were combined and lyophilized to obtain PHA·HCl as a slightly grayish powder (9 mg, 88% yield).[14]

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Registry No. DOPA, 59-92-7; PHA, 13380-36-4; EHA, 92695-07-3; MTL, 14810-93-6; lincomycin A, 154-21-2; lincomycin B, 2520-24-3; tyrosine, 60-18-4; glucose, 50-99-7; methionine, 63-68-3; glycerol, 56-81-5; pyruvic acid, 127-17-3.

Biosynthesis of the Lincomycins. 2. Studies Using Stable Isotopes on the Biosynthesis of Methylthiolincosaminide Moiety of Lincomycin A

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Abstract: Lincomycin is an antibiotic produced by *Streptomyces lincolnensis* and consists of a unique aminooctose moiety, α-methylthiolincosaminide (MTL), attached via an amide linkage to a propylhygric acid unit. The biosynthesis of the MTL moiety of lincomycin has been investigated by using both specifically carbon-13 labeled substrates and uniformly carbon-13 labeled D-glucose. In the latter case ^{13}C–^{13}C spin coupling patterns in lincomycin and MTL were used to determine those carbon atoms from glucose that remained intact during their conversion to the antibiotic. By combination of the biosynthetic information obtained from the ^{13}C–^{13}C spin coupling patterns with that from those carbon atoms in MTL which were enriched from carbon-13 specifically labeled molecules, conclusions can be drawn about likely pathways and intermediates between glucose and MTL. The C_8-carbon skeleton of MTL is assembled through condensation of a pentose unit (C_5) and a C_3 unit. The C_5 unit can be assembled in two ways. Either it is derived from glucose via the hexose monophosphate shunt (HMPS) as an intact unit or it is assembled from condensation of a C_3 unit (glyceraldehyde 3-phosphate) with a C_2-unit donor such as sedoheptulose 7-phosphate (SH7P) via a transketolase reaction. The C_3 unit, which combines with the C_5 unit, is likely contributed from a suitable donor molecule such as SH7P via a transaldolase reaction. Dependent upon the origin of the C_3-unit donor, this unit may consist either of an intact C_3 unit or a C_2 unit combined with a C_1 unit. The octase produced from condensation of a C_5 unit and a C_3 unit can then be converted by unexceptional means to MTL.

Lincomycin (**1**) is a clinically important antibiotic produced by *Streptomyces lincolnensis* (Figure 1). Structurally, lincomycin is an aminooctose glycoside. The aglycone moiety, propylhygric acid, is biosynthetically derived from tyrosine most probably via 2,3-extradiol ring cleavage of DOPA, while methionine contributes the two additional C-1 units.[1] The aminooctose moiety, α-methylthiolincosaminide (**2**) (MTL), is a structurally unique compound, which has previously defied efforts to elucidate its biosynthetic origin.[2] Recently, a totally synthetic route to lincosamine (**3**) has been published.[3] The commercial importance of lincomycin has prompted an attempt to improve this fermentation through genetic approaches. However, these efforts have so far been hampered by insufficient information on the biosynthetic pathway and intermediates that lead from glucose to MTL. The availability of D-($^{13}C_6$)glucose and the ability to analyze complex ^{13}C–^{13}C spin coupling patterns prompted us to attempt to use this substrate as a means to gain insight into how this C_8 unit was assembled from glucose. The existence of a fermentation medium in which glucose could be used as a sole carbon source was also important, so that sufficient enrichment could be achieved to observe the complex coupling patterns. While this work was in progress, the power of this method has been illustrated in studies using D-($^{13}C_6$)glucose in combination with analysis of ^{13}C–^{13}C spin coupling patterns in the antibiotics pactamycin,[4] streptonigrin,[5] geldanamycin,[6] and naphridinomycin.[7] The results described in this manuscript have been presented in preliminary form.[8]

Results and Discussion

The strategy behind our approach to elucidating the manner in which D-($^{13}C_6$)glucose might provide the precursors of the C_8 unit in lincomycin was to first determine the patterns of connectivity of units derived from glucose in MTL. Since glucose served as the sole carbon source during the antibiotic production phase, the D-($^{13}C_6$)glucose was diluted 1 in 10 with nonisotopically labeled glucose prior to addition to the fermentation medium. After isolation of the antibiotic from the fermentation broth, either the parent antibiotic or MTL, obtained by alkaline hydrolysis of

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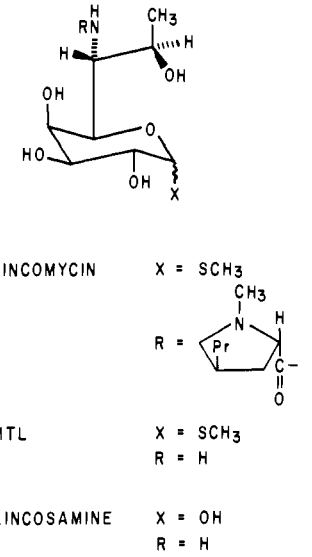

Figure 1. Structures of lincomycin, MTL, and lincosamine.

