Table III. Formation of Adenosine 5′-*N*,*N*-Dimethylphosphoroamidate

Base	Conditions Temp, °C	Time, hr	AMP, %	Amidate, %	
	rt	24	91.5	8.5	
	100	1	85.5	6.2	8.3 not identified
	150	1	12.5	25.0	18.7 adenine 43.8 ADP ?
Pyridine	rt	24	76.0	10.0	14.0 not identified
Triethylamine	rt	24	71.0	29.0	
4-Morpholine[a]	rt	24	70.0	30.0	

[a] 4-Morpholine *N*,*N*′-dicyclohexylcarboxamidine.

to be 85% [λ_{max} 254 mμ (ε 12,950)] at pH 7.0. Another product was guanosine 5′-phosphate (15%).

At early stages of this reaction (1.5 hr), paper electrophoresis showed three spots: guanosine 5′-phosphate (25%), P^1,P^2-diguanosine pyrophosphate (36%), and guanosine 3′,5′-cyclic phosphate (38%).

In cases where *N*-benzoylguanosine 5′-phosphate was used in place of guanosine 5′-phosphate, the procedure used in the preparation of adenosine 3′,5′-cyclic phosphate was applied. After the *N*-benzoyl group was removed by treatment with 95% ethyl alcohol and concentrated ammonia (1:2 v/v) and the liquid heated in a sealed tube at 100° for 2 hr, guanosine 3′,5′-cyclic phosphate was obtained in 85% yield.

Adenosine 5′-*N*,*N*-Dimethylphosphoroamidate. The amidate was obtained by treating adenosine 5′-phosphate with excess HMPA at temperatures ranging from 20 to 150°. The amount of amidate increases as the reaction temperature rises or with addition of bases. The results are summarized in Table III. This amidate was stable in neutral solution but in acidic medium (pH 1) it decomposed to adenosine 5′-phosphate (about 70% in 1 day): λ_{max} 258 mμ (ε 15,400); R_f 0.66 (solvent I), electrophoretic mobility relative to AMP 0.40 (buffer I).

Acknowledgment. The authors wish to express their hearty thanks to Dr. Tsujiaki Hata for his advice.

Solvolysis of Adenine Nucleosides. I. Effects of Sugars and Adenine Substituents on Acid Solvolyses

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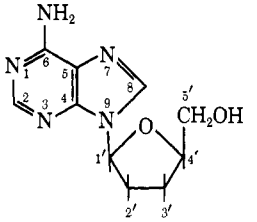
Abstract: The acidic solvolyses of 2′,3′-dideoxy-2′,3′-didehydroadenosine > 2′-deoxyadenosine > 9-β-D-psicofuranosyladenine ≫ 3′-deoxyadenosine > 8-bromoadenosine > adenine arabinoside ~ 2-chloroadenosine ~ N^6-methyladenosine ≳ adenosine ~ 2-methyladenosine > 1-methyladenosine ~ N^6-dimethyladenosine ≳ adenine xyloside > 8-methoxyadenosine ~ 2′*C*-methyladenosine result in the respective sugar and stable adenine moiety except in the case of 1-methyladenosine where the resultant 1-methyladenine is more slowly transformed into 5-aminoimidazole-4-*N*′-methylcarboxamidine. The typical ranking of relative activities are given above for 80° in 0.10 *M* HCl. Studies have been conducted at various acid and buffer concentrations, and at various temperatures for many of these compounds. The facts that only specific acid catalyzed solvolyses of the protonated and nonprotonated species were observed and that there was no maximum in solvolysis rate in the low pH region supported the argument against a Schiff base intermediate subsequent to ethereal oxygen attack. The probability of an A-1 mechanism for solvolyses of diprotonated adenine nucleosides with protons on the nitrogens in the 1 and 7 positions is favored by the fact that the entropies of activation, $\Delta S^{\pm}$, are close to zero. Although the inductive effect of the 2′ hydroxyl on the sugar moiety of adenosine inhibits acid solvolysis, a less significant increase in reactivity is introduced by the substitution of a hydrogen for the 3′ hydroxyl. The effects of substituents on the pyrimidine ring of the adenine moiety lead to only minor effects in reactivity whereas substitution of a bromine or methoxyl group on C-8 of the imidazole portion has a more pronounced effect consistent with the appropriate order for inductive effects aiding protonation. This and other evidence is consistent with the presumption that hydrogen ion attack of the protonated purine nucleoside to form a solvolyzing dication by an A-1 mechanism is on the imidazole moiety and most probably at the 7 position rather than on the ethereal oxygen. The fact that the 1-methyladenosine cation solvolyses in acid at about the same rate as the adenosine cation strongly suggests that it is the 1-protonated form of the latter that reacts with a second proton to result in a solvolyzing dication.

