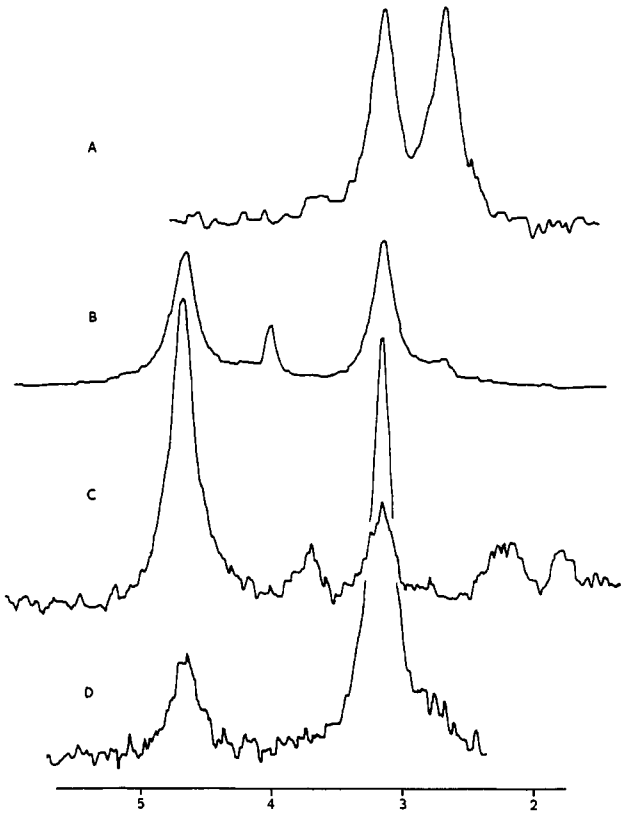
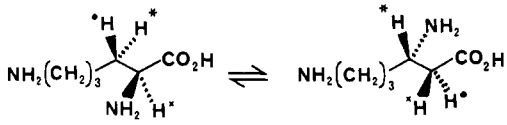


Figure 2. 41.44-MHz ^2H NMR spectra (Bruker HX270), 4000 data points, 90° pulse, line broadening 0.5 Hz; samples in 1.5 mL of $CHCl_3$ with internal $CDCl_3$ reference, δ 7.27: (A) synthetic (2*RS*)-**3c**-d_1 + **3d**-d_1, 394 transients; (B) biosynthetic product **3b**, from **1b**, 1675 transients; (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 β-lysine derivative produced biosynthetically from **1b** is labeled as in **3b**, since the C-2 *deuterium* resonance appeared at δ 3.18. Thus, transfer of deuterium from C-3 of α-lysine to C-2 of β-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-β-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 α-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 β-lysine mutase reaction in which the 6-amino group of L-β-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]

Steven J. Gould*[,2] and T. K. Thiruvengadam

School of Pharmacy
Section of Medicinal Chemistry and Pharmacognosy
The University of Connecticut, Storrs, Connecticut 06268

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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 β-lysine (**2**) formation. Evidence has been reported for incorporation of α-lysine (**3**) into the β-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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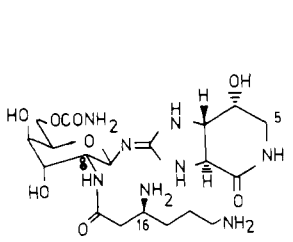

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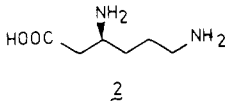

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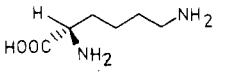


3

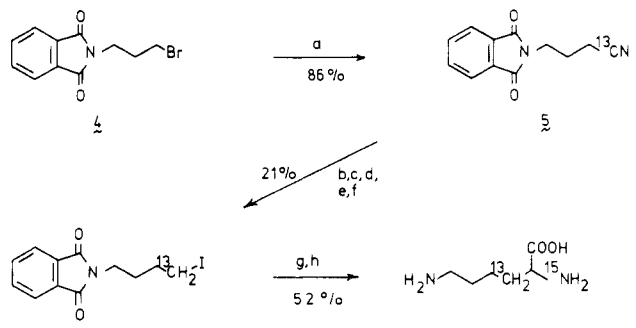
^{15}N label was retained when [2-^{15}N]-**3** was fed and the product analyzed by mass spectrometry.[8] In addition to these anabolic pathways of aerobic *Streptomyces*, **2** has also been identified as the first product in the anaerobic degradation of **3** by various species of *Clostridium*.[9] The *Clostridium* (*S*)-α-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 μmol) and L-[U-^{14}C]lysine[18] (15 μCi) 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 δ 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 β-amino acids are known.[23] Although the formation of β-arginine[24] has not yet been studied,[25] reports on β-alanine,[26] β-leucine,[27,28] β-tyrosine,[29] and β-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 α-lysine 2,3-aminomutase reactions but are epimeric at the β-amino carbon; the biosynthesis of these metabolites is currently under study.

