Biosynthesis of the Lincomycins. 1. Studies Using Stable Isotopes on the Biosynthesis of the Propyl- and Ethyl-L-hygric Acid Moieties of Lincomycins A and B

Nanda M. Brahme,[†] Jose E. Gonzalez,*[‡] Jim P. Rolls,*[‡] Ed J. Hessler,[‡] Steve Mizsak,[‡] and Laurence H. Hurley*[†]

Contribution from Fermentation Research and Development, The Upjohn Company, Kalamazoo, Michigan 49001, and The Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712. *Received May 14, 1984*

Abstract: The biosynthesis of the propyl-L-hygric acid and ethyl-L-hygric acid moieties of lincomycins A and B has been examined by using deuterated and carbon-13 labeled precursors in combination with carbon-13 NMR and mass spectral analysis. The results, using specifically deuterated tyrosine, DOPA, and methionine, demonstrate that tyrosine is converted via DOPA to an intermediate that undergoes aromatic ring cleavage most probably via a 2,3-extradiol cleavage reaction. An experiment using D-($^{13}C_6$)glucose in combination with analysis of the ^{13}C–^{13}C spin-coupling patterns in lincomycin A and propyl-L-hygric acid permits the determination of those carbon atoms that remain together during the biosynthesis of lincomycin A. Glucose is converted via glycolysis and the hexose monophosphate shunt to phosphoenolpyruvate and erythrose 4-phosphate, respectively, which are in turn converted via the shikimic acid pathway to tyrosine and hence into DOPA. The subsequent reactions after DOPA are consistent with the 2,3-extradiol cleavage pathway and an addition of a C-1 unit from methionine to give rise to the terminal methyl group of the propyl side chain. These results are now consistent with those obtained for the C_2- and C_3-proline moieties found in anthramycin, tomaymycin, and sibiromycin that are also biosynthetically derived from tyrosine.

The antibiotic-producing strain *Streptomyces lincolnensis* produces two closely related antibiotics, lincomycins A and B (Figure 1). While both antibiotics carry an identical unusual sugar moiety, namely, α-methylthiolincosaminide (MTL), they differ in the amino acid moiety; lincomycin A having a propyl-L-hygric acid (PHA) moiety (C_3-proline) while lincomycin B has an ethyl-L-hygric acid (EHA) moiety (C_2-proline). Earlier biosynthetic studies on the lincomycins published by scientists at The Upjohn Co. in the 1960s and early 1970s[1-3] provided the first biosynthetic example of a C_2- or C_3-proline unit formed from L-tyrosine. However, some inconsistencies emerged between this work and subsequent work on the origin of the biosynthetically similar C_2- and C_3-proline units found in the pyrrolo[1,4]-benzodiazepine antibiotics, anthramycin,[4] tomaymycin,[5] and sibiromycin.[6] In recent reviews[7,8] one of us had postulated a common biosynthetic pathway arising from tyrosine leading to a common branch-point compound, which can be converted by similar mechanisms to either a C_2-proline unit (lincomycin B, tomaymycin) or a C_3-proline unit (lincomycin A, anthramycin and sibiromycin). The results of experiments described in this article provide firm evidence that discriminates between the earlier postulate[1] and the more recent unified postulated[7,8] that encompasses all known representatives of the C_2- and C_3-proline type of biosynthetic unit. The accompanying paper[9] provides the first insight into how the MTL unit of the lincomycins is assembled from glucose. Some of the results described in this paper were previously presented as an abstract.[10]

Results and Discussion

The alternative postulates for the biosynthetic pathways leading from tyrosine to the C_2 and C_3-proline units of the lincomycins are shown in Figures 2 and 3. Whereas the original Upjohn postulate proposed two different types of ring cleavage reaction (2,3-extradiol cleavage of *cyclo*-DOPA or 3,4-intradiol cleavage of *cyclo*-DOPA) leading to intermediates each requiring the addition of a C-1 unit from methionine, the later revised postulate[3] and the unified postulate for C_2- and C_3-proline units in the lincomycins and the pyrrolo[1,4]benzodiazepines requires a single type of ring cleavage (2,3-extradiol cleavage of DOPA) and the addition of a C-1 unit *only* in the case of the C_3-proline unit. Experiments were designed to discriminate between these alternative pathways using both specifically deuterated tyrosine and DOPA molecules in combination with ^{13}C NMR, and using deuterated methionine in combination with mass spectral analysis. The second portion of this paper describes the results of an experiment using D-($^{13}C_6$)glucose in combination with analysis of ^{13}C–^{13}C coupling patterns in PHA, to trace the path of glucose carbons through the shikimic acid pathway to tyrosine and finally into lincomycin A.

