in molecules in aqueous solutions. We are thus led to conclude that our LMO-VCD and absorption intensities would be in closer absolute agreement if alanine were sampled in a nonaqueous solution.

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Registry No. Alanine-N-d_3, 19470-97-4; alanine-C^*-d_1-N-d_3, 27539-86-2; alanine-C-d_3-N-d_3, 73674-54-1.

Mass Spectrometry of Nucleic Acid Constituents. Electron Ionization Spectra of Selectively Labeled Adenines

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Abstract: The unimolecular decomposition of adenine following electron ionization has been studied on the basis of extensive isotopic substitution to establish the extent of site selectivity in fragment ion formation. Carbon-13 labeling at C-2, with earlier published work on [^{15}N]adenines, shows that elimination of HCN from the molecular ion is more than 90% derived from N-1 and C-2. Loss of NH_2 and NH_3 is predominantly from N-1, with complete retention of N-7 and N-9. $C_2H_4N_3^+$ (m/z 70) is formed by a rapid process with quantitative retention of N-1, C-2, N-3, C-4, and N-9. These results permit the assignment and estimation of ^{13}C and ^{15}N within the adenine nucleus, of potential value in studies of purine biosynthesis and metabolism. The adenine molecular ion is shown to be structurally identical with the m/z 135 fragment ion from adenosine on the basis of their collision-induced decomposition mass spectra. Equations are given for calculation of isotopic incorporation levels in fragment ions of adenine.

In recent years mass spectrometry has assumed an important role in the characterization of synthetic and natural nucleic acid bases and nucleosides.[1] In terms of systematic examination of detailed fragmentation processes, as opposed to largely descriptive reports of spectra, the greatest effort has been expended on nucleosides rather than the corresponding bases.[1-3] This has been due largely to the greater difficulty of introduction of isotopic labels into selected positions of the heterocyclic nucleus and to the inherently complex nature of heteroaromatic decomposition processes,[4,5] which further strengthens the need for selectively labeled models. In addition to purposes of structural characterization, study of the unimolecular decomposition processes of purines and pyrimidines serves to further expand the knowledge of the behavior of polynitrogen heterocycles, an area of gaseous ion chemistry that has received generally superficial attention with respect to mechanistic details. In the case of purines such as adenine and guanine, the establishment of site selectivity in the formation of fragment ions is of potential value for the mass spectrometric location of stable isotopes in the purine nucleus, in studies of purine biosynthesis and metabolism.[6]

The mass spectrum of adenine was first reported by Shannon and Letham,[7] and independently by Rice and Dudek,[8] both of whom outlined the principal ionic decomposition routes. In subsequent work, Occolowitz[9] studied the sequential expulsion of two HCN molecules using [8-^{14}C]adenine and N-deuterated adenines, while Leonard and Henderson examined the same reaction series on the basis of ^{15}N labels in two positions[10] and later in each of the five possible positions.[11] The inclusion of N-1 in the first loss of HCN was demonstrated by ^{15}N labeling,[11] contrary to expectations resulting from ^{2}H- and ^{14}C-labeled adenines.[9]

We have studied the fragmentation processes of adenine on the basis of its high-resolution mass spectrum, on decomposition pathways established by metastable ion measurements, and on extensive isotopic substitution. The five isomeric ^{15}N-labeled adenines have been synthesized and examined, along with [2-^{13}C]-, [8-^{14}C]-, [2-^{2}H]-, and [8-^{2}H]adenine, to establish the extent of positional selectivity in the formation of five sets of fragment ions that have not previously been examined.

Experimental Section

General Remarks. [^{15}N]Ammonia (several lots, >99 atom %) used in syntheses was purchased from Mound Laboratories, Miamisburg, OH. [2-^{13}C]Adenine was obtained from Merck Isotopes, St. Louis, MO, and [8-^{14}C]adenine from I.C.N., City of Industry, CA. [2-^{2}H]Adenine was prepared by back exchange of D-8 (100 °C, 1 h) in [2,8-$^2H_2$]adenine, which had earlier been prepared by a catalytic exchange procedure.[12]

Starting materials for the [^{15}N]adenines were prepared by literature procedures; however, the reaction conditions reported in the following sections commence with the first introduction of the ^{15}N isotope. An authentic unlabeled sample of each compound was used for direct TLC comparison to corroborate structural assignments of the labeled adenines and of all intermediates. Ultraviolet spectra were recorded with a Beckman Acta C III spectrophotometer and infrared spectra were recorded on a Beckman IR 100 spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected.

