croprobe of an 80-MHz Varian CFT-20 in hexadeuteriobenzene, 35 000 scans with 5-s delay) showed nine peaks at δ 195.14, 135.09, 102.14, 24.60, 22.53, 22.14, 21.32, 21.03, and 20.35. Structure III would be expected to have three proton NMR signals in the regions indicated. Structure IV should have four signals, but the trienylmethyls are so similar that they are quite likely to be accidentally isochronous. Cumulenes typically have a band at about 2010 cm^{-1} in the IR spectrum, whereas allenes are generally seen to absorb at 1995 cm^{-1}. The δ 195 carbon peak is in the range invariably encountered for the sp carbon atom in allenes;[14] on these various grounds, one might favor structure III. On the other hand, the presence of six high field and three low field peaks in the ^{13}C NMR spectrum seems in better accord with structure IV (expected;[15] six and four, respectively) than with III (five and five). The missing 10th peak must be attributed to isochronous signals or unusual relaxation characteristics of one of the carbon atoms. Our present work focuses on the *tert*-butyl substituted carbene; it is hoped that this type of substitution will lead to more stable derivatives.

In any case, proof that the title carbene (or some loosely bound complex mimicking it) has been captured seems unassailable. This opens further possibilities for experimental study, among them the question whether it has the same dipolar characteristics as dimethylallenylidene and whether extension to still longer chains is possible, perhaps with *tert*-butyl group stabilization. Our work continues along these lines.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

(14) R. H. A. M. Janssen, R. J. J. C. Lousberg, and M. J. A. de Bie, *Rec. Trav. Chim. Pays-Bas*, **100**, 85 (1981).
(15) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972, Chapter 3.

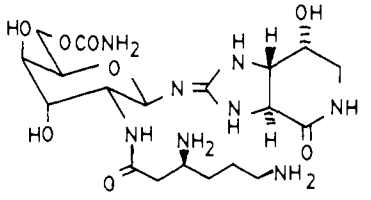
Studies of Nitrogen Metabolism Using ^{13}C NMR Spectroscopy. 2. Incorporation of L-[*guanido*-^{13}C,$^{15}N_2$]Arginine and DL-[*guanido*-^{13}C,2-^{15}N]Arginine into Streptothricin F[1]

Steven J. Gould,*[2] Kazys J. Martinkus, and Chou-Hong Tann

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

Received March 10, 1981

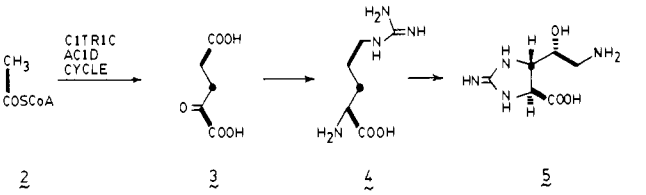
We have recently reported the labeling of streptothricin F (**1**), a broad-spectrum antibiotic produced by various *Streptomyces*, by sodium [1,2-$^{13}C_2$]acetate.[3] As shown in Scheme I, the labeling



1

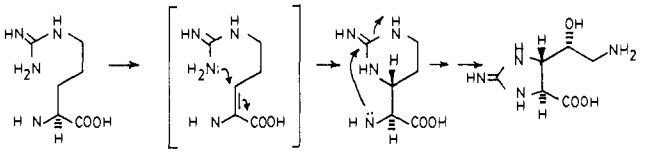
pattern of the heterocyclic (streptolidine) portion of the antibiotic was consistent with the incorporation of acetyl-CoA (**2**) via α-ketoglutarate (**3**) and arginine (**4**). This interpretation eliminated

Scheme I



2 **3** **4** **5**

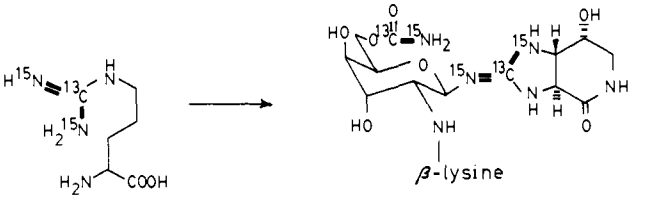
Scheme II



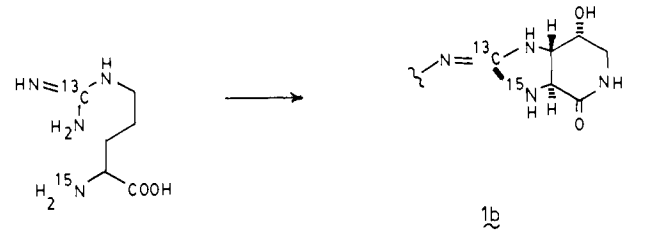
4 **5**

the need to invoke two fundamentally different metabolic pathways for the formation of the streptolidine moiety (**5**), as had been claimed by previous workers.[4,5]