lincomycin, was subjected to ^{13}C NMR analysis. Those carbon atoms remaining connected through the biosynthetic process are readily detected by their coupling patterns. For example, an intact C_3 unit should be recognizable by three sets of signals. The two external carbon atoms should each show a doublet around the noncoupled singlet resonance signal. The central carbon resonance should show a doublet of doublets (or a triplet when the coupling constants are identical) on each side of the noncoupled singlet resonance signal. Similarly, a four- or five-carbon unit will consist of exterior carbon atoms showing a doublet around a singlet and interior carbons showing a doublet of doublets around a singlet. More complex patterns will result from situations where a constituent C_3, C_4, or C_5 unit can in some cases be derived from smaller units. Identification of the *largest fragments* that could be incorporated into the C_8 unit was of utmost importance, since this presumably would provide information on the most direct pathway to form the aminooctose moiety of lincomycin. Smaller subunits of these larger fragments would presumably arise via more circuitous pathways.

Presuming that we were able to identify the carbon atom size of the constituent fragments from the experiment with D-($^{13}C_6$)glucose, we would still lack evidence on the identity, orientation within C_n units, and origin of the precursors. Consequently, we also designed experiments with carbon-13 specifically enriched glucose and C_3 units. Although the results from these later experiments alone would be unlikely to yield definitive information on the probable identity of precursors of the C_8 unit, when used in combination with the ^{13}C–^{13}C spin coupling data from the D-($^{13}C_6$)glucose experiment they should provide firmer conclusions.

Analysis of the MTL and Lincomycin Carbon-13 NMR Spin Coupling Patterns from an Experiment with D-($^{13}C_6$)Glucose. D-($^{13}C_6$)Glucose (98% enriched) diluted 1 in 10 with nonisotopically labeled glucose was fed to flasks of *S. lincolnensis* producing lincomycin. Following isolation of the ^{13}C-enriched lincomycin from the fermentation broth, its ^{13}C NMR was recorded. This sample was then hydrolyzed to MTL, and a second ^{13}C NMR was obtained. ^{13}C NMR analysis and the spectra are presented in Figure 2A,C. The carbon-13 NMR assignments for MTL were made primarily on the basis of the previously assigned spectra of lincomycin A[9] and by using specific ^{13}C–^{1}H decoupling for each carbon. Also included in Table I is the multiplicity observed for ^{13}C–^{13}C spin coupling patterns and the coupling constants. While the multiplicity of carbon atoms 1–6 and 8 could be clearly observed in the ^{13}C NMR spectra of MTL (see Figure 2A,B). However, since the complexity for carbon atom 7 was considerably reduced in the lincomycin spectrum, this spectrum was used for the analysis of this resonance signal (Figure 2C).

The multiplicity observed in Figure 2A–C and tabulated in Table I show that carbon atoms 1, 3, 4, and 8 each shows a doublet

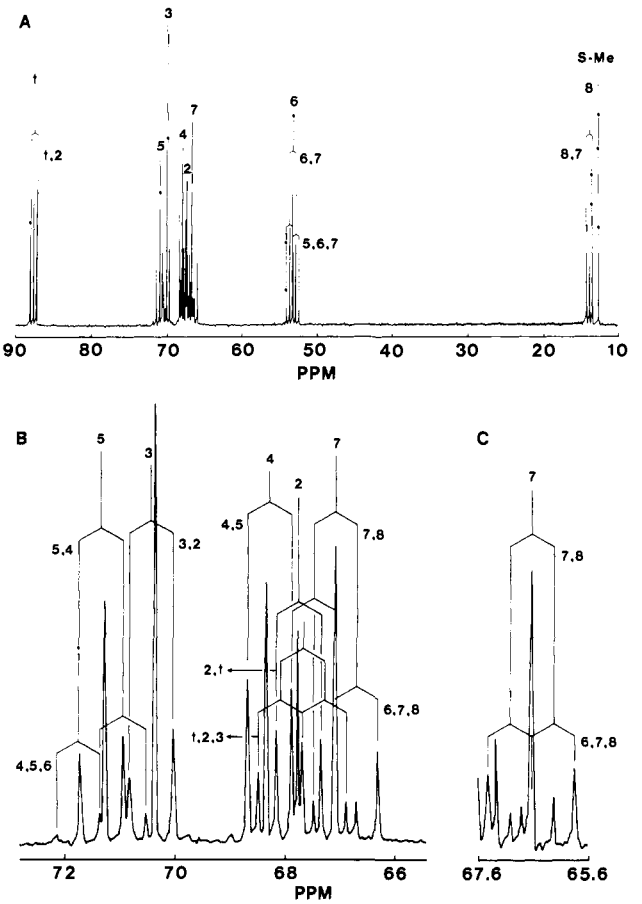

Figure 2. ^{13}C NMR spectrum of MTL derived from an experiment with D-($^{13}C_6$)glucose (A). ^{13}C NMR spectrum of expanded regions of carbon atoms 2–5 and 7 for MTL (B). ^{13}C NMR spectrum of expanded region of carbon atom 7 in lincomycin (C).