[1-^{15}N]Adenine (3). A mixture of 4(5)-amino-5(4)-cyanoimidazole[13] (1, 48 mg) and diethoxymethyl acetate[14] (DEMA, 3.5 mL) was stirred

(1) Hignite, C. In "Biochemical Applications of Mass Spectrometry"; Waller, G. R., Dermer, O. C., Ed.; Wiley-Interscience: New York, 1980; pp 527-566.
(2) Hignite, C. In "Biochemical Applications of Mass Spectrometry"; Waller, G. R., Ed.; Wiley-Interscience: New York, 1972; pp 429-447.
(3) McCloskey, J. A. In "Basic Principles in Nucleic Acid Chemistry", Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. I, pp 209-309.
(4) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. "Mass Spectrometry of Organic Compounds"; Holden-Day: San Francisco, 1967; pp 582-595.
(5) Porter, Q. N.; Baldas, J. "Mass Spectrometry of Heterocyclic Compounds"; Wiley-Interscience: New York, 1971.
(6) *Adv. Exp. Med. Biol.* **1980**, *122B*, 1-86.
(7) Shannon, J. S.; Letham, D. S. *N. Z. J. Sci.* **1966**, *9*, 833-842.
(8) Rice, J. M.; Dudek, G. O. *J. Am. Chem. Soc.* **1967**, *89*, 2719-2725.
(9) Occolowitz, J. L. *Chem. Commun.* **1968**, 1226-1227.
(10) Leonard, N. J.; Henderson, T. R. *J. Am. Chem. Soc.* **1975**, *97*, 4990-4999.
(11) Barrio, M. C. G.; Scopes, D. I. C.; Holtwick, J. B.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3986-3988.
(12) Kinoshita, T.; Schram, K. H.; McCloskey, J. A. *J. Labelled Compd. Radiopharm.*, in press.
(13) Ferris, J. P.; Orgel, L. E. *J. Am. Chem. Soc.* **1966**, *88*, 3829-3831.

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and heated at 115–120 °C for 6 h. The solvents were removed by evaporation under reduced pressure to yield 66 mg (90.6%) of 5(4)-cyano-4(5)-[(ethoxymethylene)amino]imidazole (**2**) as an oil. A solution of **2** (60 mg) in absolute ethanol (8 mL) was placed in a glass tube (2.2 cm in diameter; 8.2 cm height) and cooled in a 2-propanol–dry-ice mixture (15–25 °C). [^{15}N]Ammonia 0.0987 g; 125 mL at NTP) was passed into this solution through a flowmeter at the rate of 10–12 mL/min, for a total of 11–12 min (the ammonia cylinder was previously cooled in a 2-propanol–dry ice bath at −5° to −10 °C for 15–20 min). The glass tube was then sealed in a stainless steel reaction vessel and heated, with stirring, at 160–65 °C for 24 h. The reaction vessel was then cooled to room temperature and the mixture evaporated to dryness under reduced pressure. The crude residue was dissolved in water (4 mL) and purified by HPLC using the solvent system H_2O–CH_3CN 96:4 (v/v) as an eluant. The combined fractions were evaporated to dryness to yield 33 mg (66.3%) of a white solid; mp >300 °C.

[3-^{15}N]Adenine (11). This synthesis follows closely the method previously reported.[11,15] The starting material, 4-bromo-5-[^{15}N]nitroimidazole (**5**), was prepared by nitration of 4-bromoimidazole (**4**) with $H^{15}NO_3$–H_2SO_4.[11] Subsequent steps in the synthesis included the following: benzylation to 1-benzyl-5-bromo-4-[^{15}N]nitroimidazole (**6**), cyanide displacement to 1-benzyl-5-cyano-4-[^{15}N]nitroimidazole (**7**), Raney nickel hydrogenation to 4-[^{15}N]amino-1-benzyl-5-cyanoimidazole (**8**), diethoxymethyl acetate condensation to 1-benzyl-5-cyano-4-[(ethoxymethylene)[^{15}N]amino]imidazole (**9**),[14] ring closure with ethanolic ammonia to 7-benzyl[3-^{15}N]adenine (**10**),[15] and sodium–ammonia hydrogenolysis to [3-^{15}N]adenine (**11**). The detailed experimental conditions for the synthesis of compounds **5–11** are reported in the supplementary material.

[6-*amino*-^{15}N]Adenine (13). This labeled adenine was prepared by the reaction of $^{15}NH_3$ with 6-chloropurine (**12**) by the method of Leonard and Henderson.[10] The experimental conditions for the synthesis and purification of **13** are presented in the supplmenetary material.

