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STUDIES ON NITROGEN METABOLISM USING ^{13}C NMR SPECTROSCOPY. 5.[1] METABOLISM OF L-α-ARGININE IN THE BIOSYNTHESIS OF BLASTICIDIN S

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Abstract: [3-^{13}C,2-^{15}N]Arginine has been converted to the β-arginine moiety in blasticidin S with retention of both labels via an intramolecular migration of nitrogen, and [3,3-$^{2}H_2$]arginine has been incorporated with retention of both labels with one deuterium migrating from C-3 to C-2.

Blasticidin S, **1**, an antibiotic isolated from cultures of Streptomyces griseochromogenes,[3] is used commercially in Japan against piricularia oryzae (causative agent of rice blast disease).[4] Preliminary biosynthetic studies by Seto, et al,[5] demonstrated that **1** is formed from D-glucose, cytosine, L-arginine, and L-methionine. Thus, the L-β-arginine moiety, **2**, is derived from the common α-arginine, **3**. Compound **2** is also found as the side-chain of the cyclic peptide antibiotic LL-BM547β.[6] We have previously found that both the L-β-lysine portion of streptothricin F[7] and free L-β-lysine produced by Clostridium subterminale[8] are derived by a rearrangement involving the original α-nitrogen and the pro-3R hydrogen of L-α-lysine. In contrast to this, Parry and Kurylo-Borowska found that the conversion of L-α-tyrosine to the L-β-tyrosyl moiety of another Streptomyces metabolite, edeine, resulted in loss of both the original nitrogen and the pro-3S hydrogen.[9] In an effort to determine the biogenetic relationships amongst the various β-amino acids, we have now studied the L-α-arginine aminomutase reaction involved in blasticidin S biosynthesis.

As a necessary step, fully assigned ^{1}H and ^{13}C NMR were obtained by the analyses of the 400 MHz ^{1}H spectrum, 100.6 MHz ^{13}C spectrum and ^{1}H-^{1}H COSY, standard DEPT, $^{1}H/^{13}C$ HETCOR and LR HETCOSY[10] experiments.[11,12]

DL-[3-^{13}C,2-^{15}N]Arginine **3a** was synthesized in 6.3% overall yield by direct analogy to our previous preparation of the similarly labeled lysine.[13] DL-[3,3-$^{2}H_2$]Arginine **3b** was prepared in two steps from **3**. The latter was first converted to [2,3,3-$^{2}H_3$]arginine **3c** (31% yield, >95% d_3 by ^{1}H NMR).[14] The deuterium at C-2 was then almost completely removed (90%) by repeated exchange with 20% aq. HCl at 180° C in a sealed tube.[15]

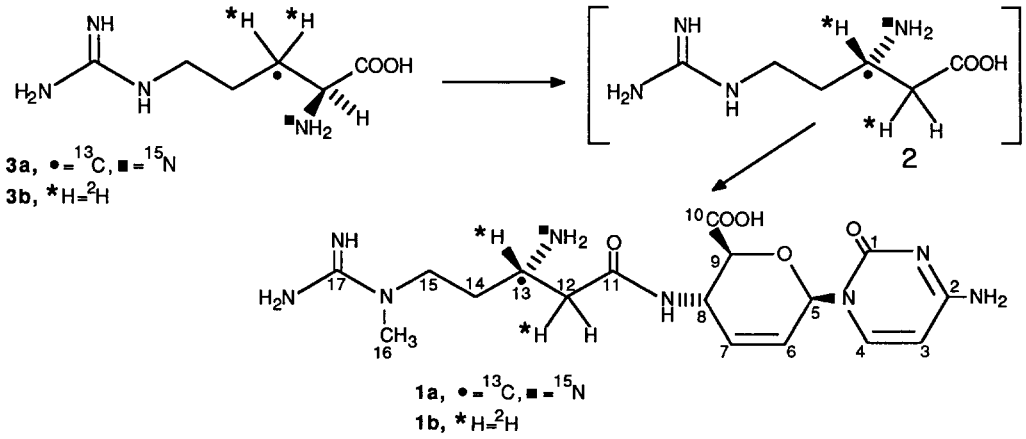
S. griseochromogenes[16] was maintained as spores on yeast-malt extract agar.[17] A seed broth[18] (50 mL in a 125 mL Erlenmeyer flask) was inoculated and grown at 29°C and 225 rpm for 48 h in a gyrotory incubator-shaker. This was then used to inoculate (2% v/v) production broths[19] (200 mL in a 1 L baffled flask[20]) that were grown under the same conditions for 120 h. Work up via ion exchange chromatographies[21] yielded crude antibiotic that was recrystallized at

pH 6.8 from CO_2-free water and MeOH to typically yield 40 mg of pure 1 (~20% recovery).[22]

Two 200 mL production broths were inoculated in standard fashion with a seed culture of S. griseochromogenes. The labeled arginine 3a·HCl (37 mg, 0.170 mmol) and DL-[1-^{14}C]arginine[23] (16.5 μCi) in 10 mL of water was divided into two equal portions and divided among the two flasks at 24 and 36 h after inoculation. After an additional 80 h, the fermentations were combined and worked up as described above to yield 77 mg of pure 1a (19% recovery). Based on the radioactivity incorporated (30% if both enantiomers were utilized), a 5.2% enrichment in ^{13}C was expected. The 100.6 MHz broad band decoupled ^{13}C NMR spectrum of 1a[24] in D_2O (pD 8.65)[25] exhibited a doublet (J_{CN}=3.2 Hz) overlapping the natural abundance singlet (1.6 Hz upfield isotope shift) for C-13 (δ46.51), revealing that the nitrogen originally at C-2 of 3a had migrated intramolecularly to the adjacent carbon atom. Normalizing the integrals against the resonance for C-15 gave an apparent enrichment of 3.8%.