Biosynthetic Experiments with L-(3′,5′-$^2H_2$)Tyrosine, L-(2′,5′,6′-$^2H_3$)DOPA, and L-(6-$^2H_3$)Methionine. An examination of the biosynthetic fate of the aromatic protons of DOPA in lincomycins A and B, when followed through the *cyclo*-DOPA (Figure 2) and 2,3-extradiol DOPA cleavage pathways (Figure 3) reveals a number of distinct differences. While the H_a proton is lost via the cyclodopa pathway in PHA and EHA, it may be retained via the 2,3-extradiol DOPA cleavage pathway in the propyl or ethyl side chains of lincomycins A and B, respectively. A similar analysis of the H_b protons for both pathways reveals a retention of just one of these protons via the *cyclo*-DOPA pathway, while there is retention of both protons via the 2,3-extradiol DOPA cleavage pathway. While one of these H_b protons is found at the same position of PHA and EHA irrespective of the type of ring-cleavage pathway, the second H_b proton retained only in the 2,3-extradiol DOPA cleavage pathway is found at the

[†] University of Texas.
[‡] The Upjohn Co.

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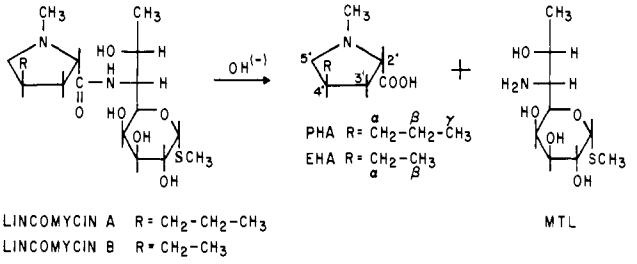

Figure 1. Structures of the lincomycins A and B and the products of alkaline hydrolysis.

5′-position of both PHA and EHA.

Biosynthetic feeding experiments with L-(3′,5′-$^2H_2$)tyrosine and L-(2′,5′,6′-$^2H_3$)DOPA and ^{13}C NMR analysis of the resulting PHA and EHA moieties in lincomycins A and B clearly discriminate between the pathways shown in Figures 2 and 3. Comparison of the broad-band proton-decoupled natural-abundance spectra of lincomycins A and B (Figure 4A and 5A) with those samples obtained from feeding experiments with L-(3′,5′-$^2H_2$)tyrosine (Figure 4B and 5B) shows both a reduction in the ^{13}C NMR resonance signal for the β-carbons of PHA and EHA (^1H–^{13}C species) and an appearance of an isotopically shifted triplet (^2H–^{13}C coupled species) at approximately the same chemical shift. This same deuterated species of PHA and EHA is found in the sample of lincomycins A and B obtained from feeding experiments with L-(2′,5′,6′-$^2H_3$)DOPA presumably arising from the deuterated H_a atom of DOPA (see Figure 4C and 5C). Additional partially deuterated carbon atoms are also clearly detectable at the 5′- and α-positions of lincomycins A and B. (Interestingly, the 4′-carbon resonance signal in lincomycins A and B appears reduced in intensity in both samples labeled from L-(2′,5′,6′-$^2H_3$)DOPA presumably broadened due to an isotope effect brought about by the cumulative presence of deuterium atoms at the 5′, α, and β-positions.) Taken together, these results clearly rule out the cyclo-DOPA pathway (Figure 2) and are in accord with 2,3-extradiol DOPA cleavage pathway (Figure 3).

An additional important discriminating difference between the pathways proposed in Figures 2 and 3 is the contribution of the

Table I. Mass Spectral Analysis of Lincomycins A and B Isolated from an Experiment in Which L-(6-$^2H_3$)Methionine[a] Was Fed to *S. lincolnensis*[a]

antibiotic	rel % of $^2H_3$ and $^2H_6$ species[b]	
	$^2H_3$	$^2H_6$
lincomycin A	17.7	82.3
lincomycin B	95.4	4.6

[a] 90% Atom enriched added at a level of 0.5 g per flask at 96 h. [b] Tri- and hexadeuterated species were estimated from the ions of m/z 158 and 177, respectively. Biemann's formula[12] was used to calculate the excess isotopic abundances.

C-1 unit from methionine to the PHA and EHA moieties of lincomycins A and B, respectively. While both moieties carry a NCH_3 unit derived from the C-1 pool via methionine,[2] the *cyclo*-DOPA pathway requires an additional methyl group at the α- or β-positions of lincomycins A and B, respectively. In contrast, the 2,3-extradiol DOPA cleavage pathway only requires an additional methyl group at the γ position of lincomycin A. Mass spectra analysis (Table I) of PHA and EHA from an experiment in which L-(6-$^2H_3$)methionine was fed to *S. lincolnensis* demonstrates that *only* in the case of PHA is there found both an *N*- and *C*-methyl unit derived from the deuterated methionine; while only one methyl unit is present in EHA. Therefore, it can be concluded that the 2,3-extradiol DOPA cleavage pathway is operative in the lincomycin biosynthetic pathway.

