

Syntheses of all singly labeled [¹⁵N]adenines: Mass spectral fragmentation of adenine

(electron impact/isotope/polynitrogen heterocycle)

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ABSTRACT Syntheses of all five of the singly labeled [¹⁵N]adenines are now provided. The presence or absence of two-bond ¹⁵N-¹H spin couplings in their ¹H NMR spectra confirm the location of the isotope in each case. The fragmentation patterns in their mass spectra are indicative of the sequential losses of HCN units and of CH₂N₂ from adenine upon electron impact.

The synthesis of [6-*amino*-¹⁵N]adenine and [9-¹⁵N]adenine provided useful information concerning the fragmentation of adenine under electron impact (1). We considered that the completion of the synthesis of all of the singly-labeled [¹⁵N]adenines would be valuable not only in the determination of the total mass spectral fragmentation patterns of adenine but also in ¹⁵N NMR spectroscopic investigations of adenine and adenine-containing molecules (2-4). Moreover, the additional ¹⁵N-labeled adenines could extend the value that has already been demonstrated in the study of mechanisms of heterocyclic ring rearrangements (1, 5, 6) and loci of alkylation (7).

MATERIALS AND METHODS

Melting points were taken on a Büchi melting-point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Cary model 15 spectrophotometer. ¹H NMR spectra in ²H₂O, p²H ≈ 8.0, were determined on Varian Associates model A-60 and HR-220 spectrometers. Chemical shifts are in ppm relative to tetramethylsilane. Low-resolution mass spectra were obtained on a Varian-MAT CH-5 spectrometer.

The ¹⁵NH₃, Na¹⁵NO₂, and H¹⁵NO₃ were obtained from Bio-Rad or Los Alamos Scientific Laboratory, University of California, Los Alamos, NM. T. L. Brown (University of Illinois) also provided us with ¹⁵NH₃ originally obtained from Mound Facility, Monsanto Research, Miamisburg, OH.

[6-*amino*-¹⁵N]Adenine and [9-¹⁵N]Adenine. These singly labeled adenines were prepared by the methods of Leonard and Henderson (1).

[1-¹⁵N]Adenine. *Method A.* 6-Benzylamino[1-¹⁵N]purine (1) (100 mg in 6 ml of *t*-butyl alcohol) was treated with 2 mol equivalents of KMnO₄ (140 mg in 5 ml of water). The reaction mixture was heated gently at 60-70°C for 20-25 min. Additional aqueous 10% (wt/vol) KMnO₄ was added, and heating was continued for 10 min. After cooling, unreacted KMnO₄ was destroyed by addition of solid NaHSO₃. The mixture was acidified to pH 2.0 with HCl and was filtered through charcoal and Celite. The colorless solution was extracted with ether to remove benzoic acid and was then neutralized. Analysis by thin-layer chromatography [acetone/water, 50:50 (vol/vol), or isobutyric acid/ammonia/water, 74:1:24 (vol/vol); silica gel] revealed only one spot, which corresponded to adenine. [1-¹⁵N]Adenine was pu-

rified by chromatography on silica gel with CH₃OH/CHCl₃, 1:10 (vol/vol) as eluant. The yield was 36 mg (60%).

Method B. A second synthesis, leading to a sample of [1-¹⁵N]adenine identical by NMR and mass spectrometry with that produced by method A, utilized an intermediate that we had used previously for the synthesis of 7-benzyladenine. 1-Benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (8) was treated with ¹⁵NH₃ (2 equivalents) in ethanol at 70°C in a bomb for 24 hr, and the 7-benzyl[1-¹⁵N]adenine obtained quantitatively was debenzylated by means of sodium (2 equivalents) and ammonia (9, 10) to give [1-¹⁵N]adenine, which was recrystallized from water.