Table I. ^{13}C NMR[a] of MTL from an Experiment in Which D-($^{13}C_6$)Glucose[a] Was Fed to *S. lincolnensis*

carbon	chemical[b] shift	method of assignment	signal pattern[d] and rel contributn, %	J_{CC}, Hz
1	87.97	9[c]	d, 68 s, 32	$J_{1,2}$ = 40.3
2	67.77	9	dd, 32 d, 34	$J_{1,2,3}$ = 40.1 $J_{2,1/3}$ = 40.8
3	70.37	^{1}H–^{13}C decoupling	d, 31 s, 69	$J_{3,2/4}$ = 40.3
4	68.34	9	d, 46 s, 54	$J_{4,5/3}$ = 40.0
5	71.30	^{1}H–^{13}C decoupling	dd, 16 d, 38 s, 16	$J_{4,5,6}$ = 40.0 $J_{5,3/6}$ = 39.6
6	53.60	9	dd, 19 d, 47 s, 34	$J_{5,6,7}$ = 41.6 $J_{6,7/5}$ = 39.3
7	67.09	9	dd, 66 d, 17 s, 17	$J_{6,7,8}$ = 39.5 $J_{7,8/6}$ = 39.0
8	14.21	9	d, 80 s, 20	$J_{8,7}$ = 38.7
SCH_3	12.98	9	s, 100	

[a] See ref 1. [b] All chemical shifts are given in parts per million relative to tetramethylsilane (Me_4Si). 1,4-Dioxane was used as the internal standard, the shifts were converted to Me_4Si by the relationship $\delta_C(CH_3)_4Si = \delta(p\text{-dioxane}) + 67.4$ ppm. [c] From ref 9. [d] s = singlet, d = doublet, dd = doublet of doublets (see text).

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Table II. Enrichments of Carbon-13 Resonance Signals of Lincomycin from Experiments with Various Carbon-13 Enriched Substrates, Relative to Nonisotopically Labeled Lincomycin Run under Similar Conditions

precursor fed[a]	enrichment of precursor	carbon atom enriched and enrichment factor[b]								
		1	2	3	4	5	6	7	8	SCH_3
D-(1-^{13}C)glucose	90.7	3.16	1.23	1.45	1.63	1.34	1.31	1.32	1.57	1.55
D-(6-^{13}C)glucose	91.9	1.32	1.18	1.22	1.16	1.07	1.24	1.14	3.00	1.88
(1-^{13}C)pyruvate	90.0	0.99	0.86	0.93	0.95	0.94	1.32	0.90	0.96	0.96
(2-^{13}C)glycerol	90.0	0.94	1.12	0.97	1.00	1.04	0.87	3.08	1.01	1.84
(1,3-$^{13}C_2$)glycerol	90.0	1.39	1.03	1.17	1.20	0.95	2.38	1.00	2.33	2.17

[a] Glucose was fed at 0 h at a level of 0.3 g/flask. Pyruvate was fed at 72 h at a level of 0.3 g/flask, while glycerol was fed at 72 h but at 0.1 g/flask. [b] Defined as the ratio of signal intensity in the ^{13}C-enriched lincomycin to that in unenriched lincomycin.

(d) as well as a singlet (s). The absence of a doublet of doublets (dd) for these signals indicates that these carbon atoms are at external positions of an intact C_n ($n \geq 2$) units; i.e., a C_3 unit is evident between carbon atoms 1, and 3 and a C_5 unit is evident between carbon atoms 4 and 8. Conversely, carbon atoms 2 and 5–7 show a "dd" in addition to a "d" and a "s" signal. Therefore, each of these must be internal carbons within carbon atom units at least three carbons in length.

An analysis of the relative percentages of each of the constituent signals within the multiplet, i.e., percentage of s, d, and dd species, provides further information on the contributions of the smaller subunits found within the previously identified C_3 and C_5 units. For carbon atoms 1–3, the percentage of dd for C-2 (32%) is a direct measure of the relative amount of C_3 vs. C_2 and C_1 species, which, together, make up the total amount of carbon species present in these carbon atoms of the antibiotic. Furthermore, since the d at C-3 (31%) occurs at almost the same percentage as the dd at C-2 (32%), then the d at C-2 (34%) must be due solely to coupling with C-1. In accord with this conclusion, the d at C-1 (68%) is almost equal to the addition of the d (34%) and dd (32%) at C-2. Therefore, the carbon atoms between C-1 and C-3 are derived from a C_3 unit (32%), a C_2 unit (C-1 and C-2) (34%), and C_1 units due to carbon atoms derived from both carbon-13 in the natural abundance D-glucose and randomized label from D-($^{13}C_6$)glucose.