We now report direct, conclusive evidence for **4** as the primary precursor of **5**, as well as evidence supporting Bycroft and King's proposal (Scheme II) for the biogenesis of **5** from **4**.[6] L-[*guanido*-^{13}C,$^{15}N_2$]Arginine (**4a**) and DL-[*guanido*-^{13}C,2-^{15}N]arginine (**4b**) now have each been incorporated into the antibiotic, yielding **1a** and **1b**, respectively. The presence and locations of both ^{13}C and ^{15}N labels were revealed in the ^{13}C NMR spectra from the heteronuclear spin couplings.[7]



4a **1a**



4b **1b**

Arginine **4a** (48.8 mg as the hydrochloride, 232 μmol, 92 atom % ^{13}C, 94 atom % ^{15}N), mixed with DL-[1-^{14}C]arginine (4.9 μCi), was added aseptically to each of two 250-mL production broths 12 h after innoculation with a vegetative seed culture of *Streptomyces* L-1689-23.[3] Standard workup[3] 36 h later afforded 126 mg of the crystalline, radiochemically pure helianthate salt of streptothricin F.

(1) This is part 2 in the series "The Biosynthesis of Streptothricin F".
(2) Career Development Awardee of the National Cancer Institute (CA00627), 1979–1984.
(3) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 2871–2872.
(4) Sawada, Y.; Kubo, T.; Taniyama, H. *Chem. Pharm. Bull.* **1976**, *24*, 2163–2167.
(5) Sawada, Y.; Kawakami, S.; Taniyama, H.; Inamori, Y. *J. Antibiot.* **1977**, *30*, 632–640.
(6) Bycroft, B. W.; King, T. J. *J. Chem. Soc., Chem. Commun.* **1972**, 652–654.
(7) (a) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707. (b) Haber, A.; Johnson, R. D.; Rinehart, K. L. *Ibid.* **1977**, *99*, 3451. (c) Burton, G.; Nordlow, H.; Hosozawa, D.; Matsumoto, H.; Jordan, P. M.; Faterness, P. E.; Pryde, L. M.; Scott, A. I. *Ibid.* **1979**, *101*, 3114. (d) Ostrander, J. M.; Hurley, L. H.; McInnes, A. G.; Smith, D. G.; Walter, J. A.; Wright, J. L. C. *J. Antibiot.* **1980**, *33*, 1167–1171. (e) Leete, E.; McDonell, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 658–662.

0002-7863/81/1503-4639$01.25/0

The helianthate salt was converted to streptothricin F trihydrochloride[3] and the 67.88-MHz proton-noise decoupled ^{13}C NMR spectrum of the sample in 2% pyridine/D_2O obtained. Only the signals from the guanido and carbamate carbons showed spin couplings indicating the presence of ^{15}N, and none of the 18 observable singlets showed enhancement of ^{13}C content when normalized to the signal of the anomeric sugar carbon and compared with the natural abundance spectrum.

The natural abundance peak of the δ 160.38 signal was obscured by the superposition of a large triplet (J_{CN} = 20 Hz) and a small doublet (J_{CN} = 20 Hz).[8] This signal can now clearly be assigned to the guanido carbon of **1**.[3] Since **4a** was not 100% enriched in ^{13}C and ^{15}N, a triplet was observed for the 81% of **1a** containing a $^{15}N{=}^{13}C{-}^{15}N$ grouping ($J_{AB} \sim J_{BC}$) and a doublet was observed for the 10% of **1a** containing either a $^{15}N{=}^{13}C{-}^{14}N$ or a $^{14}N{=}^{13}C{-}^{15}N$ grouping.[9] A small doublet (J_{CN} = 26 Hz) flanked the carbamate signal at δ 155.44, indicating the specific—though much more distant—derivation of this moiety from arginine, too.[10]

Measurement of the guanido and carbamate signals in the ^{13}C NMR spectrum of **1a** indicated enrichments of 26% and 0.4%, respectively, when normalized to the signal of the lactam carbonyl and compared with the natural abundance spectrum.

