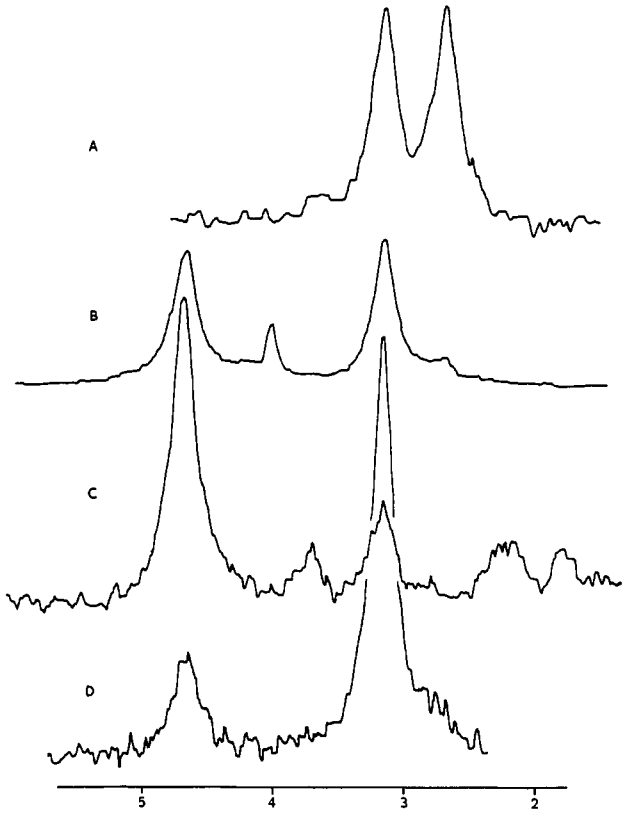
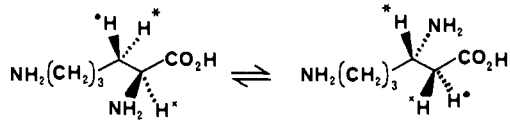



**Figure 2.** 41.44-MHz $^2H$ NMR spectra (Bruker HX270), 4000 data points, 90° pulse, line broadening 0.5 Hz; samples in 1.5 mL of $CHCl_3$ with internal $CDCl_3$ reference, $\delta$ 7.27: (A) synthetic (2*RS*)-**3c**-$d_1$ + **3d**-$d_1$, 394 transients; (B) biosynthetic product **3b**, from **1b**, 1675 transients; (C) biosynthetic product **3f**, from *1d*, 1250 transients; (D) biosynthetic product **3c**, from **1c**, 4682 transients.

From this signal assignment, it therefore follows that the analogous $\beta$-lysine derivative produced biosynthetically from **1b** is labeled as in **3b**, since the C-2 *deuterium* resonance appeared at $\delta$ 3.18. Thus, transfer of deuterium from C-3 of $\alpha$-lysine to C-2 of $\beta$-lysine proceeds with *inversion* of configuration at C-2.

We then turned our attention to the identification of the hydrogen (deuterium) atom transferred from C-3 to C-2. For this purpose (2*RS*, 3*R*)-lysine-*3*-$d_1$ (**1c**) and (2*RS*, 3*S*)-lysine-*3*-$d_1$ (**1d**) were synthesized (Scheme II). Ethyl 4-chlorobutyrate (**9a**) was reduced ($LiAlD_4$, ether, −10 °C) to 4-chloro-1-butanol-*1,1*-$d_2$ (**9b**) which was then oxidized (pyridinium chlorochromate[27]) to 4-chlorobutyraldehyde-*1*-$d_1$ (**9c**). This was reduced with either (+)- or (−)-pinanyl-9-BBN[28,29] to yield (1*S*)-4-chloro-1-butanol-*1*-$d_1$ (**10a**) or (*1R*)-4-chloro-1-butanol-*1*-$d_1$ (**10b**), respectively. The absolute configurations of **10a** and **10b** were assigned by NMR analysis of the corresponding (−)-camphanate esters, which also showed that their configurational purities were ca. 90%.[30] The alcohols were then converted to the corresponding mesylates, **10c** or **10d**, which, in turn, were treated with the sodium salt of ethyl acetamidocyanoacetate to give in moderate yield the condensation products **11a** or **11b**, mp 92–94 °C, respectively.[31] These were converted with NaI/acetone into the 6-iodo analogues, **11c** or **11d**, mp 94–95 °C, and thence with potassium phthalimide into the 6-phthalimido derivatives[32] **11e** or **11f**, respectively. Finally, acidic hydrolysis gave the required lysines **1c** and **1d**. Incubation of these as before with a cell-free extract from *Clostridium SB4* yielded, after workup, samples of di-*N*-phthaloyl-$\beta$-lysine ethyl ester whose deuterium NMR spectra (Figure 2C,D) establish that they are labeled primarily as shown in **3c** (from (3*R*)-lysine-$d_1$) and **3f** [from (3*S*)-lysine-$d_1$ (**1d**)]. The fact that both products show some deuterium at both C-2 (pro-*S*) and C-3 is probably a result of incomplete stereospecific labeling in the precursors. In any case it is clear that for the great majority of the product formed, the 3 pro-*R* hydrogen of $\alpha$-lysine is transferred to C-2, and the 3-pro-*S* hydrogen retained at C-3. Thus, replacement of the transferred hydrogen by the amino group occurs with inversion of configuration at C-3, **12** ⇄ **13**. The stereochemical course of the lysine