Acknowledgment. This work was supported by Public Health Service Research Grant GM 25996 from the National Institutes of General Medical Sciences. The potassium [^{15}N]phthalimide was provided by the Stable Isotopes Resource at the Los Alamos

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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 η^2-CO Complex as an Intermediate in the Proton-Induced Reduction of CO

K. H. Whitmire and D. F. Shriver*

Department of Chemistry, Northwestern University Evanston, Illinois 60201

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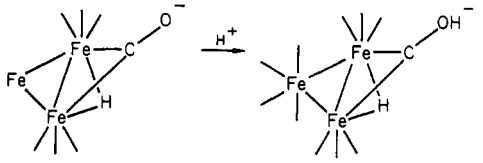
In their original NMR spectroscopic investigation of metal carbonyls in acid media, Wilkinson and co-workers demonstrated that protonation occurs on the metal.[1] Subsequent diffraction studies have confirmed this general concept and provided detailed structural information on the variety of bonding 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, η^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 μL 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]

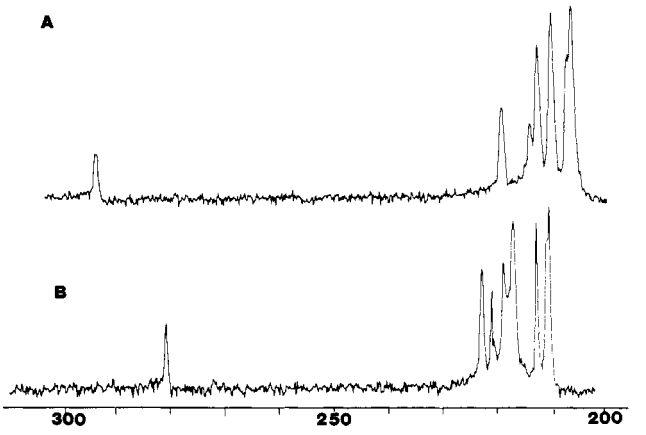


Figure 1. ^{13}C NMR spectra of (A) $(\mu\text{-H})Fe_4(CO)_{12}(\eta^2\text{-COH})$ (I) and (B) $[PPN][HFe_4(CO)_{13}]$ (II). These spectra were observed at 20 MHz on a Varian CFT-20 spectrometer at -90 °C in CD_2Cl_2.

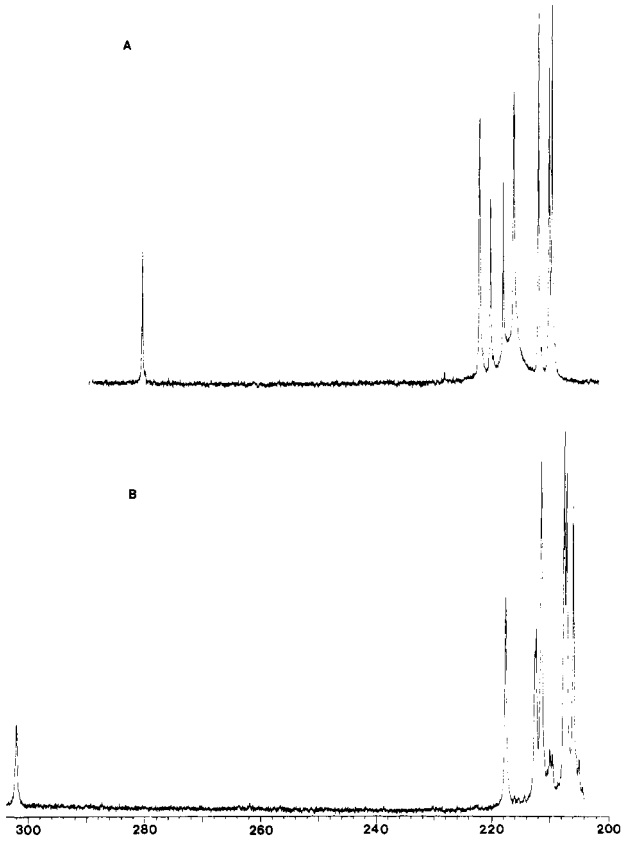


Figure 2. ^{13}C NMR spectra of (A) $[PPN][HFe_4(CO)_{13}]$ (II) and (B) $(\mu\text{-H})Fe_4(CO)_{12}(\eta^2\text{-}COCH_3)$ (III). Spectra were obtained at 90 MHz on a Nicolet NT-360 spectrometer at -90 °C in CD_2Cl_2.

The η^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 η^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 η^2-CO to 294 ppm, indicating that protonation has occurred

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