Biosynthetic Experiments Using D-($^{13}C_6$)Glucose. As a consequence of experiments using specifically carbon-13 labeled glucose, glycerol, and pyruvate designed to provide biosynthetic information on the origin of MTL, it was deduced that the shikimic acid pathway could be implicated in the origin of PHA in lincomycin A.[3] This was expected in view of the demonstrated intermediacy of tyrosine in PHA biosynthesis. Primarily as means to provide new insight into the biosynthetic origin of MTL, but also to follow the path of carbon from glucose through the shikimic acid pathway to tyrosine, we have now analyzed samples of lincomycin A biosynthetically labeled from D-($^{13}C_6$)glucose. A fermentation medium was used in which glucose was the sole carbon source during the antibiotic-producing phase and the

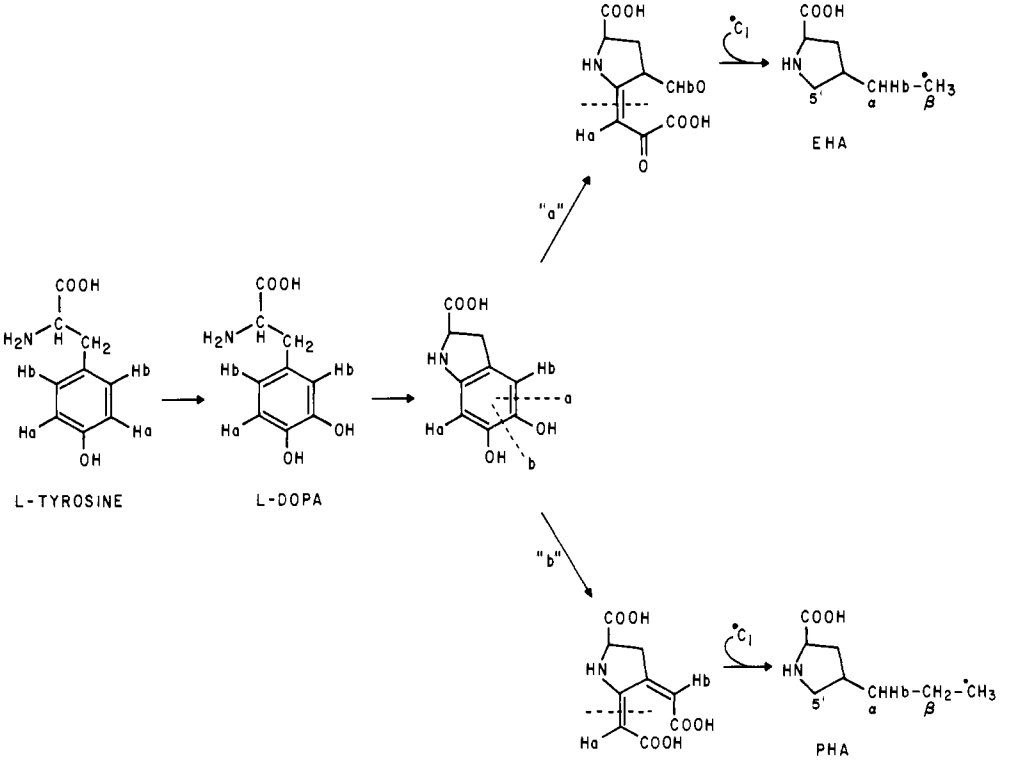

Figure 2. Proposed parallel biosynthetic pathways leading to PHA and EHA via 2,3-extradiol cleavage of *cyclo*-DOPA and 3,4-intradiol cleavage of *cyclo*-DOPA.

