not a precursor of lasalocid A but that the two compounds probably arise from a common precursor. A compound with the ability to cyclize to either of the two isomers is the epoxide **5** proposed in Figure 6. Microorganisms such as *Pseudomonas oleoverans* are known to epoxidize alkenes enzymatically (38) in the presence of NADH and molecular oxygen. The olefinic precursor (**3**) of the diepoxide **4** could arise by dehydration of the secondary alcohol at C-23 (in **2**) at some stage during the formation of the carbon skeleton of lasalocid A by *S. lasaliensis*. Acid cyclization of the epoxide **5** in Figure 6 should

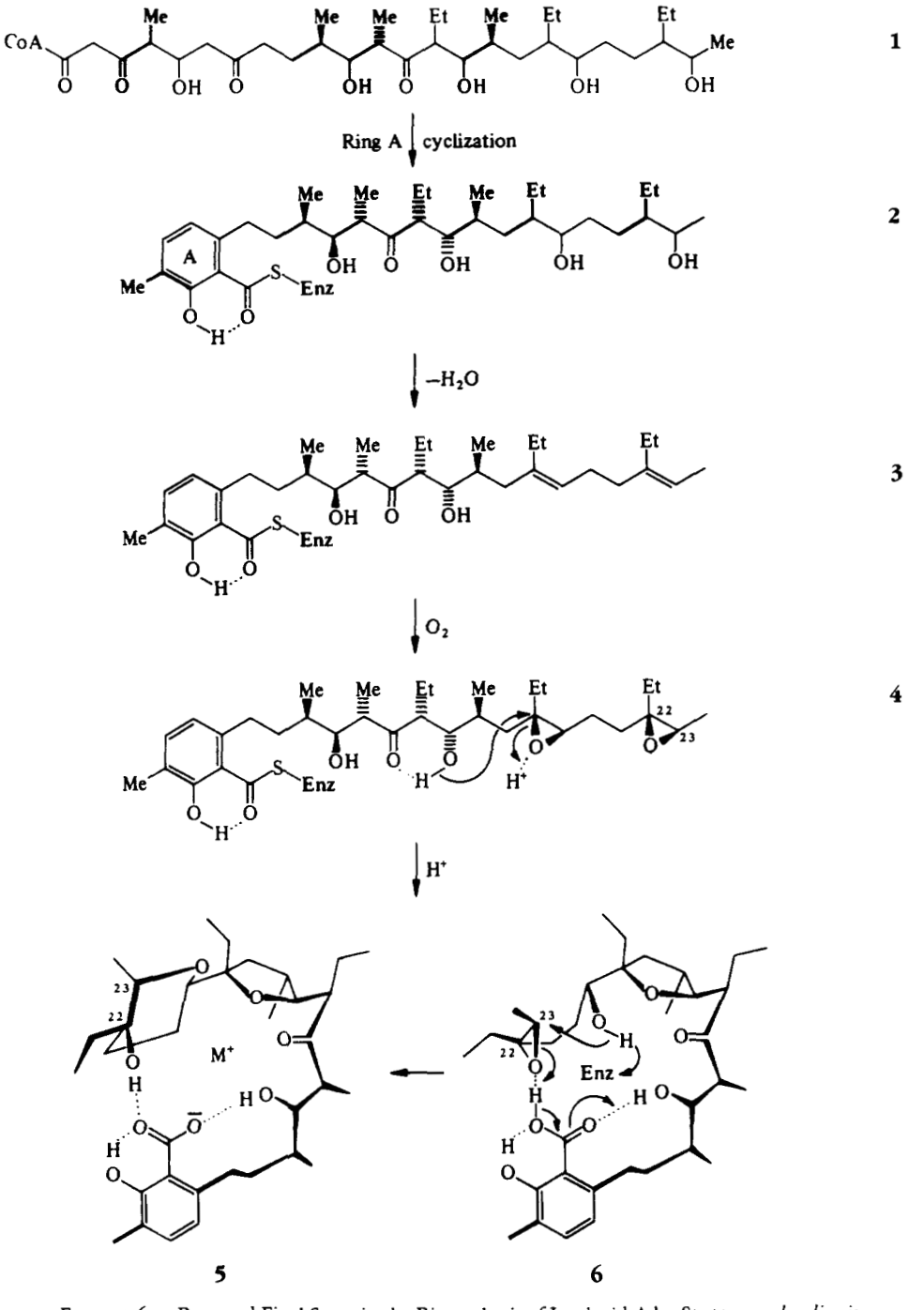


FIGURE 6. Proposed Final Steps in the Biosynthesis of Lasalocid A by *Streptomyces lasaliensis*

lead to selective cleavage (39) of the C-O bond to the more substituted carbon (C-22) according to the Markonikoff rule. On protonation of the epoxide oxygen, bond breaking is more advanced than bond formation, giving C-22 a fractional positive charge that gains stability from the ethyl substituent. An extrapolation of this hypothesis can be applied to the formation of the other tetrahydrofuran ring in iso-lasalocid A. Thus, a C-

18(*R*),19(*R*),22(*R*),23(*R*)-diepoxide (**4**) under acidic conditions could lead by a concerted mechanism to iso-lasalocid A.