We next synthesized DL-[*guanido*-^{13}C,2-^{15}N]arginine **4b**.[11] A portion of this material (11.15 mg as the hydrochloride, 52 μmol, 90 atom % ^{13}C, 98 atom % ^{15}N), mixed with 2.23 μCi of DL-[5-^{14}C]arginine, was fed to each of four 250-mL production broths. Pure helianthate (231 mg) was obtained in standard fashion. The 67.88-MHz ^{13}C NMR spectrum of pure streptothricin F trihydrochloride (**1b**) now revealed a new doublet (J_{CN} = 12.0 Hz)[12] flanking the guanido signal.

This doublet, showing a small upfield shift (1.05 Hz)[13] and measuring for a 2.9% enrichment, clearly demonstrated the formation of the new C–N bond predicted in Scheme II. Thus, all three guanido nitrogens of **1** are derived from arginine. Since the two labels in **4b** were separated by a potentially labile bond, this result along with that obtained from the incorporation of **4a** confirms the intact incorporation of arginine into **5** and gives strong support for the validity of the pathway outlined in Scheme II.

Recognizing that arginine is incorporated into **5** intact, the strategic inclusion of DL-[5-^{14}C]**4** in the L-**4a** feeding can now be used to show that only L-arginine is utilized in the biosynthesis of **1**. On the basis of the amount of antibiotic present at the end of the fermentation (218 mg),[14] 8.2% of the radioactivity fed had been incorporated. This would predict an 11.8% total enrichment of ^{13}C had both D- and L-arginine been utilized whereas utilization of only L-arginine would have yielded a 23.6% enrichment. The observed total enrichment was 26.4%.

Future work will examine further details of streptolidine and β-lysine biosynthesis by using additional $^{13}C/^{15}N$- as well as $^{13}C/^{2}H$-labeled precursors.

Acknowledgment. This work was supported by Public Health Service Research Grant GM 25996 from the National Institute of General Medical Sciences. The L-[*guanido*-^{13}C,$^{15}N_2$]arginine and potassium [^{15}N]phthalimide were provided by the Stable Isotopes Resource at the Los Alamos Scientific Laboratory, jointly supported by the U.S. Department of Energy and the NIH (RR-00962-02, Division of Research Resources). We are grateful to Dr. Donald Borders, Lederle Laboratories, Pearl River, NY, for generous gifts of streptothricin F and *Streptomyces* L-1689-23. Peter Demou of the Chemistry Department, Yale University, is thanked for obtaining the ^{13}C NMR spectra. The Bruker HX-270 NMR instrument facility at Yale University used in this work was supported by National Institutes of Health Grant RR 01077.

(8) These observed coupling constants are nearly identical with the J_{CN} = 21.3 Hz observed for **4a**; see ref 7b.

(9) This value is arrived at by multiplying the enrichments at each of the three positions of **4a**.

(10) See ref 7b. Also see: Hornemann, U.; Eggert, J. H. *J. Antibiot.* **1975**, *28*, 841–843 for previous labeling of carbamates by arginine.

(11) Arginine **4b** was synthesized from potassium [^{15}N]phthalimide and [^{13}C]urea by adapting the procedures of Murray and Williams, Kurtz, and Janus (Murray, A., III; Williams, D. L. "Organic Synthesis with Isotopes"; Interscience: New York, 1958; pp 1781–1785. Kurtz, A. C. *J. Biol. Chem.* **1949**, *180*, 1253–1267. Janus, J. W. *J. Chem. Soc.* **1955**, 3551–3552).

(12) The position of the carbon–nitrogen double bond in **1** is still not unequivocal. The position we use comes from the X-ray structure of streptolidine obtained by hydrolysis of **1**;[6] since many subtle factors influence the magnitude of ^{13}C–^{15}N spin couplings, our data do not necessarily indicate otherwise.

(13) Others have reported such shifts: see ref 7d,e, and references cited therein. Of the numerous compounds we have made, this is only the second time we have observed an isotope shift.