4,6-Diamino-5-[^{15}N]nitrosopyrimidine (15). A solution of 4,6-diaminopyrimidine (**14**) in 1 N HCl (5 mL) was cooled (0–5 °C) in a 2-propanol–dry ice bath. Sodium [^{15}N]nitrite (75 mg; 1.07 mmol) in 2 mL of water was added over a period of 5 min with cooling. The solution was stirred for 1 additional h, with the temperature being allowed to rise to room temperature. The pH of the solution was adjusted to 8 with solid $NaHCO_3$. The blue nitroso compound was collected by filtration and washed with a small amount of cold water. The crude solid was recrystallized from an ethanol–water (9:1, v/v) mixture to yield 90 mg (78.9%) of **15**.

[4,6-^{14}N,5-^{15}N]Triaminopyrimidine (16). A solution of **15** (85 mg) in absolute ethanol (30 mL) was reduced at 35 psi of hydrogen at room temperature in the presence of commercial Raney nickel (0.52 g). The catalyst was separated by filtration through Celite, washed with a small amount of ethanol, and then evaporated to dryness in vacuo under reduced pressure to yield 58 mg (75.4%) of **16**: mp 256–258 °C.

[7-^{15}N]Adenine (17). A mixture of **16** (55 mg; 0.43 mmol) and diethoxymethyl acetate (5 mL) was stirred and heated at 115–120 °C for 10 h. The solvents were then removed under reduced pressure. The residue was dissolved in water (5 mL) and purified by HPLC using the solvent system H_2O–CH_3CN (96:4, v/v) as the eluant. Fractions containing the product were combined and evaporated to dryness to afford 39 mg (63.8%) of a white solid; mp >300 °C.

[4-^{15}N,5-^{14}N]Diamino-6-chloropyrimidine (19) and [4,6-^{15}N,5-^{14}N]-Triaminopyrimidine (19a). A suspension of 5-amino-4,6-dichloropyrimidine[16] (**18**, 0.5 g) in 10 mL of absolute ethanol in a Pyrex glass tube (2.2 cm in diameter and 8.2 cm long) was cooled in an acetone–dry ice bath. A stream of [^{15}N]ammonia was passed into the suspension until 0.2 g of the ammonia had dissolved in the ethanol. The glass tube was sealed with a flame, cooled, and placed in a stainless steel reaction vessel. Ethanol was added to the vessel to cover one-third of the glass tube. The reaction vessel was sealed and heated at 150 °C in an oil bath for 4 h. The reaction vessel was cooled, and solid crystals separated in the glass tube. This solid was collected and recrystallized from ethanol; mp 242–245 °C, yield 0.344 g (98%). A mass spectrum of the crystalline product showed it to be 94% of **19** and 6% of **19a**.

6-Chloro-[9-^{15}N]purine (20). The mixture of **19** and **19a** (0.344 g), was dissolved in 3 mL of 1:1 acetic anhydride–triethyl orthoformate. The solution was heated under reflux for 1.5 h, then cooled, and evaporated to dryness at 1–2-mm pressure. The residue was warmed at 40 °C for 15 min with 4 mL of 10% NaOH solution. The solid dissolved, and the solution was clarified with carbon and filtered. The filtrate was acidified to pH 5 and partially evaporated under reduced pressure. The solid that separated was collected, dried, and repeatedly crystallized from a mixture of ethanol and water to yield 0.175 g (51%) of **20**; mp >300 °C.

[9-^{15}N]Adenine (21). **20** (0.175 g) was added to 5 mL of 1-butanol containing 0.35 g of dissolved NH_3. The solution was sealed in a Pyrex glass tube and placed in a stainless steel reaction vessel containing 3–4 mL of 1-butanol. The reaction vessel was sealed and heated in an oil bath at 150 °C for 20 h. After cooling, the glass tube was opened, and the contents evaporated to dryness under reduced pressure. The resulting solid was crystallized seven times from hot alcohol to give a small yield of pure **21**, mp >300 °C, which gave one spot on a TLC silica plate, developed in a solvent system consisting of the upper phase collected from a vigorously shaken mixture of ethyl acetate–*n*-propyl alcohol–water 4:1:2.