The general conclusions derived from the isolation of iso-lasalocid A from the *S. lasaliensis* fermentation were:

1. Epoxides such as **4** and **5** are likely precursors in the biosynthesis of lasalocid A and by extrapolation, other polyether antibiotics.
2. The terminal, tetrahydropyran ring of lasalocid A is the last to form of the three cyclic systems present in the antibiotic. According to Lynen (40), cyclization of 6-methylsalicylic acid follows immediately on completion of the polyketide chain, and, therefore, the first ring system to form in lasalocid is probably the aromatic chromophore.
3. Unlike all the pther polyether antibiotics, in the crystalline state iso-lasalocid A lacks the cyclic conformation referred to earlier as characteristic of the class. It was therefore proposed (Figure 6) that during the biosynthesis of lasalocid A, the precursor molecule **5** assumes a cyclic conformation, which is a crucial step in the formation of the tetrahydropyran ring. There is strong evidence (41) supporting the intramolecular catalytic properties of the carboxyl group (anomalous alkylation of the tertiary alcohol), and an intermediate, such as **5**, would be more subject to conformational control than the electronic (Markonikoff) factors, which favor formation of iso-lasalocid A.

Recent support for a polyene-polyepoxide biosynthetic route for all these antibiotics has come from Cane and his co-workers (42,43) in their further studies of monensin biosynthesis (44). Incorporations of [1-^{13}C, ^{18}O] substrates have established that O-1, O-2, and O-3 of monensin are derived from the carboxylate oxygens of propionate whereas O-5, O-6, and O-10 originate from the corresponding acetate oxygens (Figure 7). On the other hand, growth in the presence of $^{18}O_2$ has demonstrated that the remaining ether oxygens O-7, O-8, and O-9 are derived from molecular oxygen. These results have been explained by suggesting that the final steps in the biosynthesis of monensin involve the all-(*E*) triene **7**, which undergoes epoxidation at each double

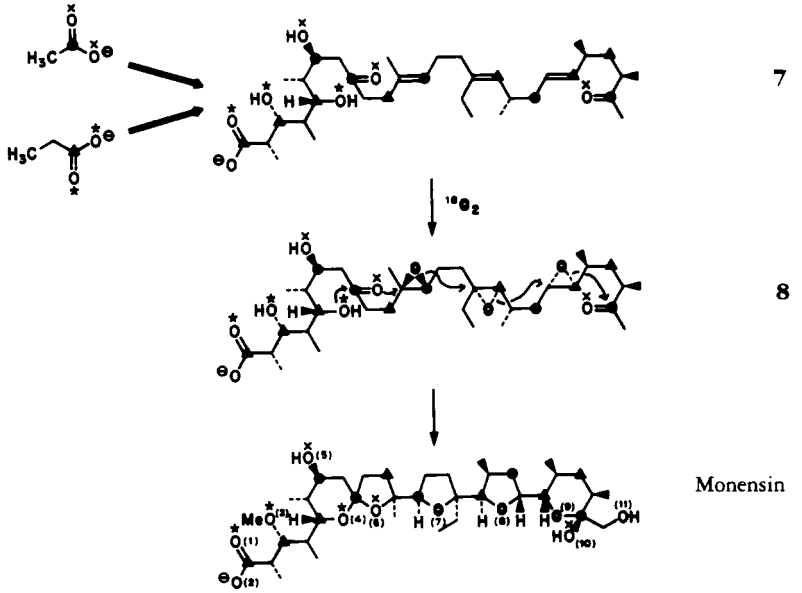


FIGURE 7. Proposed Final Steps in the Biosynthesis of Monensin Based on the Incorporation of Oxygen Atoms (42,43)

bond to form the (12*R*,13*R*,16*R*,17*R*,20*S*,21*S*)-triepoxide **8**. Attack of the C-5 hydroxyl of **8** at the C-9 carbonyl initiates a concerted cyclization to generate all five ether rings of monensin in a similar fashion to that proposed earlier for iso-lasalocid A.

The hypothetical triepoxide model for the biosynthesis of monensin has now been extended (35c) to account for an additional eighteen polyether antibiotics, all of which have the same four starting subunits in their biosynthesis, namely, acetate-propionate-propionate-acetate (APPA). Their structural and stereochemical regularity summarized in Figure 8 is therefore a reflection of the more fundamental regularity already evident in their putative triene precursors (Figure 7).

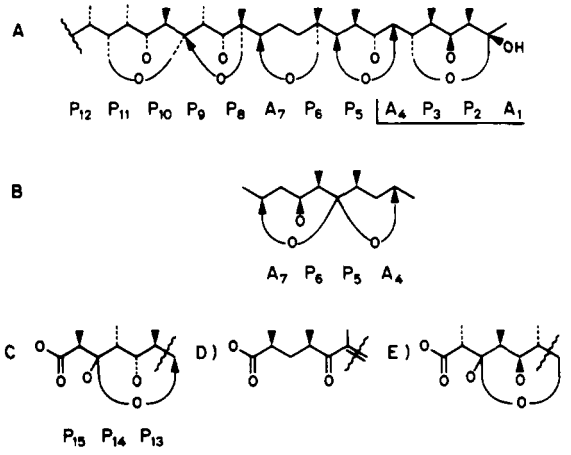


FIGURE 8. Stereochemical Model for the Eighteen APPA Group Polyethers for Subunits 1-12 (A) with a Dianemycin Variant (B) and for the Terminal Subunits 13-15, a Lonomycin Type Variant (C), a Dianemycin Type (D), and a Carriomycin Type (E).

In addition to the APPA polyethers and lasalocid, the polyene polyepoxide hypothesis can now be extended to all the compounds of this class in a unified stereochemical model of polyether antibiotic structure and biogenesis.

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