Analysis of carbon atoms 4–8 leads to conclusions on how this unit may be derived. The dd signals for carbon atoms 5–7 provide compelling evidence for a C_5 unit. While the dd for C-5 (16%) and C-6 (19%) carry approximately equal percentage values, the dd for C-7 (66%) occurs at a much higher value. On the basis of these data alone, we can conclude that a C_5 unit contributes at about a level of 16–19% to this set of carbons. The percentage of dd for C-7 in excess of that needed for the C_5 unit (66 − 16 = 50%) must be due to a C_3 unit comprised of C-6, C-7, and C-8. This is supported by ds at C-6 (47%) and C-8 (80%). The excess d percentage at C-8 (80 − (50 + 16) = 14%) is then due to a C_2 unit (C-7, C-8), also evidenced by a doublet at C-7 (17%). Since the d and dd at C-7 can be completely accounted for through the C_3 unit (C-6, C-7, and C-8) and the C_2 unit (C-7 and C-8), evidence for a C_2 unit between C-6 and C-7 is lacking. The d at C-4 (46%) is more than fully accounted for by contributions to the C_5 unit (16–19%) and a C_2 unit (C-4 and C-5) (38%). Contributions of C_1 units to carbon atoms between C-4 and C-8 vary from a high of about 50% (C-4 and C-5) to a low of about 20% (C-7 and C-8) and arise due to randomized label from D-($^{13}C_6$)glucose and from carbon-13 in natural abundance D-glucose. A diagrammatic representation of the relative contributions of C_1–C_5 units to the MTL moiety of lincomycin is shown in Figure 3.

Carbon-13 Enrichments in MTL Resulting from Feeding Experiments with Specifically Labeled Substrates and Identification of Probable Precursors of MTL. The ^{13}C–^{13}C spin coupling data in the MTL isolated from an experiment in which D-($^{13}C_6$)glucose had been added to the fermentation broth had provided conclusive data on the pattern of carbon units incorporated into this C_8 sugar. However, evidence on the identity or orientation of probable precursors that constitute these C_2, C_3, or C_5 units was lacking. To provide biosynthetic information, which would identify likely candidates for the constituent subunits of this aminooctose, experiments with specifically enriched carbon-13 labeled substrates were conducted. Carbon atoms in MTL found to be enriched with carbon-13 relative to the corresponding atoms in a nonisotopically enriched sample of MTL are shown in Table II.

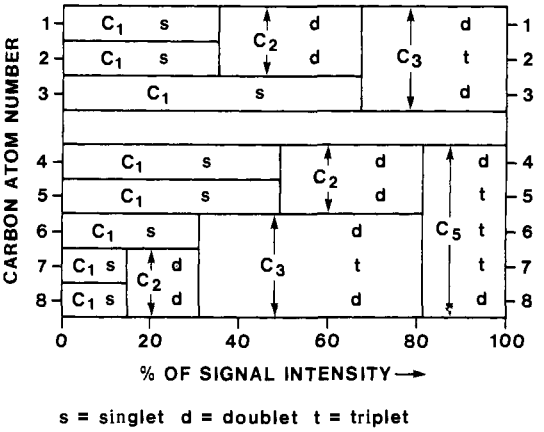

Figure 3. Diagramatic representation of the relative contributions of various subunits that may combine to ultimately produce the C_8 unit of MTL.

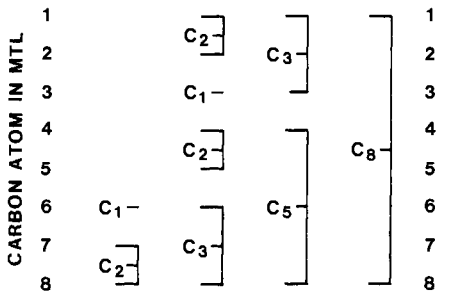

Figure 4. Stepwise condensation of subunits that lead to the oculose precursor of MTL. The relative contributions of each signal have an error factor of ±10% *of the calculated value.* This results from the concentration dependency of the chemical shift values of resonance signals that are in close proximity. These concentration dependent changes cause errors in signal heights which consequently affect the calculated values of relative contributions of various pathways to the octose.

The aminooctose (C_8 unit) can be formed from condensation of a C_5 unit (carbon atoms 4–8) and a C_3 unit (carbon atoms 1–3). Furthermore, the C_5 unit can be either supplied intact or from condensation of a C_3 unit (carbon atoms 6–8) and a C_2 unit (carbon atoms 4–5). Finally, the two C_3 units (carbon atoms 1–3 and carbon atoms 6–8) may each be contributed as an intact C_3 unit or by condensation of a C_2 unit (carbon atoms 1–2 and carbon atoms 7–8) with a C_1 unit (carbon 3 and carbon 6, respectively). This is shown dramatically in Figure 4. Significantly, the ratios of the relative percentages of the C_2 units to the C_1 units, which comprise the two C_3 units, is quite different for the C_3 unit found in carbon atoms (1–3 (ratio 1:1) to the C_3 unit found in carbon atoms 6–8 ratio 1:2). This suggests that these two C_3 units are derived from different precursors.