[3-¹⁵N]Adenine. A procedure in this laboratory that used 7-benzyladenine as an intermediate (8, 11) was adapted to ¹⁵N labeling. The starting material, 4-bromo-5-[¹⁵N]nitroimidazole, was prepared as described for the unlabeled compound (12). 4-Bromoimidazole (2.33 g) in absolute ethanol (12 ml) was cooled at 0°C and carefully treated with 70% aqueous H¹⁵NO₃ (1 ml). The solution was evaporated *in vacuo*, and the residue was treated with concentrated sulfuric acid (12 ml) and heated at 90°C for 1 hr. The cooled reaction mixture was diluted carefully with cold water, and the off-white solid that deposited was collected by filtration and dried (2.51 g). Subsequent steps (8) included the following: benzylation to 1-benzyl-5-bromo-4-[¹⁵N]nitroimidazole; cyanide displacement to 1-benzyl-5-cyano-4-[¹⁵N]nitroimidazole; Raney nickel hydrogenation to 4-[¹⁵N]amino-1-benzyl-5-cyanoimidazole; ethyl orthoformate condensation to 1-benzyl-5-cyano-4-ethoxymethylene[¹⁵N]aminoimidazole; ring closure with ethanolic ammonia (8) to 7-benzyl[3-¹⁵N]adenine, NMR [(C²H₃)₂SO] (purine protons) δ 8.41 (1, s, 8-H), 8.19 (1, d, J = 15 Hz, 2-H); and sodium/ammonia hydrolysis (9, 10) to [3-¹⁵N]adenine.

[7-¹⁵N]Adenine. The route was patterned after nitrosation and reduction steps that were described by Evans *et al.* (13), and three trial runs were carried out initially with unlabeled material. [4,6-¹⁴N,5-¹⁵N]Triaminopyrimidine was prepared as follows. To a solution of 4,6-diaminopyrimidine hydrochloride (489 mg) in 1 M HCl, cooled to between 0°C and -5°C, was added a solution of Na¹⁵NO₂ (345 mg) in water (3.7 ml) over a period of 20 min. The solution was stirred for an additional hour and was allowed to come to room temperature before addition of NaHCO₃ to ca. pH 7.5. The 4,6-diamino-5-[¹⁵N]nitrosopyrimidine was collected by filtration, washed with cold water, and suspended in water (7 ml) at 60°C. Sodium dithionite (1.2 g) was added in portions, giving a clear orange solution. This was heated to boiling, acidified with 50% (vol/vol) aqueous sul-

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Table 1. Mass spectra of ^{15}N -labeled adenines

Ratios of m/e values	Fragmentation pattern					
	Ade, %	[$1\text{-}^{15}\text{N}$]Ade,* %	[$3\text{-}^{15}\text{N}$]Ade, %	[$6\text{-amino-}^{15}\text{N}$]Ade, %	[$7\text{-}^{15}\text{N}$]Ade, %	[$9\text{-}^{15}\text{N}$]Ade, %
M^+ ($\text{C}_5\text{H}_5\text{N}_5$) 136/(135 + 136)	8	97	93	91	93	92
[M-HCN] $^+$ 109/(108 + 109)	12	13	85	78	91	89
[M-2HCN] $^+$ 82/(81 + 82)	3	19	63	57	70	30
[M-HCN- CH_2N_2] $^+$ 67/(66 + 67)	38	20	43	50	36	41
[M-3HCN] $^+$ 55/(54 + 55)	15	21	40	45	51	21
	Average % of base peak					
m/e values						
136	9.2	100	100	100	100	100
135	100	2.7	7.5	9.4	7.3	8.7
109	6.8	4.1	28	35	30	30
108	50	28	4.8	9.8	2.9	3.8
82	0.7	2.6	8.8	10	10	4.8
81	21	11	5.2	7.5	4.2	11
67	6.2	1.7	4.1	4.4	2.0	4.3
66	10	6.6	5.5	4.4	3.6	6.1
55	3.7	3.3	7.3	8.1	10	4.1
54	21	12	11	10	9.5	15

* Made from 99% [^{15}N]ammonia.

furic acid, boiled for a few minutes, filtered hot to remove particulate matter, and cooled to room temperature. The precipitate was collected by filtration, washed sequentially with water, ethanol, and ether, and dried to give 306 mg of the sulfate salt. A solution of [4,6- ^{14}N , 5- ^{15}N]triaminopyrimidine sulfate (300 mg) in formic acid (10 ml) was heated at reflux under nitrogen. After 6 hr, sodium formate (500 mg) was added; the total reflux time was 60 hr. Formic acid was removed *in vacuo*. The residue was dissolved in a minimum amount of water, and the solution was adjusted to pH 7.8 and concentrated to half volume. The precipitated [7- ^{15}N]adenine was collected. Recrystallization from water yielded 106 mg of [7- ^{15}N]adenine (24% over-all yield).