Mass Spectrometry. Low-resolution mass spectra were recorded by using an LKB 9000S instrument, with samples introduced by direct probe, ionizing energy 70 eV and ion source temperature 250 °C. The resulting relative-ion abundance data, given in the supplementary material, represent the mean of ten consecutive scans. Compositions of the major ions reported by Rice and Dudek[8] were confirmed, and compositions were established for m/z 27–29, 40–43, 55, 56, 65, 68–70, and 92–94 from measurements of exact mass made with a Varian MAT 731 mass spectrometer, by peak matching and photographic recording. The complete composition list for all ions is given in the supplemental material. Metastable ion measurements were made with the Varian instrument using two techniques. A linked scan at constant B/E, where B is the magnetic field and E is the sector voltage, records all daughter ions that are formed in the first field-free region (between ion source and electric sector) by the decay of a given precursor ion. The second method involves scanning the accelerating voltage with fixed electric sector voltage and fixed magnetic field. This method detects the precursors of a given daughter ion formed in the first field-free region. Collision-induced decomposition (CID) mass spectra of adenine and adenosine were recorded at the Midwest Center for Mass Spectrometry with a Kratos-MS-50 triple analyzer instrument, under the following conditions: sample introduction by direct probe, ionizing energy 70 eV, accelerating voltage 8 kV, resolution ($M/\Delta M$) 3000. Collisional activation of m/z 135 ions was carried out by using He at pressure sufficient to cause 50% attenuation of the incident beam. CID spectra were determined by scanning the third sector, which is an electrostatic analyzer.

Results and Discussion

Scheme I shows the principal unimolecular decomposition routes and ion compositions, summarized from earlier work[7,8] and the present study.[17] The major pathways, as represented by metastable ions, are indicated, while additional minor and alternate pathways are not shown.

Calculations of ^{13}C or ^{15}N Retention. Measurement of ^{13}C (or other isotopic) compositions of ions can be made, with high-resolving power[10] to distinguish ^{13}C vs. ^{12}CH or ^{15}N vs. ^{14}NH differences in ion groups that contain several species which differ by H in unlabeled adenine. We have used an alternative method that requires nominal mass resolving power and knowledge of ion abundances and compositions from the unlabeled compound. An example of the method is given below for the first loss of HCN from the molecular ion. Expanded forms of the calculation for the second and third sequential losses of HCN and for M − HCN − NH_2CN, M − NH_2, and M − NH_3 in the mass spectrum of adenine are given in the supplementary material. The equations are mostly expressed in terms of ^{13}C but are applicable to any isotopic label, such as ^{15}N or ^{2}H.

Loss of hydrogen cyanide from [^{13}C]adenine leads to the following species, where an asterisk denotes the presence of ^{13}C or other heavy isotope: m/z 107, M* − H_2CN*, M − H_2CN; m/z 108, M* − HCN*, M* − H_2CN, M − HCN; m/z 109, M* − HCN. Ions M − HCN and M − H_2CN represent contributions from unlabeled adenine in the synthetic material (typically several percent) and are readily corrected for. Relative intensity values

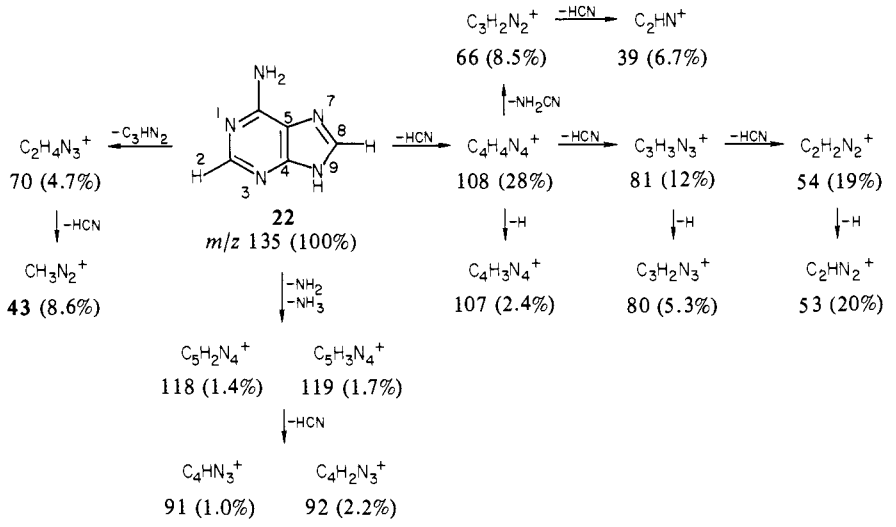
(14) Montgomery, J. A.; Fitzgibbon, W. E. In "Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods, and Techniques", Townsend, L. B., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1978; Vol. 2, 995–997.

(15) This synthesis follows the experimental procedures reported by: Leonard, N. J.; Carraway, K. L.; Helgeson, J. P. *J. Heterocycl. Chem.* **1965**, *2*, 291–297.