**12** **13**

2,3-aminomutase reaction thus parallels the cryptic stereochemistry elucidated for the coenzyme-$B_{12}$-dependent $\beta$-lysine mutase reaction in which the 6-amino group of L-$\beta$-lysine replaces the C-5 pro-*S* hydrogen to form (3*S*,5*S*)-3,5-diaminohexanoic acid with inversion of configuration at C-5.[33,34]

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## Studies of Nitrogen Metabolism Using $^{13}C$ NMR Spectroscopy. 3. Synthesis of DL-[3-$^{13}C$,2-$^{15}N$]Lysine and Its Incorporation into Streptothricin F[1]

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One of the major questions we have addressed in our study of streptothricin F (**1**) biosynthesis[3,4] concerns the mechanism of $\beta$-lysine (**2**) formation. Evidence has been reported for incorporation of $\alpha$-lysine (**3**) into the $\beta$-lysine portion of streptothricin,[3,5] viomycin,[6] and the polymycins.[7] In the last case, much of the

(1) This is part 3 in the series "Biosynthesis of Streptothricin F".
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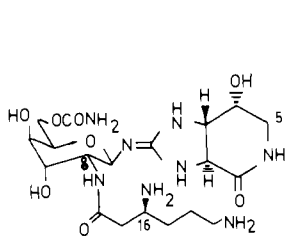

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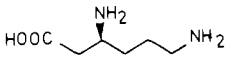

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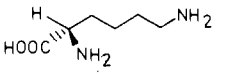


3

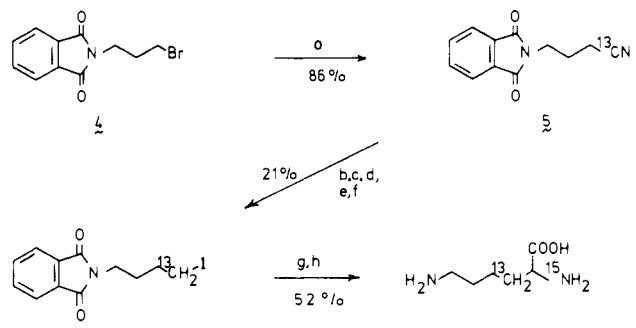
$^{15}N$ label was retained when [2-$^{15}N$]-**3** was fed and the product analyzed by mass spectrometry.[8] In addition to these anabolic pathways of aerobic *Streptomyces*, **2** has also been identified as the first product in the anaerobic degradation of **3** by various species of *Clostridium*.[9] The *Clostridium* (*S*)-$\alpha$-lysine 2,3-aminomutase has been isolated and cofactor requirements reported;[10] Aberhart has recently demonstrated the stereospecific migration of the 3-pro-*R* hydrogen of **3** and inversion of configuration occurring at both C-2 and C-3 in this reaction.[11] We now report that the mutase reaction occurring in the biosynthesis of **1** occurs with an intramolecular migration of nitrogen from C-2 to C-3.

DL-[3-$^{13}C$,2-$^{15}N$]Lysine **3a**[12] was synthesized as shown in Scheme I. Treatment of (bromopropyl)phthalimide (**4**)[13] with Na$^{13}CN$ gave the nitrile **5**, which was reduced catalytically to the amine hydrochloride and immediately converted to the sulfonamide.[14] Thermal rearrangement[15] of the *N*-nitroso[16] derivative afforded the tosylate, which was converted to the iodide **6**. The iodide was then coupled with diethyl [$^{15}N$]phthalimidomalonate,[17] and the diphthalimide hydrolyzed in acid to give **3a** in 9% overall yield.

Four 250-mL production broths were innoculated in standard fashion with a seed culture of *Streptomyces* L-1689-23.[3] The labeled lysine **3a**·2HCl (45 mg, 200 $\mu$mol) and L-[U-$^{14}C$]lysine[18] (15 $\mu$Ci) in 12 mL of water were divided into three equal portions and each divided equally amongst the four broths at 12, 20, and 30 h after innoculation. After an additional 18 h the fermentations were combined and worked up as previously described[3] to yield 210 mg of the pure helianthate salt of **1a**.[19] On the basis of the radioactivity incorporated (15%), a 4.3% enrichment of $^{13}C$ was expected in the antibiotic if only the L enantiomer were utilized.