A mechanistic explanation of the acid solvolysis of the nucleosides to a base and a sugar has been proposed based on the analogous solvolysis of the simpler glycosylamines.[1] The essential steps were considered to be the protonation of the sugar ring oxygen, sugar ring opening, followed by water attack on the Schiff base to yield the heterocyclic base and sugar.[2-4] If the heterocyclic base could be protonated elsewhere, the ease of proton transfer to the sugar ring oxygen was considered to be an important factor. However, the validity of this mechanism for nucleoside solvolysis, based on the analogy to the acid solvolysis of the simple glycosylamines, has been seriously questioned.[2,5,6]

The kinetics and mechanisms of solvolyses of var-

(1) G. W. Kenner in "The Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1957, p 312.
(2) E. R. Garrett, J. K. Seydel, and A. J. Sharpen, *J. Org. Chem.*, 31, 2219 (1966).
(3) C. A. Dekker, *Annu. Rev. Biochem.*, **29**, 453 (1960).
(4) T. L. V. Ulbricht, *Comp. Biochem.*, **8**, 196 (1963).
(5) B. Capon, *Chem. Rev.*, **69**, 407 (1969).
(6) R. Shapiro and S. Kang, *Biochemistry*, **8**, 1806 (1969).

Table I. Ultraviolet Absorption Maxima and Spectrophotometric pK_a' of the Adenine Nucleosides and Products of Their Solvolysis



Compound and source	pK_a'[a] (μ = 0.10)	—0.10 *M* HCl— λ_{max}, nm	$10^{-3}\epsilon$	—0.10 *M* NaOH— λ_{max}, nm	$10^{-3}\epsilon$
Adenosine (AR)[b]	3.62 ± 0.05	257	14.6	259	15.4
2′-Deoxyadenosine (A_2dR)[b]	3.80 ± 0.04	258	14.1	260	15.2
3′-Deoxyadenosine (A_3dR)[c]	3.71 ± 0.05	258	14.0	260	14.2
2′,3′-Dideoxy-2′,3′-didehydroadenosine ($A_{2,3}$ddR)[d]		258	13.5	260	13.9
2′-*C*-Methyladenosine (2′-C-MeAR)[e]		258	15.1	260	14.9
9-β-D-Arabinofuranosyladenine or adenine arabinoside (AAr)[e]		257	12.6	259	13.9
9-β-D-Xylofuranosyladenine or adenine xyloside (AXy)[f]		258	14.5	260	15.3
1-Methyladenosine (1-MeAR)[a]		257	13.7	257	14.6
2-Methyladenosine (2-MeAR)[g]		(258)	(12.5)	263	14.5
2-Chloroadenosine (2-ClAR)[g]		265	14.6	265	15.3
N^6-Methyladenosine (6-MeAR)[h]	(4.0)	262	16.5	266	15.9
N^6,N^6-Dimethyladenosine (6-Me_2AR)[h]	(4.5)	268	18.2	276	19.0
8-Bromoadenosine (8-BrAR)[d]	4.02 ± 0.04	262	18.9	264	17.5
8-Methoxyadenosine (8-OMeAR)[d]	3.85 ± 0.05	261	13.5	259	14.6
Adenine (Ad)[b]	4.15 ± 0.05 9.90 ± 0.05	262	13.2	269	12.3
1-Methyladenine (1-MeAd)[i]	(7.20, 11.0)	259	11.6	270	14.4
2-Methyladenine (2-MeAd)[i]	(5.1)	(266)	(12.9)	271	10.8
2-Chloroadenine (2-ClAR)[i]				272	12.8
N^6-Methyladenine (N-MeAd)[h]	(4.2, 10.0)	267	15.2	273	15.8
N^6,N^6-Dimethyladenine (6-Me_2Ad)[h]	(3.9, 10.5)[i]	277	15.6	281	17.8
8-Bromoadenine (8BrAd)[b]		262	15.8	270	15.7
8-Methoxyadenine (8-OMeAd)[i]		(270)	(13.9)	(271)	(14.3)
4,5,6-Triaminopyrimidine (TAP)[h]	(1.47, 5.78)[i]	265[k]	10.2[k]	277	7.8
5-Aminoimidazole-4-*N*′-methylcarboxamidine[i]	9.40 ± 0.05	281	(12.8)	290	(15.4)