(16) Lin, H. H.; Price, C. C. *J. Org. Chem.* **1961**, *26*, 264–265.

(17) Ion abundance data from [2-^{13}C]adenine and the five mono-^{15}N-labeled adenines studied, as well as a complete compositional list of ions in the adenine mass spectrum that do not contain natural heavy isotopes, are given in the supplementary material.

Scheme I



(I) representing loss of HCN and H_2CN can be represented by eq 1. The contribution from $I_{(M^\bullet - H_2CN)}$ in m/z 108 can then

$$(I_{108}/I_{107})_{adenine} = [(I_{M^\bullet - HCN} + I_{M^\bullet - HCN^\bullet})/(I_{M^\bullet - H_2CN} + I_{M^\bullet - H_2CN^\bullet})]_{adenine^\bullet} \quad (1)$$

$$I_{M^\bullet - H_2CN} = (I^*_{109} + I^*_{108} - AI_{107})/(A + 1) \quad (2)$$

be represented in terms of relative abundances (eq 2), where $A = (I_{108}/I_{107})$ for adenine. The fraction of label remaining after the first loss of HCN is then

$$(M^* - HCN)/[(M^* - HCN) + (M^* - HCN^*)] = I^*_{109}/(I^*_{109} + I_{108} - I_{M^\bullet - H_2CN}) \quad (3)$$

After substitution of $I_{M^\bullet - H_2CN}$ from eq 2, the percent of ^{13}C retained can be calculated from eq 4,

$$\%\ ^{13}C\ \text{retained} = I^*_{109}(A + 1)/[A(I^*_{109} + I^*_{108} - I^*_{107})] \times 100 \quad (4)$$

using experimentally determined ion abundance data.

Isotope retention values can also be calculated simply from peak ratios due to labeled and unlabeled species.[11] However, failure to take into account all contributions to a given ion type (m/z 107, 108, and 109, as opposed to m/z 108 and 109, in the preceding example) can lead to errors of varying magnitude, depending on the relative abundances of the ions involved. For example, consideration of m/z 80 (M - 2(HCN) - H, Scheme I) in addition to m/z 81 and 82 in [9-^{15}N]adenine gives an ^{15}N retention value of 37% (present study), while omission of m/z 80 gives only 30%, the same value reported by using the shorter method.[11] Our value of 38% agrees well with the inherently more accurate value of 39% obtained with high-resolution mass spectrometry[10] in which all isotopic species are resolved. It is noted that the use of the more detailed method we describe would not have qualitatively altered the conclusions reached earlier,[11] but if these results are to be used for quantitative measurements of isotopic retention, the more accurate method should be used.

Sequential Expulsion of HCN. The sequential elimination of HCN is a major process in the mass spectra of polynitrogen heterocycles and constitutes the principal reaction sequence in many purine derivatives,[5] including purine itself.[18]

Previous examinations of the five ^{15}N-labeled adenines demonstrated approximately 87% loss of N-1 in the first expulsion of HCN, with much less specificity found in the second and third losses.[11] The spectrum of [2-^{13}C]adenine shows that 98% of the carbon in the first HCN is from C-2 (Table I), rather than C-6 or other positions that would indicate restructuring of the skeleton following ring opening. Taken together, the ^{15}N (ref 11) and ^{13}C data indicate that the first loss is over 90% site specific for N-1 and C-2, and 6–8% for C-2 and N-3.[19] Initial ring opening may thus be assumed to occur at 1,6 (structures a, b) or 2,3 (c, d). An earlier mechanism[7] depicting the loss of C-6 and N^6, made without the benefit of skeletal labeling, is therefore incorrect. The conclusion by Occolowitz that 55% loss of the first HCN originates from C-6 and N^6 and 45% from C-2 and N-1, N-3 was based in part on deuterium labeling (evidently at N^6, N-9, and C-8).[9] Our measurement of [2-^{2}H]adenine and [8-^{2}H]adenine shows 43% and 10% loss of D from C-2 and C-8 in the first loss, which points to the error in the earlier conclusion.[9] Indications as to which *skeletal atoms* are involved in the reaction, based on deuterium labeling, are not reliable due to extensive rearrangement of hydrogen prior to expulsion of HCN.

Table I. Isotopic Composition of Ions from [2-^{13}C]Adenine

ion	m/z	% ^{13}C retained[a]
M	135	100
M – HCN	108	2
M – 2(HCN)	81	13
M – 3(HCN)	54	9
M – HCN – NH_2CN	66	33
M – C_3NH_2	70	100

[a] Corrected for naturally occurring heavy isotopes and for 11% residual ^{12}C at C-2.