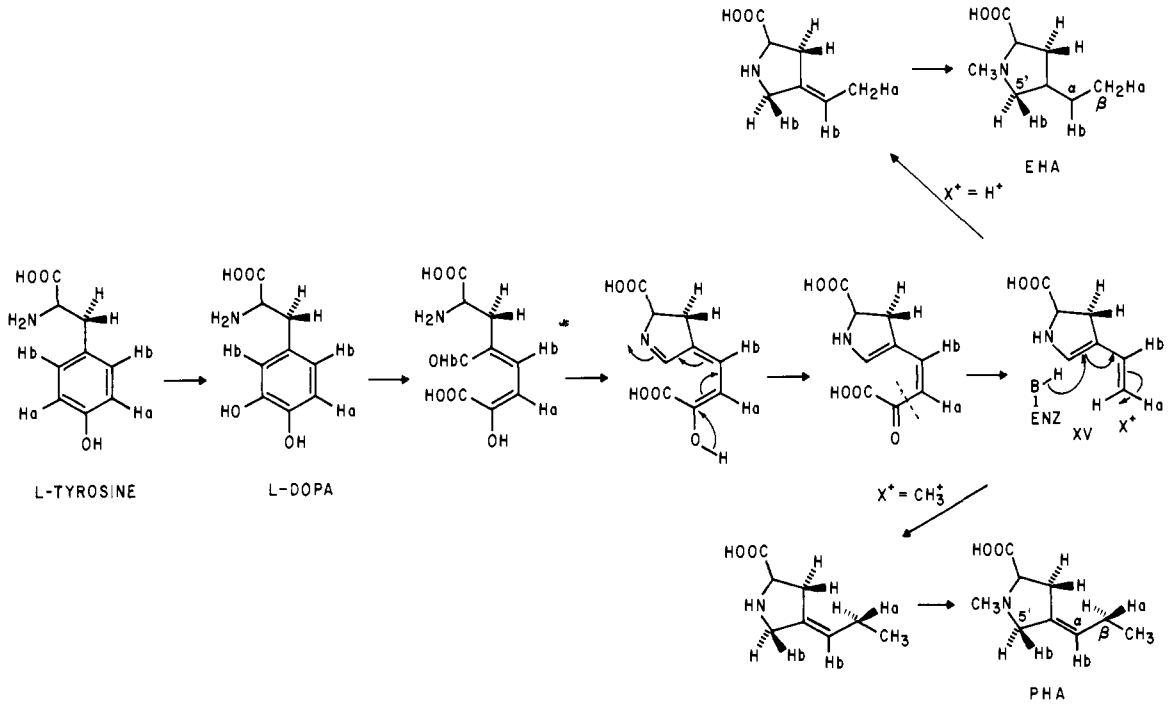

Figure 3. Proposed parallel biosynthetic pathways leading to PHA and EHA via 2,3-extradiol cleavage of DOPA.[7,8] A referee has suggested an alternative and attractive mechanism for the addition of X^+ (SAM or H) in which alkylation (protonation) of an enamine followed by decarboxylation to quench the immonium ion is proposed. We thank this referee for this suggestion.

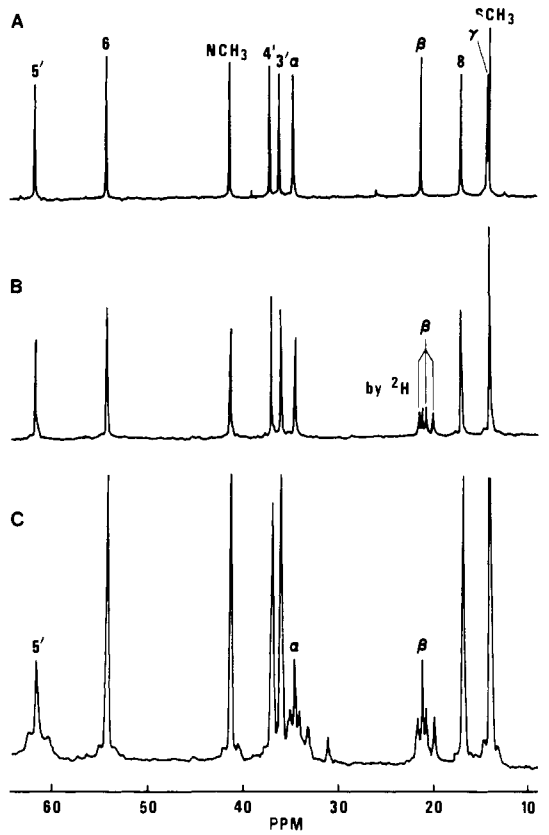

Figure 4. Partial ^{13}C NMR spectra (10–65 ppm region) of natural abundance lincomycin A (A), samples of lincomycin A obtained from feeding experiments with L-(3′, 5′-$^2H_2$)tyrosine (97% $^2H_2$) added at a level of 0.5 g per flask at 72 h (B), and L-(2′,5′,6′-$^2H_3$)DOPA (79% $^2H_3$) added at a level of 0.5 g per flask at 96 h (C).