Several lines of evidence point toward glyceraldehyde 3-phosphate (G-3-P) being the source of the C_3 unit comprising carbon atoms 6–8. First, (1-^{13}C)pyruvate, (2-^{13}C)glycerol, and (1,3-$^{13}C_2$)glycerol all enrich one or more of the carbon atoms between C-6 and C-8 (see Table II). The specific enrichment of

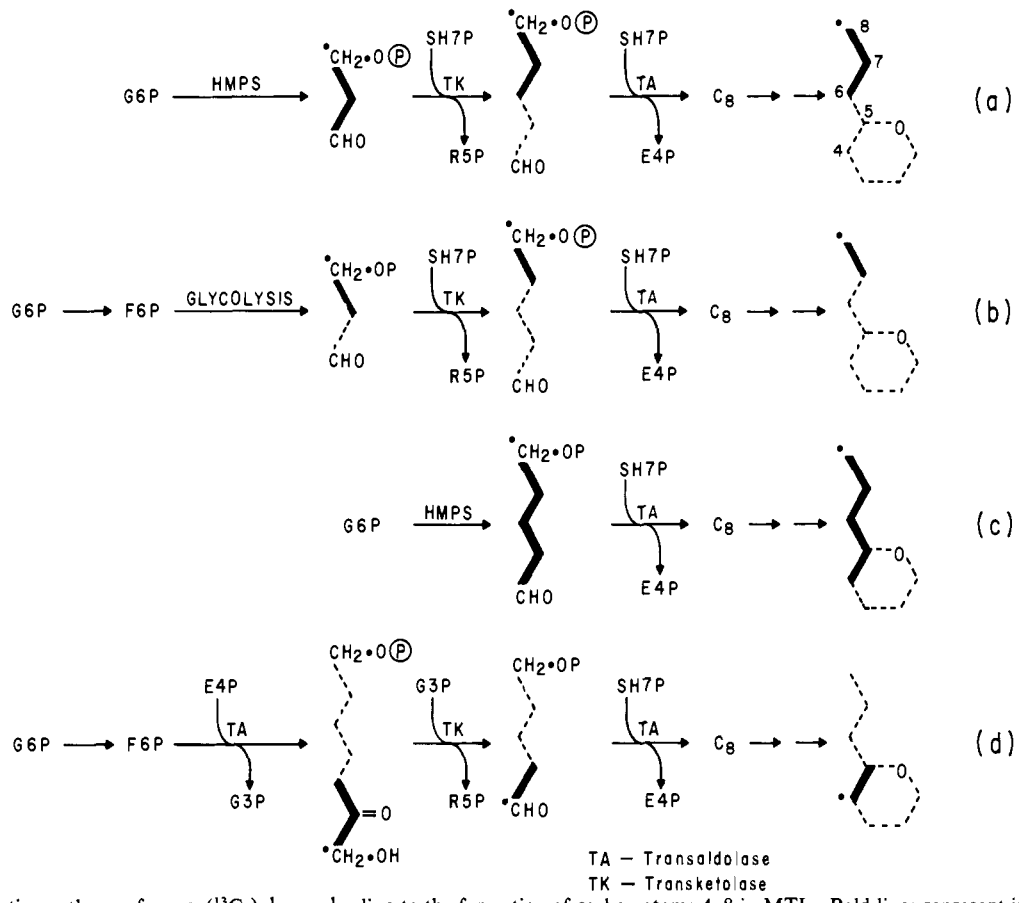

Figure 5. Alternative pathways from D-($^{13}C_6$)glucose leading to the formation of carbon atoms 4–8 in MTL. Bold lines represent intact carbon atom units retaining ^{13}C–^{13}C spin coupling.

C-6 of MTL from (1-^{13}C)pyruvate is in accord with the expected conversion of pyruvate to G-3-P. Similarly, specific enrichments of C-7 of MTL from (2-^{13}C)glycerol and C-6 and C-8 of MTL from (1,3-$^{13}C_2$)glycerol are also as expected. Second, while D-(6-^{13}C)glucose significantly enriches C-8 of MTL, D-(1-^{13}C)glucose enriches this carbon of MTL to a much lower extent. Glucose is converted by glycolysis to the trioses, G-3-P and dihydroxyacetone phosphate, which, because they are interconvertible by triose isomerase, equilibrate the label between C-1 and C-6 of glucose. Therefore, if glycolysis was the sole source of G-3-P incorporated into this C_3 unit, we would expect equivalent enrichments at C-8 of MTL from D-(1-^{13}C)- and D(6-^{13}C)glucose, which is clearly not the case. To explain the greater enrichment of C-8 by D-(6-^{13}C)glucose, we suggest that the hexose monophosphate shunt (HMPS) also contributes significantly to the pool of G-3-P which may serve as a precursor of carbon atoms 6–8 in MTL. Unlike glycolysis, the HMPS does not equilibrate C-1 of glucose with C-6 and can generate G-3-P exclusively from C-4–C-6 of glucose via the transketolase reaction. Since this series of reactions involving the HMPS provides G-3-P in which C-3 is enriched from C-6 of glucose, this would help explain the greater enrichment of C-8 of MTL from D-(6-^{13}C)glucose rather than from D-(1-^{13}C)glucose (Figure 5, pathway a). Another factor contributing to the observed enrichment pattern is the derivation of a MTL precursor pentose from glucose by the HMPS-mediated elimination of carbon 1 of glucose.

As shown in Figure 3, the C_3 unit comprising carbon atoms 6–8 in MTL can also be derived through a minor pathway in which a C_2 unit (C-7 and C-8) and a C_1 unit are constituents. A combination of steps in the HMPS and glycolysis can explain this pattern of subunits for the C_3 unit. The HMPS can generate D-fructose 6-phosphate (F-6-P) in which carbon atoms 1 and 2 are retained as a C_2 unit. F-6-P can then enter into glycolysis to produce C-3-P in which carbon atoms 2 and 3 remain intact and become the C_2 unit comprising carbon atoms 7 and 8 of MTL (see Figure 5, pathway b).