It is of interest that more ^{15}N is apparently retained in the M – 2(HCN) ion (19%) than in the M – HCN (13%).[11] This points to the existence of a population of M – 2(HCN) ions not derived from M – (N-1)HCN, but rather by pathways involving initial HCN loss from the other four nitrogens. This interpretation is supported by the spectrum of [2-^{13}C]adenine, which shows 13% retention of C-2 in M – 2(HCN) compared with 2% retained in M – HCN. A similar divergence of pathways is found in the M – HCN – NH_2CN ion (m/z 66, Table I), in which the ^{13}C content is greater than in M – (N-1,C-2)HCN. The main population of m/z 66 ions is therefore not derived from the principal M – HCN species in which N-1,C-2 has been lost. The elimination of the third HCN (m/z 54) is relatively nonselective, both with regard to C-2 (Table I) and to the five nitrogen atoms.[11]

Loss of NH_3 and NH_2: m/z 118 and 119. The loss of NH_3 and NH_2 from the molecular ion (Scheme I) constitutes small but significant decomposition processes. Equations used for calculations of ^{15}N retention are given in the supplementary material. Contrary to the expectation that both reactions would involve cleavage of the C-6–N^6 bond with loss of N^6, the dominant process involves loss of N-1 (Table II) and either 0% (M – NH_3)

(18) Goto, T.; Tatematsu, A.; Matsuura, S. *J. Org. Chem.* **1965**, *30*, 1844–1846.

(19) Nitrogen-15 retention data for the first three losses of HCN, calculated using the method described, agree qualitatively with those reported earlier,[11] and are listed in the supplementary material (Table 4).

Table II. Isotopic Composition of Ions from Selectively Labeled [^{15}N]Adenine

ion	m/z	% ^{15}N retained[a]				
		N-1	N-3	N^6	N-7	N-9
M	135	100 (96.4)[b]	100 (96.1)	100 (97.1)	100 (97.8)	100 (96.0)
M – NH_2	119	55	84	78	100	98
M – NH_3	118	18	75	100	100	100
M – C_3HN_2	70	100	100	0	0	100

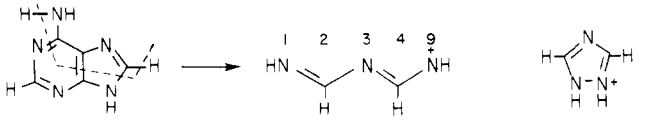
[a] Corrected for naturally occurring heavy isotopes and for residual ^{14}N at the labeled site. [b] Values in parentheses show mol % ^{15}N introduced by synthesis, measured from the molecular ion.

or 22% (M – NH_2) of N^6. Essentially complete retention of nitrogen in the imidazole ring is observed, providing a useful means of distinguishing the pyrimidine and imidazole nitrogens in labeled adenines. Further expulsion of HCN from ions *m/z* 118 and 119 produces the minor ions *m/z* 91–94. Their low abundances precluded accurate measurement of ^{13}C or ^{15}N retention.

It is interesting that NH_3 is lost from adenine in high yield following UV irradiation.[20] Little effect was noted by irradiation at wavelengths longer than 230 nm, so that adenine is probably susceptible only to ionizing radiation below 200 nm. On the basis of similar reactions of hypoxanthine, the corresponding nucleoside inosine, and other models, it was concluded that the nitrogen lost is from N-1 and/or N-3.

When considered in addition to the results from the first loss of HCN (from C-2, N-1), the present results imply that a significant fraction of decomposing molecular ions may exist in noncyclic states. It is notable in this regard that ^{13}C and ^{15}N labeling of 2-, 3-, and 4-cyanopyridines revealed that expulsion of HCN from the molecular ion occurs predominantly from the ring rather than from the cyano substituent, through an open-chain intermediate.[21] The loss of NH_3 from N-1 requires the rearrangement of three hydrogens from the dominant (in solution) tautomer **22**, so that the imino structure b is favored over other forms as an initial intermediate.