We next determined the fate of the hydrogen atoms at C-3 of the precursor arginine. Again using 2x200 ml production broths, 97 mg (0.460 mmol) of 3b·HCl was fed in mixture with 10.9 μCi of DL-[1-^{14}C]arginine. After the standard work up, 70 mg (15% recovery) of pure 1b was obtained. The 61.4 MHz ^{2}H NMR spectrum in deuterium depleted water[26] revealed two resonances in addition to those from residual HOD (δ4.90) and from t-BuOH (δ1.28) added as a chemical shift reference and for quantitation of the deuterium incorporation. Thus deuterium was observed at C-12 (δ2.7) and at C-13 (δ3.7) in nearly equal amounts (46:54), indicating that hydrogen had also migrated in the rearrangement.

The arginine-2,3-amino mutase reaction so far exhibits the same characteristics as the lysine-2,3-amino mutase reaction,[8] and both are clearly different from the formation of β-tyrosine.[9] Of the remaining β-amino acids, β-alanine is derived from aspartic acid;[27] β-leucine formation from potato extracts involves a B_{12}-dependent rearrangement[28] while that from Andrographis paniculata does not[29] (no actual isotope labeling experiments have been reported



in either case); the formation of neither N,N-dimethyl-β-phenylalanine[30] nor N-methyl-β-glutamic acid[31] has been studied. Although an initial examination of the structures of these metabolites would suggest a biogenetic relationship, the accumulating experimental evidence clearly indicates this is not necessarily the case.

The stereochemistry of hydrogen migration in the arginine-2,3-aminomutase reaction is currently being studied and efforts are also underway to isolate the responsible enzyme to determine the cofactor requirements of this intriguing reaction.

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11. ^{1}H NMR (D_2O): δ 2.03 (H-14,dd,J=9.3,6.6), 2.62 (H-12b,dd,J=16.2,8.1), 2.74 (H-12a, dd,J=16.2,4.8), 3.02 (H-16,s), 3.46 (H-15,quintet,J=6.9), 4.10 (H-9,d,J=9.3), 4.73 (H-8, ddd,J=9.3,2.4,1.8), 5.85 (H-7,dd,J=10.2,2.4), 6.02 (H-3,d,J=7.5), 6.09 (H-6,ddd,J=10.2, 1.8,0.9), 6.46 (H-5,d,J=0.9), 7.59 (H-4,d,J=7.5).
12. ^{13}C NMR (D_2O): δ 3.13 (C-14), 37.8 (C-16), 39.1 (C-12), 47.6 (C-8), 48.6 (C-15), 48.7 (C-13), 79.7 (C-9), 81.8 (C-5), 98.7 (C-3), 128.2 (C-7), 135.0 (C-6), 144.9 (C-4), 158.5 (C-1), 159.6 (C-2), 168.3 (C-17), 172.9 (C-11), 177.1 (C-10).
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16. Obtained from Kaken Chemical Co., Japan (Strain ATCC 21024).
17. The agar consisted of yeast extract (0.4 g), malt extract (1.0 g), dextrose (0.4 g), agar (2.0 g) and double distilled water (100 ml). The pH was adjusted to 7.3.
18. Kaken seed medium: glucose (1.0 g), beef extract (0.5 g), Polypeptone (BBL) (0.5 g), NaCl (0.1 g) and double distilled water (50 ml) and the adjusted to pH 7.5.
19. Kaken production medium: sucrose (10 g), soybean flour (2.0 g), wheat embryo (5.0 g) dried brewers yeast (5.0 g), NaCl (1.2 g), double distilled water (200 ml) and adjusted to pH 7.0.
20. Production increased by four-fold with baffled flasks (Belco, Stock No. 2547).
21. A similar procedure as in Ref. 5b was followed. The harvested broths were centrifuged, the supernatant was adjusted to pH 3 and the resulting precipitate was removed by centrifugation. The dark brown liquid was adjusted to pH 7 and passed through a Bio-rad AG2-X8 (OH^-) column. The nonbound material and the water washings were collected and simultaneously carefuly neutralized with 6N HCl during collecting in order to avoid decomposition of blasticidin at the very high pH (~13). The resulting solution was adjusted to pH 5.5 and loaded onto a Bio-rad AG-50W-X2 (H^+) column, then washed with deionized water, 5% pyridine, and eluted with 1.2% NH_4OH. Initial pyridine-containing fractions were discarded and subsequent fractions showing 1 by TLC (silica gel, solvent nBuOH:MeOH:NH_3:H_2O = 5:2:2:1) were collected, concentrated in vacuo to remove NH_3, and then lyophilized.
22. The antibiotic was quantitated by bioassay using B. circulans.
23. Obtained from ICN.
24. Spectral width 25000, aquisition time 1.3 sec, 30,600 transients, 17 mg of **1a** in 0.5 ml D_2O, pD adjusted to 8.25 using NaOD, chemical shifts based on CH_3 resonance (1.3 ppm) of CH_3CN.
25. At neutral pH, the peaks of C-13 (48.7 ppm) and C-15 (48.6 ppm) were too close to clearly observe the splitting due to ^{13}C-^{15}N coupling of C-13. To move them sufficiently apart, the spectrum was obtained at pD = 8.65; the two peaks were separated by 1.02 ppm (C-13 at 46.51 ppm and C-15 at 47.53 ppm).
26. Obtained from Aldrich, deuterium depletion = 99.5%. Spectral parameters: sweep width 500 Hz, acquisition time 2.04 sec., 18,302 transients.
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