THE BIOSYNTHESIS OF MAGNAMYCIN, A MACROLIDE ANTIBIOTIC*

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The successful elucidation of the chemical architecture of the macrolide antibiotics has stimulated considerable interest in the biosynthesis of these novel compounds. Magnamycin is an interesting member of this class of compounds characterized by a large lactone ring to which is attached the disaccharide mycaminosylmycarose. The lactone ring consists of a 17 carbon backbone with a branching methyl group, a branching aldehyde group, three hydroxyl substituents, as well as a carbonyl and an epoxide ring (1). These features taken together present unique biochemical problems and it has been suggested by Woodward that the macrolide antibiotics may be formed from both acetate and propionate (1). In this communication we report the incorporation of both propionate and acetate residues into the lactone moiety of magnamycin.

Streptomyces Halstedii was grown on a rotatory shaker for 60 hours in a medium consisting of the following components: starch, yeast extract, casamino acids, and $CaCO_3$. The mycelia were then separated by centrifugation, resuspended in a medium of the above composition, and again placed on the shaker. Under these

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conditions the accumulation of magnamycin in the medium begins at 8 to 10 hours after resuspension and reaches a maximum at approximately 40 hours. The labelled compounds (24 mmoles per liter) were added two hours after the accumulation had started and the fermentation was terminated 20 hours later. The magnamycin formed was isolated essentially by the method of Tanner et al. (2). The degradation of magnamycin to carimbose and to the "C-12 acid" followed the general procedures worked out during the elucidation of the structure of magnamycin (3,4,5) (Fig. 1). All compounds were purified to constant melting point and to constant activity. The purity of the compounds was also established by infrared and ultraviolet spectra. Samples were counted at infinite thickness on stainless steel dishes and sufficient counts were taken to give a probable error of 5 percent. The results are expressed as counts per minute multiplied by the molecular weight of the compound and are summarized in the Table.

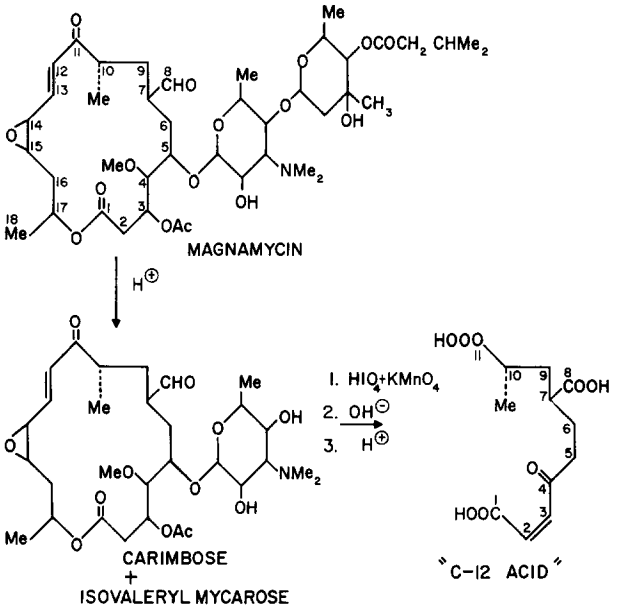

Fig 1: Scheme for the degradation of Magnamycin

Incorporation of Acetate-2-C^{14}, Propionate-2-C^{14} and Propionate-1-C^{14} into Magnamycin and its Degradation Products

Compound	Activity in the various experiments (c.p.m. x M.W.)		
	Acetate-2-C^{14}	Propionate-2-C^{14}	Propionate-1-C^{14}
Precursor	357 x 10^4	316 x 10^4	474 x 10^4
Magnamycin	293 x 10^4	156 x 10^4	265 x 10^4
Carimbose	265 x 10^4	151 x 10^4	258 x 10^4
"C-12 acid"	105 x 10^4	--	255 x 10^4
Fatty acids* (Lithium salts)	4060	878	258

* Figures represent counts per minute.