RESULTS AND DISCUSSION

Syntheses. Syntheses of [6-*amino*- ^{15}N]adenine and [9- ^{15}N]adenine have been described (1). For the synthesis of [1- ^{15}N]adenine, we found that we could destroy the sidechain of 6-benzylamino[1- ^{15}N]purine (1) selectively by oxidation with potassium permanganate in *t*-butyl alcohol/water. In this case and for the other isotopically labeled adenines that were prepared, the location of the label was established by the route of synthesis and was checked by the ^{15}N - ^1H nuclear magnetic spin coupling through two bonds. The extent of ^{15}N labeling in the adenine products was determined by mass spectrometry. A second synthesis of [1- ^{15}N]adenine was based on the treatment of 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (8) with $^{15}\text{NH}_3$ in ethanol in a bomb, followed by debenylation of the intermediate 7-benzyl[1- ^{15}N]adenine with sodium and ammonia.

For the synthesis of [3- ^{15}N]adenine, the pyrimidine ring was fashioned onto an appropriately substituted imidazole (8, 9). The external nitrogen of the product of nitration of 4-bromoimidazole with H^{15}NO_3 , namely, 4-bromo-5-[^{15}N]nitroimidazole, became the N-3 of the labeled adenine by the substantiated route outlined. To make [7- ^{15}N]adenine, the imidazole ring was fashioned onto [4,6- ^{14}N , 5- ^{15}N]triaminopyrimidine. In this intermediate, labeling of the central nitrogen among the three amino groups was achieved by nitrosation of 4,6-diaminopyrimidine with H^{15}NO_2 , followed by reduction with sodium dithionite (13).

^1H NMR Spectra. The chemical shifts relative to 2,2-dimethyl-2-silapentane-5-sulfonate of the H-2 and H-8 protons of the singly labeled [^{15}N]adenines in $^2\text{H}_2\text{O}$, $\text{p}^2\text{H} \approx 8.0$, were identical for the five compounds, within the limits of instrumental error, and were singlets except where they were adjacent to ^{15}N . Then they were doublets as follows: [1- ^{15}N]adenine, 2-H, $J = 14$ Hz; [3- ^{15}N]adenine, 2-H, $J = 13$ Hz; [7- ^{15}N]adenine, 8-H, $J = 12.5$ Hz; and [9- ^{15}N]adenine, 8-H, $J = 12.5$ Hz—all in $^2\text{H}_2\text{O}$ at $\text{p}^2\text{H} \approx 8.0$. The coupling constant previously reported (1) for H-8, 10 Hz, for the last compound was in $(\text{C}^2\text{H}_3)_2\text{SO}$ solution. The H-2 of [6-*amino*- ^{15}N]adenine was a singlet, with no suggestion of an attendant doublet, indicating that there had been no appreciable rearrangement during the process of synthesis used for this compound (1). The difference in magnitude between the two-bond ^{15}N - ^1H spin couplings for H-2 and H-8 in these labeled adenines, as determined in $^2\text{H}_2\text{O}$, was not as great as the differences reported in $(\text{C}^2\text{H}_3)_2\text{SO}$, although the sign of the difference was the same (1).

Mass Spectrometry Results. The results of the fragmentation of the five ^{15}N -labeled adenines at 70 eV in the mass spectrometer are given in Table 1. The data were obtained as computer output, and the m/e ratios were averaged over three determinations. They represent relative amounts of incorporation of one ^{15}N atom for key fragment ions, if allowance is made for the m/e ratios to be expected for adenine itself as a norm (left column). Because the isotope ratios have not been determined at high resolution in this work, there are slight numerical variations from the results reported earlier for two of the [^{15}N]adenines (1). However, the conclusions with regard to the fragmentation patterns for adenine are reinforced and are now extended by the inclusion of the other three ^{15}N -labeled adenines, with reference to Table 1, as follows: (i) the loss of the first HCN from the molecular ion involves principally N-1, whereas N-7 and N-9 are completely retained; (ii) the loss of the second HCN involves N-9 to about half the extent of N-3, N-7, and N⁶ together; (iii) the loss of three HCN units leaves some of the original N-3, N-7, and N⁶; and (iv) the loss of HCN and CH_2N_2 from the molecular ion leaves some of the same nitrogens in the fragments of m/e 67(66), whereas the geminal nitrogens N-3 and N-9 are lost to approximately the same extent by this route.

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