[a] Determined at 25.0°. [b] Calbiochem. [c] Dr E Walton, Merck, Sharp and Dohme Research Laboratories. [d] Dr. R. K. Robins, formerly of the University of Utah. [e] Dr. H. E. Kaufman, University of Florida. [f] Dr. H. B. Wood, Jr., Cancer Chemotherapy National Service Center (CCNSC). [g] Dr. J. A. Montgomery, Southern Research Institute. [h] Cyclo Chemical Corporation. [i] Prepared from 1-MeAR. [j] Literature data given in parentheses. [k] In 1.8 *M* hydrochloric acid (doubly protonated species). For the singly protonated species at pH 3.5 (λ_{max} 287 nm (ϵ = 10,200)). The uncharged species at pH 8.0 has ϵ 7760 at 277 nm.

iously substituted quanosines have been considered[7] recently in great detail and the arguments have been summarized that contradict the above postulated mechanisms for these compounds. These mechanisms have also been excluded for deoxycytidine and deoxyuridine derivatives.[6,8] The arguments presented[6–8] are consistent with an A-1 mechanism for these studied nucleosides where the protonated nucleoside undergoes *N*-glycosyl bond rupture in the rate determining step.

Kinetic studies on the solvolyses of adenine nucleosides[7,9–12] have been limited. The specific aims of these studies were to determine the products, kinetics, and mechanisms of solvolyses when changes were made in the sugar moiety at the 9 position on the adenine ring and when selected substituents were introduced into the 1, 2, 6, and 8 positions of the adenine ring of nucleosides. The premises of the possible mechanisms of solvolysis need to be challenged by studies of various compounds since it has been suggested that different nucleosides may undergo hydrolysis by different mechanisms.[5,7,9] This first paper in this series will consider the acid solvolyses of these compounds to their respective purines and sugars and the determination of their thermodynamic parameters.

Experimental Section

The compounds D-ribose and 2-deoxy-D-ribose were purchased from Calbiochem.

1-Methyladenine was prepared in the following manner.[13] 1-Methyladenosine (50 mg) in 1 ml of 0 50 *M* hydrochloric acid was heated on a steam bath for 45 min, and the solution was cooled to room temperature. The pH of the solution was adjusted to pH 7–8 with concentrated aqueous ammonia. The solution deposited white crystals on standing for about 4 hr. The crystals were washed with water by decantation, recrystallized from hot water, and dried overnight in a vacuum oven at 40.0°. The recrystallized sample softened and melted with decomposition at 295.0–300.0° (lit, values:[13] softened at 290.0–295.0°; and melted with mp 295.0–299.0° dec). Other compounds, their spectral characteristics, and acknowledged sources are listed in Table I.

(7) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970).
(8) R. Shapiro and M. Danzig, *Biochemistry*, 11, 23 (1972).
(9) E. R. Garrett, *J. Amer. Chem. Soc.*, **82**, 827 (1960).
(10) E. R. Garrett and L. J. Hanka, *J. Amer. Pharm. Ass., Sci. Ed.*, **49**, 526 (1960).
(11) H. Venner, *Z. Physiol. Chem.*, **339**, 14 (1964). The reported Arrhenius energies of activation (6–9 kcal/mol) for the acid solvolyses were based on only two temperatures. Recalculation of the reported data in 0.1 *M* HCl indicated that the actual values are 22.9 kcal/mol for 2′-deoxyadenosine and 22.6 kcal/mol for adenosine.
(12) H. Venner, *ibid.*, 322, 122 (1960).
(13) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 193 (1963).

These compounds were used without further purification. All the common chemicals used in these investigations were of analytical reagent grade.

Spectrophotometric Determinations of pK_a'. A weighed amount of the compound (approximately 0.5 mmol) was dissolved in nitrogen-purged distilled water (100 ml) to give a 5.0×10^{-4} *M* solution. Equal volumes of this solution and an appropriate buffer solution of ionic strength 0.20 were mixed to obtain 2.5×10^{-4} *M* solutions of the compound in the buffer solutions (final ionic strength 0.10). The spectrophotometric absorbance of each of these solutions was measured against the appropriate buffer blank at a wavelength where the charged and uncharged species showed a maximum difference in absorbance. The pH of each solution was measured on a Beckman Expanded Scale pH meter using a Sargent Combination Electrode No. S-30070-10. The pH meter was standardized at the required temperature before and after each set of measurements.