The fatty acids of the cells were also isolated as lithium salts in the various experiments and their activities are presented in the Table. From the results it is clear that acetate is efficiently incorporated into the fatty acids. The incorporation of propionate-2-C^{14} into fatty acids adduces evidence to the probable existence of the methylmalonyl CoA → succinate pathway in this organism. The small but yet significant activity from propionate-1-C^{14} would seem to suggest the occurrence of some fatty acids which incorporate propionate.

The incorporation of label from acetate-2-C^{14} into magnamycin is approximately twice that from propionate-1-C^{14} and propionate-2-C^{14}. The similarity in the percent incorporation of propionate-1-C^{14} and -2-C^{14} into magnamycin and carimbose suggests that

propionate is introduced as an intact three-carbon unit. The "C-12 acid" obtained on degradation of magnamycin derived from propionate-1-C^{14} has the same molar activity as the magnamycin (Column 3). This indicates that propionate is not incorporated to any significant extent into the sugars or isovalerate and also that the "C-12 acid" must contain the propionate residues. While this work was in progress, Grisebach and Achenbach (6) showed that the acetic acid obtained from the Kuhn Roth oxidation of the "C-12 acid" from magnamycin isolated from a CT_3-$C^{14}H_2$-COOH experiment had the same activity as the "C-12 acid". Thus our experiments are in agreement with the observations of Grisebach and Achenbach and it is reasonable to suggest that the "C-12 acid" contains only <u>one</u> propionate residue, the branching methyl group of magnamycin being derived from the methyl of propionate.

The degradation of the carimbose to the "C-12 acid" from the acetate-2-C^{14} experiment sheds light on the biosynthesis of the rest of the carbon skeleton of the lactone ring. The "C-12 acid" contains only 40 percent of the activity of carimbose. This would imply that the remainder of the activity (i.e. 60 percent of the activity of carimbose) must reside in carbon atoms 12 to 18 and in the acetyl group lost during the degradation. This indicates that acetate is incorporated into the "C-12 acid" as well as into ring carbons 12 to 18. The lactone ring consists of 17 carbon atoms, one branching methyl group, one aldehyde group and an O-acetyl group. If one assumes that the branching methyl group and carbons 10 and 9 of the lactone ring are derived from propionate, then one is left with 16 carbon atoms of the lactone ring (including the

aldehyde group) plus one O-acetyl group. If these 18 carbon atoms arise from the two carbon units of acetate, then a loss of 56 percent (i.e. 5/9 x 100) of the activity of carimbose* derived from acetate-2-C^{14} should occur during its degradation to the "C-12 acid". The actual figure obtained in our experiments is 60 percent. It is therefore tempting to suggest that the rest of the carbon atoms of the lactone ring may owe their origin to acetate.**

On the basis of our experimental findings as well as that of Grisebach and Achenbach (6) we would like to advance the following mode of formation of the lactone ring of magnamycin as a working hypothesis (Fig. 2). This scheme takes into account the oxygen functions as well as the position of the double bond observed in magnamycin. The reduction of a carboxyl to an aldehyde is not uncommon in biological reactions. However, the carbon-carbon condensation between C-6 and C-7 raises interesting questions. An altern-

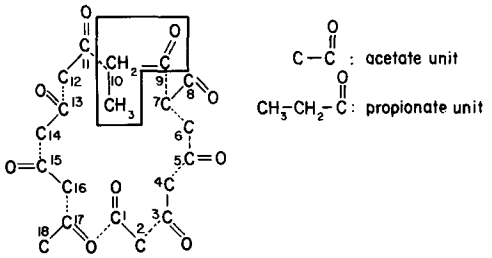

Fig 2: Hypothetical Scheme for the biosynthesis of the lactone ring of Magnamycin

* Since acetate and propionate are poorly incorporated into the mycarose moiety of magnamycin, it is reasonable to assume that mycaminose is also not derived from these precursors.

** Our current understanding of the biosynthesis of fatty acids suggests that acetate and propionate will probably participate as malonyl-CoA and methylmalonyl-CoA, respectively, in the biosynthesis of magnamycin.

ative possibility that carbon atoms 5,6,7 and 8 may be derived from a four carbon moiety which in turn is formed from acetate cannot be ruled out by our experiments at the present time. Investigations are in progress to answer these intriguing problems.

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