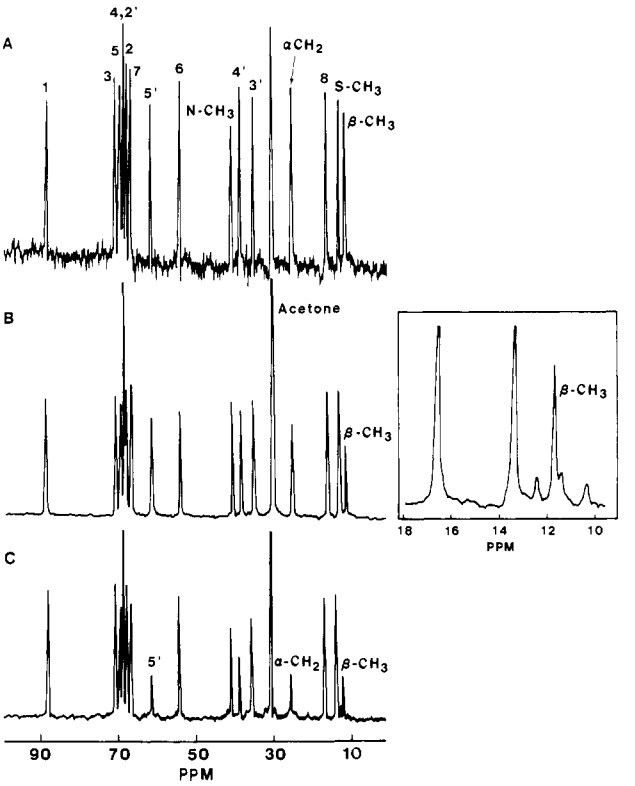

Figure 5. Partial ^{13}C NMR spectra (10–95 ppm region) of natural abundance lincomycin B (A), samples of lincomycin B obtained from feeding experiments with L-(3′,5′-$^2H_2$)tyrosine with an expanded region around β-carbon showing detail (B), and L-(2′,5′,6′-$^2H_2$)DOPA (C). Feeding conditions as stated in legend for Figure 4.

contiguously carbon-13 labeled glucose was diluted one to nine with unlabeled glucose. The resulting carbon-13 enriched lincomycin A was purified and hydrolyzed to MTL and PHA (Figure 1). Analysis of the MTL spectrum from this experiment is given in the accompanying paper,[9] while the PHA results are presented in this manuscript. The analysis of ^{13}C–^{13}C spin coupling patterns in the biosynthetic product was used to deduce pathways through which the carbon atoms remain connected during conversion of the contiguously labeled glucose to the final product. Dilution of the carbon-13 labeled substrate with unlabeled material is necessary so that those carbon atoms that remain connected can be discerned through their ^{13}C–^{13}C coupling pattern from those

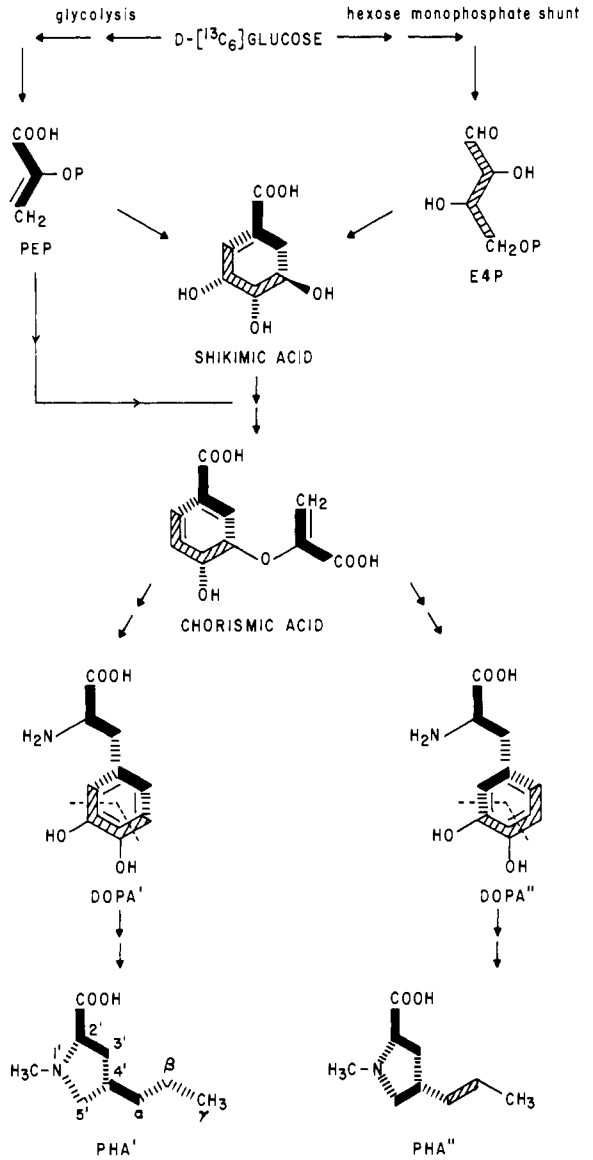

Figure 6. Summary of the proposed biosynthetic pathway leading from glucose to the PHA unit of lincomycin A. Thickened solid bands indicate intact carbon units derived from PEP, whereas the cross-hatched bands correspond to intact carbons units derived from E4P.

which are separated and reconnected again at a later step. Thus, neighboring carbons derived from glucose as an intact unit retain ^{13}C–^{13}C coupling; while those separated and rejoined have a 90% probability of losing their coupling. Such an approach to solving biosynthetic problems using contiguously labeled substrates has been described before.[11] (For a more elaborate discussion of the derivation of the coupling patterns possible from D-($^{13}C_6$)glucose see the following paper in this issue.)