(14) Determined by bioassay with *Bacillus subtilis* ATCC 6633 grown on brain-heart infusion agar.

Optical Resolution of Metal Chelates by Use of Adsorption on a Colloidal Clay

Akihiko Yamagishi*

Department of Chemistry, Faculty of Science Hokkaido University, Sapporo 060, Japan

Mitsuyuki Soma

National Instutute of Environmental Studies Tsukuba, Ibaraki 305, Japan

Received March 10, 1981

We wish to report the utilization of a colloidal clay for the optical resolution of racemic metal chelates. This research was motivated by the following findings about the racemic adsorption of *d*- and *l*-iron(II) tris(1,10-phenanthroline) chelates, [Fe(phen)$_3$]$^{2+}$, on a clay surface. In 100 mL of distilled water, 87 mg of sodium montmorillonite (Na^+M^-) (Kunipia-G, Kunimine Ind. Co., Japan) was dispersed. The resultant solution contained 1.0×10^{-3} M cation-exchange site. When Na^+M^- was added to a $(+)_{510}$-iron(II) tris(1,10-phenanthroline) bis(antimony *d*-tartrate) [(+)[Fe(phen)$_3$]$^{2+}$·(+)L^{2-}], the red solution was instantly tinted with pale pink. The absorption spectrum was measured without any serious interference by scattering. Curve a in Figure 1 shows the dependence of the increase of the apparent extinction coefficient at 530 nm ($\Delta\epsilon_{530}$) on the ratio of Na^+M^- to the metal chelate. $\Delta\epsilon_{530}$ leveled off at about [Na^+M^-]/[(+)-[Fe(phen)$_3$]$^{2+}$·(+)-L^{2-}] = 2. The same results were obtained for a solution of $(-)_{510}$-iron(II) tris (1,10-phenanthroline) bis(antimony *l*-tartrate) [(-)-[Fe(phen)$_3$]$^{2+}$·(-)-L^{2-}]. In both cases, more than 90% of the Na^+M^- was consumed for the binding with the metal chelate at [Na^+M^-]/[metal chelate] = 2.[1] Thus, each metal chelate occupies two cation-exchange sites on a clay surface when it is adsorbed from a solution of a single enantiomer.

The results were different, however, when Na^+M^- was added to the racemized solution of (+)-[Fe(phen)$_3$]$^{2+}$·(+)-L^{2-}[$^1/_2$-(+)-[Fe(phen)$_3$]$^{2+}$ + $^1/_2$(-)-[Fe(phen)$_3$]$^{2+}$·(+)-L^{2-}. As shown in curve b in Figure 1, $\Delta\epsilon_{530}$ attained a maximum value at [Na^+M^-]/[$^1/_2$(+)-[Fe(phen)$_3$]$^{2+}$ + $^1/_2$(-)-[Fe(phen)$_3$]$^{2+}$·(+)-L^{2-}] = 1 and decreased gradually with further increase of Na^+M^-. The results imply that each metal chelate occupies one cation-exchange site when it is adsorbed from a racemic solution. These conclusions also hold when (+)-L^{2-} is replaced with $2ClO_4^-$ (Figure 1).[2]

From X-ray diffraction measurements of the wet precipitates of clay–metal chelate adducts, the basal spacing of a clay sheet was determined to be 17.9 and 14.8 Å for the 1:2 (+)-[Fe(phen)$_3$]$^{2+}$/M^- and 1:1 ($^1/_2$(+)-[Fe(phen)$_3$]$^{2+}$ + $^1/_2$(-)-[Fe(phen)$_3$]$^{2+}$)/M^- adducts, respectively.[3] The former value is close

(1) The free Na^+M^- was titrated against the acridine orange cation (details will be described in a later paper).

(2) So far the racemic adsorption has been observed for [Ni(phen)$_3$](ClO$_4$)$_2$, [Fe(phen)$_2$(CN)$_2$], and [Fe(byp)$_3$(CN)$_2$]. The results on these metal chelates will be published elsewhere.

(3) For the sake of charge neutrality, $^1/_2$ equiv of (+)-L^{2-} should be included in the 1:1 ($^1/_2$(+)-[Fe(phen)$_3$]$^{2+}$ + $^1/_2$(-)-[Fe(phen)$_3$]$^{2+}$)/M^- adduct.

0002-7863/81/1503-4640$01.25/0