Ions *m/z* 70, 43, and 28. The mass 70 ion was found by measurement of exact mass to be $C_2H_4N_3^+$. Further decomposition by elimination of HCN produces *m/z* 43. No detectable precursor of *m/z* 70 was found, based on either of the two metastable ion measurement techniques described in the Experimental Section. Further, the collision-induced decomposition spectrum produced from the adenine molecular ion (Figure 1) shows *m/z* 70 as a sharp peak. These characteristics suggest its formation to be fast, an interpretation borne out by an exceptionally clear isotopic retention pattern: total absence of N^6 and N-7, and complete retention of C-2, N-1, N-3, and N-9. Hydrogens H-2 and H-8 are likewise completely retained without evidence of randomization or scrambling as in the case of other ions. From the mass spectrum of [8-^{14}C]adenine, it was established that C-8 is lost. These data thus define the composition of the *m/z* 70 ion with regard to every atom in the molecule: the contiguous skeletal atoms, N^1–C^2–N^3–C^4–N^9, plus all hydrogens except one active hydrogen are present. These data do not permit assignment of ion structure, but both open and cyclic stable forms are possible, as shown below. Unaccompanied by related ion



species differing in mass by 1, this ion should be useful for analytical differentiation of carbon and nitrogen isotopes within two major atomic groupings: N-1, N-3, N-9 vs. N^6, N-7; and C-2, C-4 vs. C-5, C-6, C-8.

The prominent low mass ion *m/z* 28 is due exclusively to H_2CN^+, as established by its exact mass. Contributions from the

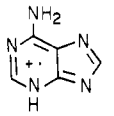
Figure 1. Collision-induced dissociation mass spectra from *m/z* 135: (a) molecular ion of adenine, (b) base + H fragment ion of adenosine.

five nitrogens are 16% N-1, 25% N-3, 21% N^6, 19% N-7, and 17% N-9 (total accounted for, 98%), reflecting a largely random distribution.

Formation of Adenine as a Fragment Ion from Adenosine. A principal and characteristic decomposition process in ribonucleosides involves production of the odd-electron free base ion by cleavage of the glycosidic bond with rearrangement of one hydroxyl hydrogen to the base, primarily from 0-2′.[22,23] Further expulsion of three molecules of HCN follows, producing an ion abundance pattern similar to that from adenine.[22] A collision-induced decomposition spectrum produced from the mass-selected adenine molecular ion (*m/z* 135), Figure 1a, was compared with that generated by the *m/z* 135 fragment ion of adenosine, Figure 1b. Under such circumstances, identical fragmentation patterns would indicate identical ion structures, independent of internal energy content and distribution due to formation from different precursors.[24] The very close correspondence of the two spectra

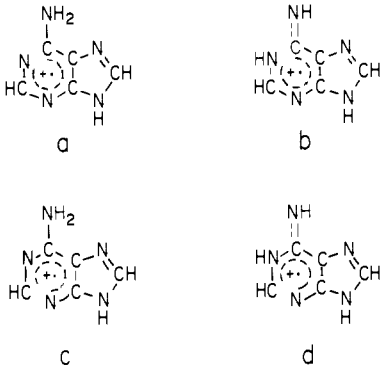
(20) Canzanelli, A.; Guild, R.; Rapport, D. *Am. J. Physiol.* **1951**, *167*, 364–374.
(21) Molenaar-Langeveld, T. A.; Vermeulen, N. P. E.; Nibbering, N. M. M.; Morgan, R. P.; Brenton, A. G.; Beynon, J. H.; Sen Sharma, D. K.; Jennings, K. R. *Org. Mass Spectrom.* **1979**, *14*, 524–531.
(22) Biemann, K.; McCloskey, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 2005–2006.
(23) Shaw, S. J.; Desiderio, D. M.; Tsuboyama, K.; McCloskey, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 2510–2522.
(24) Levsen, K.; Schwarz, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 509–568.

thus shows structural equivalence of the populations that contribute to m/z 135. Examination of a space-filling CPK model of adenosine shows that N-9 is sterically inaccessible to the transfer of labeled hydrogen directly from O-2′ in the absence of ribose ring opening, suggesting that the initial common structure of m/z 135 may be **23** rather than **22**. Other interconverting forms are



23

also possible, including ring-opened species (a–d) through which dissociation occurs.



As a consequence of their identical molecular ion structures, there exists the possibility that adenine- and adenosine-produced m/z 135 ions may follow sufficiently similar decomposition paths to permit extrapolation of established label-retention patterns in adenine to those of m/z 135 from adenosine, or other adenine nucleosides. This question would best be pursued by examination of one or more [^{15}N]adenosine models but is not presently being investigated.