An outline of the biosynthetic route from glucose to the PHA moiety of lincomycin A is shown in Figure 6. Glucose is converted via glycolysis and the hexose monophosphate shunt to phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E-4-P), respectively. One molecule of each of these primary metabolites is then channeled through the shikimic acid pathway to chorismic acid, now containing a second PEP molecule. The chorismic acid is converted via prephenic acid to tyrosine and hence into a secondary metabolic pathway via DOPA to give rise finally to PHA

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Table II. Summary of Information from ^{13}C NMR Spectrum of ^{13}C-Enriched PHA Obtained from the Experiment with D-($^{13}C_6$)Glucose

carbon	chemical shift,[a] ppm	multiplicity[b]	J_{C-C}, Hz
carbonyl	171.8	s + d	$J_{CO,2'} = 57.0$
2′	68.4	s + dd	$J_{2',3'} = 32.5$ $J_{2',CO} = 57.2$
3′	34.0	s + d	$J_{3',2'} = 32.4$
4′	36.2	s + d + d	$J_{4',5'} = 34.0$ $J_{4',\alpha} = 35.4$
5′	61.3	s + d	$J_{5',4'} = 33.6$
NCH_3	40.7	s	
α	33.8	s + d + d	$J_{\alpha,4'} = 35.6$ $J_{\alpha,\beta} = 34.0$
β	20.3	s + d	$J_{\beta,\alpha} = 33.9$
γ	13.1	s	

[a] Chemical shifts of the singlets are listed. Assignments are made by comparison with the reported spectrum.[13] 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 $\delta_C(CH_3)_4Si = \delta(1,4\text{-dioxane}) + 67.4$ ppm. [b] Multiplicity and coupling constants are obtained from the spectra shown in Figures 7 (A and B); s = singlet, d = doublet, dd = doublet of doublet.

in lincomycin A. Those carbons remaining connected are indicated by thick solid lines or cross-hatched lines. The dissymmetric rotamers of L-DOPA should be expected to give rise to equal amounts of PHA′ and PHA″ (Figure 6).

The analysis of the biosynthetic pathway shown in Figure 6 demonstrates which neighboring carbons originally derived from glucose may be incorporated as intact carbon units in the product. For example, a three-carbon unit derived via PEP could be incorporated intact into the carbonyl carbons and 2′ and 3′-carbon atoms of PHA. PEP may also contribute an intact two-carbon unit to either carbon atoms 4′–α of PHA′ or 5′–4′ of PHA″. Lastly, a two-carbon unit derived from E-4-P could be incorporated into carbon atoms α–β of PHA.

In order to resolve a number of overlapping carbon resonance signals in the sample of lincomycin A, which was derived from a feeding experiment with D-($^{13}C_6$)glucose, the antibiotic was hydrolyzed to PHA and MTL (Figure 1). Analysis of the ^{13}C–^{13}C spin coupling pattern in PHA and a portion of the lincomycin A spectrum is presented in this article, while the MTL analysis is found in the accompanying paper.[9] Owing to the complexity of the coupling pattern, the spectra are presented as expanded regions of the pertinent carbon atoms which were assigned on the basis of chemical shift, specific proton decoupling, and ^1H–^{13}C coupling patterns.[13] These results are presented in Figure 7 and Table II.

Carbon atom 2′ occurs either as a singlet (18%) or a doublet of doublets (82%) coupled simultaneously to both the carbonyl and 3′-carbon atoms. Little if any species of a doublet that would be coupled with either carbonyl carbon or 3′, but not both, is present. This eliminates the significant contribution of two-carbons units that might arise from cycling of pyruvate through the TCA cycle.

The carbon signals 5–4′–α–β show the ^{13}C–^{13}C spin coupling characteristics of three two-carbon units. Carbons 5′ and β show resonance signals for a singlet (54% and 53% for 5′ and β, respectively) and doublet (46% and 47% for 5′ and β, respectively) due to coupling with carbon atoms 4′ and α, respectively. The internal carbons 4′ and α, show a resonance signal for a singlet (17% and 22% for 4′ and α, respectively) and also show two doublets due to coupling between each other (39%) and with 5′ (43%) and β (44%), respectively.

The results obtained in this investigation using both deuterated amino acids and carbon-13 enriched glucose clearly support the previously proposed unified biosynthetic pathway[7] leading from