A portion of the helianthate was converted to the amorphous trihydrochloride, and the 67.88-MHz proton-noise-decoupled $^{13}C$ NMR spectrum of the sample in 2% pyridine/$D_2O$ was obtained.[20]

Scheme I[a]



4 5 6 3a

[a] Na$^{13}CN$, $Me_2SO$, 60 °C; (b) $PtO_2$, $H_2$, EtOH, HCl; (c) TsCl, $CH_2Cl_2$, $Et_3N$; (d) $NaNO_2$, HOAc, $Ac_2O$; (e) $CCl_4$, $Na_2CO_3$, 60 °C; (f) NaI, $Me_2CO$, reflux; (g) Sodium diethyl [$^{15}N$]phthalimidomalonate, 155 °C; (h) HCl, HOAc, reflux.

The spectrum exhibited a doublet ($J_{CN}$ = 3.4 Hz)[21] at $\delta$ 45.9, sufficiently large to completely encompass the natural abundance singlet of C-16, revealing the formation of a new $^{13}C$–$^{15}N$ bond in **1a**. A comparison of normalized integrals for the C-5 and C-8

3a 1a

singlets and the C-16 doublet indicated a 4.7% enrichment of $^{13}C$, in good agreement with the $^{14}C$ data for utilization of only the L enantiomer of **3a** in the biosynthesis. More importantly, the doublet clearly indicates that the nitrogen migration was intramolecular, since a doublet resulting from an intermolecular process would have only occurred in 0.3% of the **1a** molecules.[22]

Five $\beta$-amino acids are known.[23] Although the formation of $\beta$-arginine[24] has not yet been studied,[25] reports on $\beta$-alanine,[26] $\beta$-leucine,[27,28] $\beta$-tyrosine,[29] and $\beta$-lysine demonstrate that these four are each formed by a different mechanism. The antibiotics negamycin[30] and 3-*epi*-deoxynegamycin[31] may also be products of $\alpha$-lysine 2,3-aminomutase reactions but are epimeric at the $\beta$-amino carbon; the biosynthesis of these metabolites is currently under study.

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(18) Obtained from ICN.
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(20) Bruker HX-270; spectral width 15 000 Hz; 30° pulse angle; 0.54-s aquisition time; 19 780 transients; 35 mg of **1a** in 0.5 mL; chemical shifts based on the middle pyridine signal, 135.5 ppm.
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(22) This value was obtained by multiplying the effective $^{13}C$ concentration (5.8%) with the effective $^{15}N$ concentration (5.1%) that accounts for both natural abundance and enrichment contributions.
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## $(\mu\text{-H})Fe_4(CO)_{12}(\eta^2\text{-COH})$: Evidence for a Protonated $\eta^2$-CO Complex as an Intermediate in the Proton-Induced Reduction of CO

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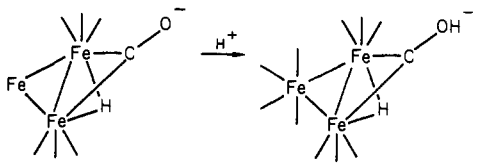
In their original NMR spectroscopic investigation of metal carbonyls in acid media, Wilkinson and co-workers demonstrated that protonation occurs on the metal.[1] Subsequent diffraction studies have confirmed this general concept and provided detailed structural information on the variety of bonding patterns between the proton and metal centers in polynuclear carbonyls.[2] In contrast to this earlier work it was recently shown that protonation of some metal carbonyl clusters also may occur at edge-bridging (eq 1)[3,4] or face-bridging carbonyl oxygens.[5]




(1)

The present research provides spectroscopic evidence for a new type of O-protonated carbonyl ligand, $\eta^2$-COH, resulting from the protonation of $[HFe_4(CO)_{13}]^-$. This new O-protonated compound appears to be a key intermediate in the recently discovered proton-induced reduction of CO in $[Fe_4(CO)_{13}]^{2-}$.[6]

In 1957, Hieber and Werner reported a compound with the empirical formula $H_2Fe_4(CO)_{13}$, which was described as soluble in ethers and benzene and stable for significant periods of time at room temperature.[7] Attempts to structurally characterize this compound in several laboratories have been uniformly unsuccessful,[8] so we have explored the possibility that, as with $HFe_3(CO)_{10}(COH)$, the anhydrous diprotonated form of the tetranuclear cluster may be stable only at low temperatures.