The information on the probable origin of the C_3-unit comprising carbon atoms 6–8 in MTL provides a starting point to track down the probable origin of the C_5 unit comprising carbon atoms 4–8 of MTL. This C_5 unit can either be derived directly or be formed by condensation of a C_3 unit (carbon atoms 6–8) and a C_2 unit (carbon atoms 4–5). The HMPS produces three pentoses (D-xylulose 5-phosphate (X-5-P), D-ribose 5-phosphate, and D-ribulose 5-phosphate), each of which can be formed as intact C_5 units derived from glucose prior to incorporation into MTL (see Figure 5, pathway c). Furthermore, molecules of X-5-P can also be generated in which carbon atoms C-1 and C-2 exist as a C_2 unit and carbon atoms C-3–C-5 exist as a C_3 unit, derived from G-3-P. Additional support that a molecule such as X-5-P can provide carbon atoms 4–8 of MTL is that the expected orientation of G-3-P in X-5-P is such that C-6 of glucose should enrich C-3 of G-3-P and subsequently C-8 of MTL, which is found in practice. The remaining carbon atoms (1 and 2) in X-5-P molecules, which are not derived from G-3-P, are transferred by a transketolase reaction from sedoheptulose 7-phosphate (SH7P). This C_2 unit donated from SH7P is originally derived from carbon atoms 1 and 2 of F-6-P, which are in turn derived from carbon atoms 1 and 2 of glucose. Consequently, carbon 4 of MTL should be more significantly labeled from glucose labeled in the 1 rather than in the 6 position, which also is found in practice (see Figure 5, pathway d).

The remaining C_3 unit comprising carbon atoms 1–3 of MTL is not labeled from pyruvate or glycerol but is more heavily enriched at the C-1 position from D-(1-^{13}C)glucose than from D-(6-^{13}C)glucose, which is opposite to the observed enrichment pattern at C-8 of MTL. In addition to an intact C_3 unit, the carbon atoms comprising C-1–C-3 of MLT may also be contributed as a C_2 unit (C-1 and C-2 of MTL) and a C_1 unit (C-3 of MTL). We therefore searched for a C_3-donor molecule that would fulfill all these requirements. SH7P meets all the criteria. A transaldolase reaction can transfer a C_3 unit (C-1–C-3 of SH7P to a pentose, such as X-5-P, to produce the octulose of C_8 unit

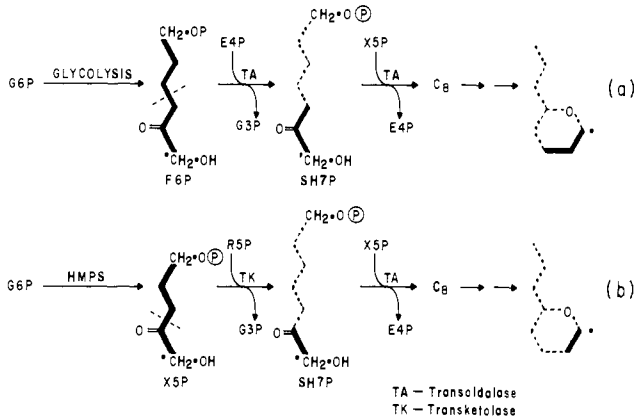

Figure 6. Alternative pathways from D-($^{13}C_6$)glucose leading to formation of carbon atoms 1–3 in MTL. Bold lines represent intact carbon atom unit retaining ^{13}C–^{13}C spin coupling.

precursor of MTL. SH7P may be formed either by a transaldolase reaction, which involves transfer of a C_3 unit from an intact C_6 unit (F-6-P) to D-erythrose 4-phosphate, or by a transketolase reaction which involves transfer of C_2 unit from an intact C_5 (X-5-P) to R-5-P (Figure 6, pathways a and b). In order that an intact C_3 unit can be transformed from F-6-P, the F-6-P must be generated via the glycolytic pathway rather than the HMPS. If equal amounts of carbon traveled through these alternative pathways, then the SH7P formed would consist of equal amounts of three-carbon atom donor molecules, containing either an intact C_3 unit (Figure 6, pathway a) or a C_2 unit and a C_1 unit (Figure 6, pathway b). This would lead to the ^{13}C–^{13}C spin coupling pattern shown in MTL. Furthermore, the enrichment in C-1 of MTL from D-(1-^{13}C)glucose can also be rationalized by assuming transaldolase formation of the three-carbon atom donor molecule (SH7P) from F-6-P and E-4-P. Providing the F-6-P utilized in this reaction is generated through the glycolytic pathway, then C-1 of SH7P is derived from C-1 of glucose. Transfer of the C_3 unit from SH7P to the C_5 unit to form the C_8 unit would result in the established enrichment of C-1 of MTL from D-(1-^{13}C)-glucose.