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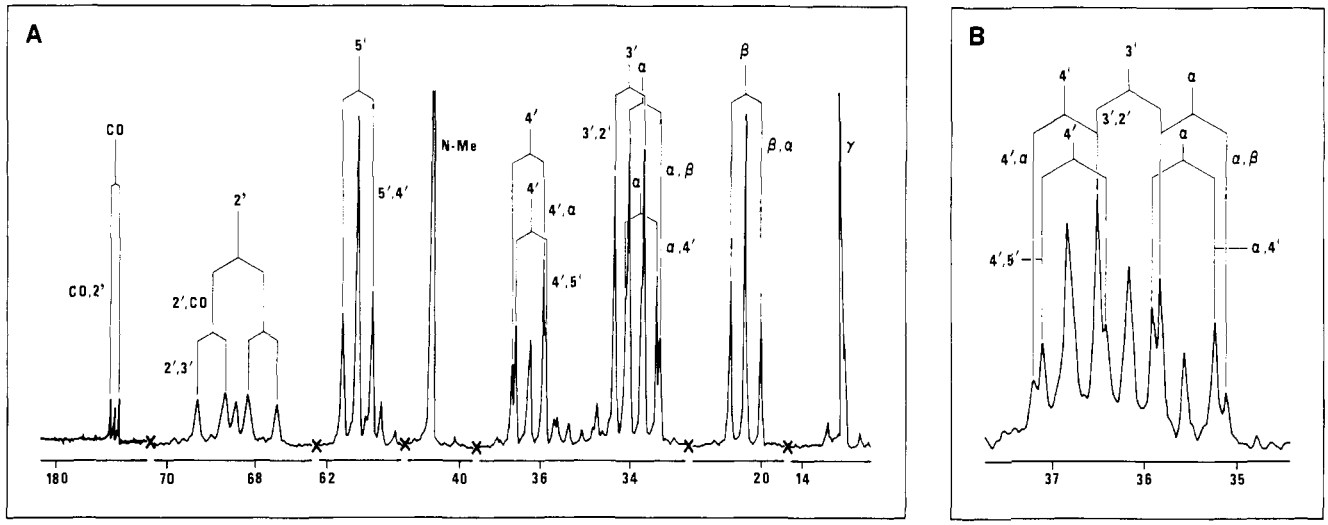

Figure 7. Partial ^{13}C NMR spectra of pertinent expanded regions (20–70 ppm) of PHA (A) and the expanded region (35–38 ppm) of lincomycin (B) obtained from feeding D-($^{13}C_6$)glucose (98.1% enrichment) to *S.lincolnensis* at a level of 0.3 g per flask at 0 h. Spectral parameters: Varian XL-200, spectral width 11 062 Hz, number of transients 462 000, data points 16K, pulse width 5 μs, acquisition time 0.74 s.

tyrosine via DOPA and involving a 2,3-extradiol cleavage pathway to produce a common precursor of the C_2- and C_3-proline units found in the lincomycin and pyrrolo[1,4]benzodiazepine antibiotics. The difference between the pathways leading to C_2- or C_3-proline units is the addition of an C-1 unit from methionine to the C_3-proline derived units.

Experimental Section

General Techniques. ^{13}C NMR spectra were recorded on a Varian CFT-20, XL-100, or XL-200 FT-NMR spectrometer. The NMR spectra were run in D_2O.

Mass spectral analysis was performed on a Varian MAT CH7 mass spectrometer and the relative enrichments of deuterium calculated using Biemann's formula.[12]

Fermentations. A high-producing strain derived from the original soil isolate of *S. lincolnensis* var. *lincolnensis*, denominated NRRL2936 in the culture collection, was maintained in Hickey–Tresner medium at liquid nitrogen temperature. The culture was grown in chloramphenicol agar slants proceeding inoculation into seeds. The three seed media (in grams/liter) used in these experiments (the three sets of numbers in parentheses refer to the three different seed media. The first set were used in the DOPA experiment, the second set for the tyrosine and methionine experiments, and the third set for the glucose experiment) consisted of Cerelose, Clinton Corn Processing Co. (5.0, 20.0, or 10.0), Hi-Starch, Illinois Cereal Mills, Inc. (20.0, 0, or 0), Yeastolac, A.F. Staley Man. Co. (10.0, 10.0, and 0.0), yeast extract, Difco Lab (0, 0, or 10.0), and N-Z Amine B, Sheffield Co. (5.0, 5.0 and 5.0). The fermentation media consisted of (grams/liter) D-glucose (30.0), $ZnSO_4 \cdot 2H_2O$ (0.001), $FeSO_4 \cdot 7H_2O$ (0.001), $MgSO_4$ (1.0), NaCl (0.5), sodium citrate dihydrate (3.0), $(NH_4)_2NO_3$ (1.6), and $K_2HPO_4 \cdot 3H_2O$ (3.3). The seed media were adjusted to pH 7.0 with sodium hydroxide prior to inoculation. Five hundred milliliter Erlenmeyer flasks were filled with 100 mL of seed or fermentation media. Typically, the culture was incubated for 2 days in one of these seed media at 28 °C while agitated at 250 rpm in a rotary shaker prior to inoculation of the fermentation at a 50-fold dilution ratio. The fermentations were usually harvested after 6 days of incubation in a rotary shaker at 28 °C and the lincomycin(s) isolated.