Anhydrous $(\mu\text{-H})Fe_4(CO)_{12}(\eta^2\text{-COH})$ (I) was prepared under an inert atmosphere by the addition of 30 $\mu$L of $HSO_3CF_3$ or $HSO_3F$ to ca. 3 mL of a frozen (-196 °C) $CD_2Cl_2$ solution containing 0.13-0.18 mmol of $[PPN][HFe_4(CO)_{13}]$ (enriched to ca. 15% $^{13}CO$) in an NMR tube. The tube was sealed under vacuum and warmed to -90 °C, and the $^{13}C$ and $^1H$ NMR spectra were determined. Additional $^{13}C$ NMR spectra were obtained on $^{13}CO$ enriched samples of $[HFe_4(CO)_{13}]^-$ (II) and $HFe_4(CO)_{12}(\eta^2\text{-}COCH_3)$ (III), both of which have been the subjects of X-ray structure determinations.[9,10]

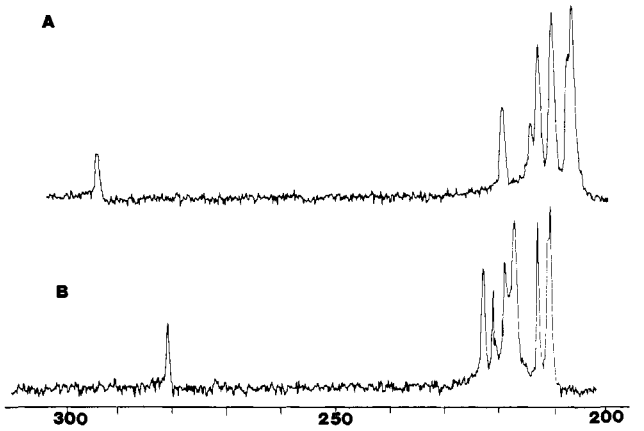



**Figure 1.** $^{13}C$ NMR spectra of (A) $(\mu\text{-H})Fe_4(CO)_{12}(\eta^2\text{-COH})$ (I) and (B) $[PPN][HFe_4(CO)_{13}]$ (II). These spectra were observed at 20 MHz on a Varian CFT-20 spectrometer at -90 °C in $CD_2Cl_2$.

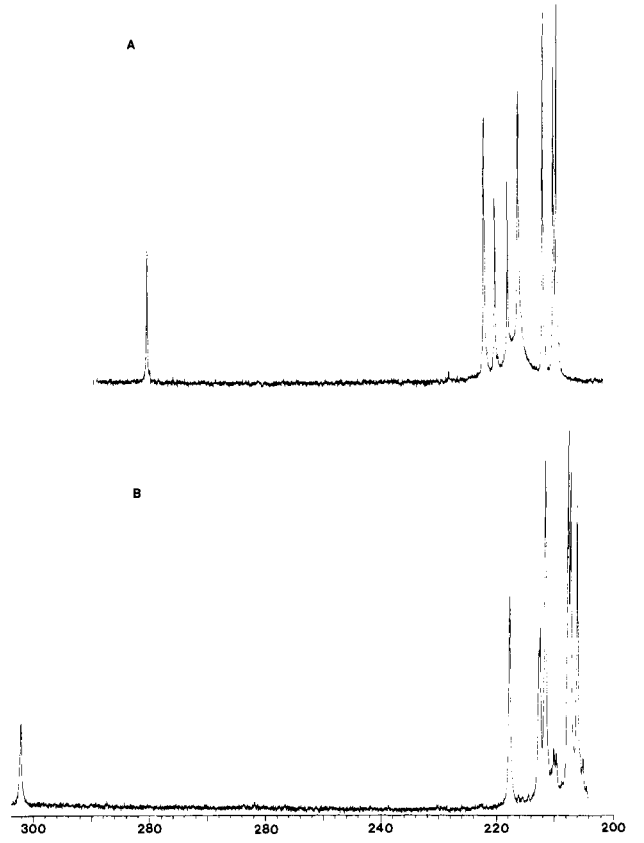



**Figure 2.** $^{13}C$ NMR spectra of (A) $[PPN][HFe_4(CO)_{13}]$ (II) and (B) $(\mu\text{-H})Fe_4(CO)_{12}(\eta^2\text{-}COCH_3)$ (III). Spectra were obtained at 90 MHz on a Nicolet NT-360 spectrometer at -90 °C in $CD_2Cl_2$.

The $\eta^2$-CO in II displays a characteristic low-field $^{13}C$ NMR feature (Figures 1 and 2) at 281 ppm relative to $Me_4Si$. Upon reaction with the methyl carbocation to produce III the resonance due to the $\eta^2$-CO shifts to even lower field, 301 ppm. Similarly, the protonation of II leads to a low-field shift of the resonance of the $\eta^2$-CO to 294 ppm, indicating that protonation has occurred

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