Conclusions

Using a combination of results, obtained from analysis of ^{13}C–^{13}C spin coupling patterns in MTL and lincomycin derived from a biosynthetic experiment with D-($^{13}C_6$)glucose, and the specific carbon-13 enrichments in MTL produced from specifically labeled substrates, we have been able to predict the probable biosynthetic intermediates and pathways, leading to a C_8 unit that can be converted into MTL by unexceptional reactions. The intermediates and pathways involved up to the production of an octulose are those associated with the HMPS and glycolysis.

In addition to the importance of these results in providing the first real evidence of how MTL is biosynthetically produced, this study further illustrates how powerful a tool ^{13}C–^{13}C spin coupling patterns can be in solving complex biosynthetic problems. It is doubtful that more traditional approaches to solving biosynthetic problems, such as those using radioactive or even single-labeled stable isotope precursors, would have revealed much useful information. The ambiguities produced as a result of alternative pathways leading to the same subunits would almost certainly have prevented any firm conclusions from being made.

The results presented in this paper and the previous one in this issue[1] provide considerable insight into the conversion of glucose to a C_8 unit and a C_2-proline unit. Whereas the pathway leading to PHA is common also to the C_3-proline units found in anthramycin and sibiromycin,[10] the C_8 unit of MTL appears so far to be unique to the lincomycin antibiotics. The terminal steps involved in the conversion of the C_8 unit to MTL and the conversion of the C_2-proline unit to PHA are still unclear. Conversion of the octulose to MTL would presumably involve isomerization

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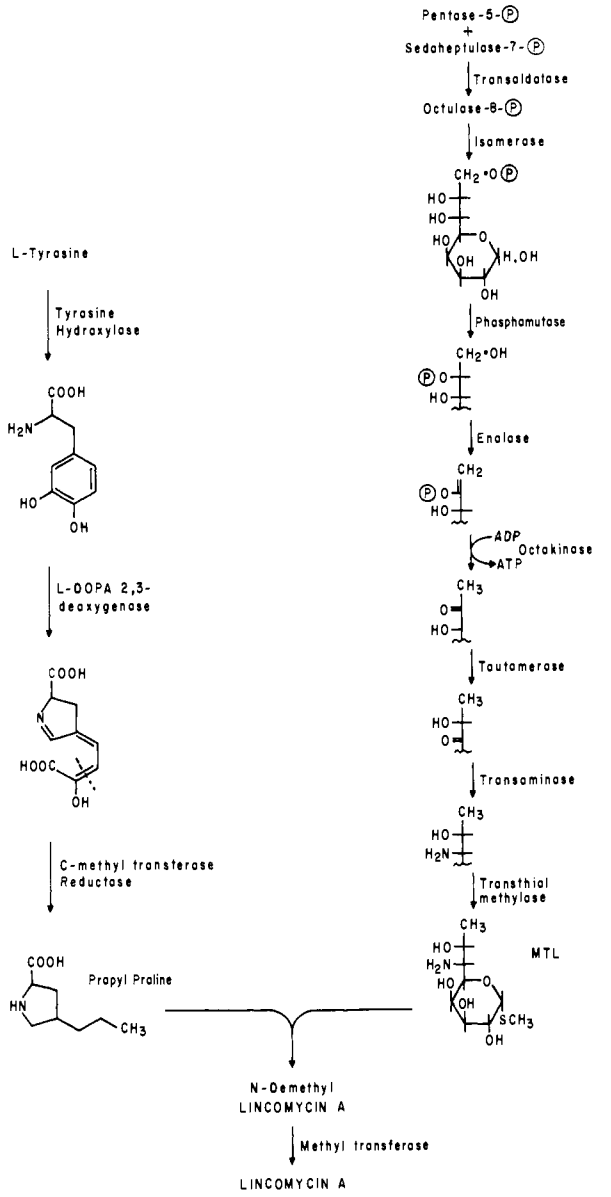

Figure 7. Proposed biosynthetic pathway leading to lincomycin A.

of the octulose to an octose, dephosphorylation and reduction of C-8, thiomethylation of C-1, and transamination of a suitable 6-ketooctose. The final steps to produce lincomycin involve condensation of MTL with propylproline and methylation of the adduct (unpublished results). This is shown in Figure 7.

Future studies will involve experiments to implicate more directly intermediates in the biosynthetic pathway to MTL. Efforts are already under way to apply the equally powerful techniques of microbial genetics to solve some of these problems.

Experimental Section

Fermentation and Isolation of Lincomycin. The fermentation conditions and procedures for isolation of lincomycin were as described previously.[1] The conditions for specific feeding experiments are listed in Table II.

NMR Experiments. ^{13}C NMR experiments were performed on a Varian XL-200 FT-NMR spectrometer as described in the previous paper.[1]

Isolation of MTL. Following the base hydrolysis of lincomycin (see previous paper[1]) MTL was isolated as follows. A mini anion-exchange resin column was prepared from 1 g of Bio-Rad AG1X2, Cl^- form, 200–400 mesh. It was washed with 1 N NaOH until free of chloride and then equilibrated with 0.01 N NaOH. The reaction solution was loaded on the column, and the column was eluted with 0.01 N NaOH. Twelve fractions of 1 mL each were collected and examined on TLC (silica gel, MeOH–$CHCl_3$–NH_4OH, 35:61:4 v/v). Fractions having MTL were combined and the pH was adjusted to 6.0.