Conclusions

More than two-thirds of the ion current produced by electron ionization of adenine is carried by fragment ions, the formation of most of which requires rupture of one or both rings. The dominant reaction sequence is initiated by expulsion of HCN, which is approximately 90% site specific for N-1, C-2. Positional selectivity is also exhibited in the formation of M – NH_2 and M – NH_3 ions (retention of N-7 and N-9) and in $C_2H_4N_3^+$ (retention of C-2, C-4, N-1, N-3, and N-9). Mass spectrometry may thus provide a useful method for location of biologically incorporated heavy isotopes in studies of purine metabolism and turnover and in detecting unusual isotopic enrichment patterns that would reflect alterations in the de novo purine biosynthesis pathway.[6,25] Although the sample for such measurements must be relatively pure and enrichments of several percent or greater must be present, the microgram-level sample requirement is more favorable by a factor of 10^3–10^4 than conventional stepwise chemical degradation methods.[26]

The complex decomposition pathways represented by ions that were found to exhibit largely random isotopic retention patterns in this and earlier work[11] are a reminder of the speculative nature of assignments of structures to fragment ions from complex heteroaromatic compounds. Numerous examples of such assignments are found in the literature.[27]

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Registry No. 1, 5098-11-3; **2**, 23142-10-1; **3**, 79364-53-7; **4**, 2302-25-2; **5**, 79364-58-2; **6**, 79364-59-3; **7**, 79364-60-6; **8**, 79364-61-7; **9**, 81602-56-4; **10**, 81602-57-5; **11**, 79364-54-8; **12**, 87-42-3; **13**, 19713-11-2; **14**, 2434-56-2; **15**, 79364-64-0; **16**, 79364-65-1; **17**, 79364-55-9; **18**, 5413-85-4; **19**, 56777-11-8; **19a**, 81602-58-6; **20**, 56777-12-9; **21**, 56777-22-1; diethoxymethyl acetate, 14036-06-7.

Supplementary Material Available: Equations for calculation of heavy isotope retention in ions M – 2(HCN), M – 3(HCN), M – HCN – NH_2CN, M – NH_2, and NH_3; compositional list of all ions in the mass spectrum of adenine; ion abundance data from [^{15}N]- and [^{13}C]adenines (Table 3); calculated ^{15}N retentions in ions M – HCN, M – 2(HCN), M – 3(HCN), M – HCN – NH_2CN (Table 4); and experimental details for preparation of [3-^{15}N]adenine and [6-*amino*-^{15}N]adenine (10 pages). Ordering information is given on any current masthead page.

(25) "Purine and Pyrimidine Metabolism", Ciba Foundation Symposium 48 (new series); Elsevier: Amsterdam, 1977.

(26) For example: (a) Smulson, M. E.; Suhadolnik, R. J. *J. Biol. Chem.* **1967**, *242*, 2872–2876. (b) Isono, K.; Suhadolnik, R. J. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 390–401.

(27) For an extensive compilation of polynitrogen heterocycle fragmentation schemes (text in Japanese) taken from the literature, see: Goto, M. In "Kagaku no Ryoiki"; Nankodo: Tokyo, 1968; Special Issue No. 85, pp 141–180.

Cis–Trans Equilibria in Aliphatic Semidiones[1]

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Abstract: Ion pairing and cis–trans equilibria of the dimethylsemidiones in Me_2SO in the presence of K^+ has been analyzed in terms of four equilibrium constants which at 25 °C are as follows: trans free ion/cis free ion = 125 ($\Delta H°$(trans–cis) = −2.5 kcal/mol); trans ion pair/cis ion pair = 2 ($\Delta H°$(trans–cis) = −1.4 kcal/mol); K(ion pairing) for the cis semidione = 250 M^{-1} ($\Delta H°$ = −1.1 kcal/mol); K(ion pairing) for the trans semidione = 4 M^{-1} ($\Delta H°$ = 0). In cyclic C_{11}–C_{15} semidiones the cis and trans isomers can be detected. The cis isomers are favored by high [K^+] whereas in the presence of K^+[2.2.2]-cryptand the trans isomers are preferred. The cyclic trans 1,2-semidiones exist in an asymmetric conformation with four magnetically nonequivalent α-hydrogen atoms which become time averaged to two pairs of hydrogen atoms at higher temperatures (>25 °C for C_{15} and >170 °C for C_{11}). Internal rotation in the trans 1,2-cyclic semidiones is quite slow but can be detected for the *trans*-cyclopentadecane-1,2-semidione at 130 °C.

Dialkyl semidiones (RC(O·)=C(O⁻)R) exhibit cis–trans equilibria which are established in a matter of seconds or less in Me_2SO at 25 °C but are slow on the ESR time scale.[3] Cis semidiones have values of a_α^H considerably greater than their trans

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