Stable Isotope Labeled Substrates. All the stable isotope compounds were purchased from MSD Isotopes, Canada with the exception of the dideuteriotyrosine and $^2H_3$-L-DOPA, which were prepared as described before.[4]

Isolation of Lincomycin. Contents of the 500-mL fermentation flask were filtered through Whatman no. 1 to remove the cells. The filtrate (approximately 100 mL) was then filtered through another filter unit (0.2 μm) to remove finer particles, and the pH of the filtrate was adjusted to 5.0. A cation-exchange column of 10-mL bed volume was prepared as below. The cation-exchange resin (Bio-Rad AG 50W-X4, 200–400 mesh size, hydrogen form) was successively washed with distilled water, 0.5% NH_3 in H_2O, and again with distilled water until the washings were neutral. The resin was slurried with distilled water and then poured into a column. The column was washed with 100 mL of 10% aqueous NaCl solution, 100 mL of 1% aqueous H_2SO_4, and finally by distilled H_2O until the pH of the eluant was neutral. The fermentation filtrate was gravity fed on the top of this column and about 20 fractions of 5 mL each were collected. These fractions were examined on TLC (silica gel, $CHCl_3$–MeOH–NH_4OH, 61:35:4 v/v) to ensure lincomycin was not passing through the column. Lincomycin was then eluted using 0.5% NH_3 in H_2O, and about 20 fractions of 5 mL each were collected. Usually, lincomycin was eluted immediately after the pH of the eluant turned basic. Fractions were examined on TLC, and those fractions containing lincomycin were combined and extracted with 6 × 10 mL of CH_2Cl_2. The pooled CH_2Cl_2 extract was washed with 20 mL of saturated brine solution, dried over anhydrous Na_2SO_4, and then evaporated to dryness. After the powder was taken up in 5 mL of distilled H_2O, the solution was lyophilized to obtain lincomycin as a white powder (15–20 mg).

Separation of Lincomycins A and B. Typically, the separation was carried out as described below. The crude lincomycin (4.2 g) obtained from 14 L of whole beer inoculated with L-(3′,5′-$^2H_2$)tyrosine and assaying ca. 4.5% lincomycin B + 95.5% lincomycin A (by GC) was separated by Craig countercurrent distribution.

The solvent/solvent system used was methylene chloride/1% aqueous sodium chloride in 200 × 100 mL tubes with 50 mL of each phase. A total of 190 transfers was carried out; Lincomycin A was located in tubes 70–100, and Lincomycin B was located in tubes 160–185 and was determined by TLC as follows: 2.5-mL CH_2Cl_2 fraction was evaporated to dryness, 0.1 mL of ethyl acetate added, and 10 μL spotted on TLC (0.25-mm silica gel G plates; 20 × 20 cm); the developing solvent was 75:25:10 methyl ethyl ketone/acetone/water, and the detection was done by I_2 vapor).

Fractions 160–185 were combined, and the phases were separated. The CH_2Cl_2 phase was concentrated to dryness and the water was concentrated to a small volume (20 g). The water phase was extracted with 4 × 200 mL of CH_2Cl_2 after addition of 8 g of potassium carbonate. The total CH_2Cl_2 extracts (including the Craig extract) were combined and concentrated to a foam. The foam was dissolved in 0.20 mL of water, 0.25 mL of acetone, and 0.05 mL of concentrated hydrochloric acid. Acetone (3.0 mL) was added dropwise at 40 °C to the solution, and after cooling first to room temperature and then to 0 °C, crystals of lincomycin B hydrochloride salt were formed. These were collected by filtration to give *152 mg* after washing with 2 × 0.5 mL of 95:5 acetone/water and drying under vacuum.

Base Hydrolysis of Lincomycin from D-($^{13}C_6$)Glucose. Typically the hydrolysis was performed as described below. Lincomycin (20 mg, 0.049 mmol) was dissolved in 1.5 mL of distilled H_2O and the solution was heated in an oil bath at 90 °C for 5 min under N_2, 0.3 mL of 50% aqueous NaOH was then added to this solution and heated at 95–100 °C under N_2 for an additional 5 h. The temperature was then raised to 110 °C and heating continued for a total of 22 h. A TLC (silica gel, $CHCl_3$–MeOH–NH_4OH, 61:35:4 v/v) showed that all the lincomycin was hydrolyzed.

Isolation of PHA from the Lincomycin Hydrolysate. Following the isolation of MTL (see the next article in this issue), the anion-exchange column was washed with distilled H_2O until the eluant pH was about 6.0. The column was then eluted with 0.1 N HCl, and 35 fractions of 2 mL each were collected. Those fractions containing PHA (no. 19–22) were identified by TLC (silica gel, $CHCl_3$–MeOH–NH_4OH, 61:35:4 v/v) and

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.

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

Nanda M. Brahme,[†] Jose E. Gonzalez,*[‡] Steve Mizsak,[‡] Jim R. Rolls,[‡] Ed J. Hessler,[‡] and Laurence H. Hurley*[†]

Contribution from Fermentation Research and Development, The Upjohn Company, Kalamazoo, Michigan 49001, and The Drug Dynamic Institute, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712. *Received May 14, 1984*

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

[†] University of Texas.
[‡] The Upjohn Co.

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