Another column was prepared using 20.0 g of ion-retardation resin (Bio Rad AG11A8 50–100 mesh) and was thoroughly washed with distilled water until free of chloride. The combined MTL fractions (pH 6.0) were passed through this column and eluted with H_2O. Twenty fractions of 4 mL each were collected and examined on TLC. The fractions having MTL were combined and lyophilized to obtain MTL as a white powder (9–11 mg).

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Registry No. 1, 154-21-2; **2**, 14810-93-6; G-3-P, 142-10-9; glucose, 50-99-7; pyruvic acid, 127-17-3; glycerol, 56-81-5.

Hieber and Bruck Revisited. The X-ray Molecular Structure and Properties of the Anionic Precursor to Hieber's Nickel(IV) Dithiolate, [K(2,2,2-crypt)][Ni($S_2CC_6H_5$)$_3$]

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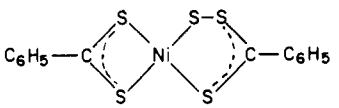
Abstract: The olive-green precursor to the sulfur-rich Ni($S_2CC_6H_5$)($S_3CC_6H_5$), originally thought to be [$Ni^{IV}S(S_2CC_6H_5)_2]_2$, has been identified as [Ni($S_2CC_6H_5$)$_3$]$^-$. This paramagnetic, six-coordinate anion, suggested by Hieber to be [$NiS_2(S_2CC_6H_5)_2]^{4-}$, readily reacts with acid to produce the sulfur-rich product. With use of [K(2,2,2-crypt)]$^+$ as a cation it has been possible to obtain the structure of the anion. For [K(2,2,2-crypt)][Ni($S_2CC_6H_5$)$_3$], a = 15.319 (4) Å, b = 18.277 (6) Å, c = 16.450 (6) Å, β = 106.00 (2)°, Z = 4. With use of 4659 Cu Kα data, the structure of the anion has been deduced to contain a distorted NiS_6 octahedron with Ni–S ≈ 2.4 Å.

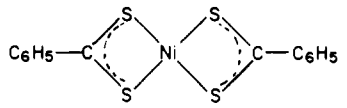
While studying synthetic methods for $Ni(CO)_4$, Hieber and Bruck published[1] evidence for the formation of a nickel complex containing only sulfur–metal coordination. This species, originally thought to contain Ni(IV), is known[2] to be I, the "sulfur-rich" Ni($S_3CC_6H_5$)($S_2CC_6H_5$). However, the olive-green anionic precursor to this complex has defied formulation to date, although Hieber[1] thought it to be $[NiS_2(S_2CC_6H_5)_2]^{4-}$.



I

Since [Ni(S_2CNR_2)$_3$]$^+$, R = alkyl, contains $Ni^{IV}S_6$ coordination[3] and, with R = CH_3 or C_2H_5, this cation rapidly decomposes in solution to oxidized ligand species and Ni(II) complexes, we decided to reexamine the related Hieber chemistry. In particular we wanted to determine whether a $Ni^{IV}S_6$ species might indeed be present, at least as an intermediate, preceding the formation of the sulfur-rich product. Our interest was further picqued by the recent observation[4] of Ni(III) species containing sulfur coordination in a CO fixing bacterial system.

Although the purple (violet) species Hieber thought to contain Ni(IV), [$SNi^{IV}(S_2CC_6H_5)_2]_2$, has been shown[2] to be I, its blue, "sulfur-poor" precursor, II, is known[1] to react with CO in benzene containing NaSH and C_2H_5OH to give $Ni(CO)_4$. Early attempts to isolate and characterize the olive-green species that lead to I failed although evidence could be obtained in solution that a species of this color was formed. Attempts to prepare crystalline products with tetraalkylammonium salts, R = methyl (Me) or *n*-butyl (Bu), also were unsuccessful, although powders were isolated. With use of 2,2,2-Crypt to encapsulate the potassium ion, a green, crystalline product was obtained. The structure of this product is reported here along with the properties of the anion.



II

Experimental Section

Materials. The reagents and solvents used in this work were purchased from commercial sources and used without further purification. The 2,2,2-Crypt (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane), $N(C_2H_4OC_2H_4OC_2H_4)_3N$, was obtained from Fluka. All liquid reagents were of research grade and were dried over 4 Å molecular sieves.

Sodium and potassium hydrogen sulfides were prepared by reacting either sodium or potassium ethoxide with H_2S gas according to procedures described in the literature.[5]

Synthesis of 1,1-Dithiolato Nickel(II) complexes. The perthiobenzoato(dithiobenzoato)nickel(II), $Ni(dtb)_2S$, and bis(dithiobenzoato)nickel(II), $Ni(dtb)_2$, were prepared and purified according to procedures previously reported.[2]

The following reactions were carried out under an atmosphere of dry nitrogen. The solvents were carefully degassed and standard Schlenk ware was used. The reactions involving 2,2,2-Crypt were carried out by using a modified procedure described by Darensbourg and co-workers[6]

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