Monitoring of Acidic Solvolyses by Paper Chromatography. The acid solvolysis of 2′-deoxyadenosine (10^{-2} *M*) in 0.50 *M* hydrocholoric acid at 25.0° was monitored by paper chromatography. Samples of the reaction mixture were taken at intervals of time, an equal volume of 0.05 *M* sodium hydroxide was added, the pH was adjusted to about 10, if necessary, with minor amounts of sodium hydroxide, and 10 μl of the solution was spotted on Whatman No. 1 paper. Ten microliters of each of the solutions (5.0×10^{-3} *M*) of 2′-deoxyadenosine, adenine, and 2-deoxy-D-ribose were also spotted on the same paper as standards. Development was carried out in the ascending manner in three different solvent systems: (a) 5% disodium hydrogen orthophosphate solution in water saturated with isoamyl alcohol, (b) methanol–water (75:25), and (c) 1-butanol–acetic acid–water (4:1:5). The development was stopped when the solvent front reached about 15 cm. The paper was removed from the development chamber, dried quickly with warm air, and examined under a short-wavelength ultraviolet (250 nm) lamp. The paper was then sprayed with Dische reagent[14] for detection of the 2-deoxy-D-ribose.

The acid solvolysis of adenosine in 0.50 *M* hydrochloric acid at 80.0° was also monitored by the same procedure.

The acid solvolysis of 1-methyladenosine in 0.10 *M* hydrochloric acid at 80.0° was followed in a slightly different way. The reaction mixture was brought to a pH of about 9.0, and the development was carried out with isopropyl alcohol–5% ammonium sulfate (1:19).

Procedures for Kinetic Studies. The nucleoside (approximately 0.05 mmol) was weighed and transferred quantitatively to a 100-ml volumetric flask. Sufficient nitrogen-purged distilled water was added to bring the volume to the mark. A 25-ml aliquot of this solution of the 5.0×10^{-4} *M* nucleoside, 50 ml of hydrochloric acid solution of double the required final molarity, and 25 ml of distilled water were mixed in a 100-ml volumetric flask to give a 1.25×10^{-4} *M* solution of the nucleoside. All of the solutions were maintained and measured at the temperature of the kinetic study. Temperatures between 20.0 and 40.0° were selected for the kinetic studies of the 2′-deoxynucleosides and between 60.0 and 80.0° for the other compounds. Aliquots (about 3 ml) were taken at intervals of time, quickly cooled to room temperature, and diluted 1:1 with a sodium hydroxide solution of appropriate molarity to adjust the pH to 12–13. Ultraviolet spectrophotometric absorbances at pertinent wavelengths using suitable reagent blanks were obtained on the Beckman DU spectrophotometer, or the spectra recorded on the Cary Model 15 spectrophotometer.

A differential spectrophotometric procedure was employed to monitor the solvolysis of the adenine nucleosides which showed an A → B type reaction. The blank was an adenine solution of the same molarity as the initial nucleoside solution. The acid solvolysis of 1-methyladenosine, which showed a sequential A → B → C type of acid solvolysis, was followed spectrophotometrically using a reagent blank only. A 10^{-4} *M* solution of the compound in hydrochloric acid of appropriate concentration was maintained at the temperature of the study and the samples were treated with sodium hydroxide solution to adjust the pH to 12–13. The acid solvolysis of 1-methyladenine in 0.10 *M* HCl at 80.0° was followed in the same manner as that of 1-methyladenosine

The acid solvolyses of 1-methyladenosine, 2-methyladenosine, 2-chloroadenosine, 8-methoxyadenosine, 8-bromoadenosine, and 2′,3′-dideoxy-2′,3′-didehydroadenosine were followed using a reagent blank only. The differential spectrophotometric procedure was used to follow the acid solvolysis of the remaining adenine nucleosides.

(14) Z. Dische, *Mikrochemie*, 7, 33 (1929).

The acid solvolyses of adenosine and 2′-deoxyadenosine were followed in 0.10 *M* hydrochloric acid at 80.0 and 25.0°, respectively, using both of the above procedures. The apparent first-order rate constants, 10^5k (sec^{-1}), obtained were 16.5 for adenosine by differential spectrophotometry and 16.2 using the reagent blank. For 2′-deoxyadenosine 10^5k was 28.2 by differential spectrophotometry, and 27.5 using the reagent blank.

The acid solvolyses of adenosine and 2′-deoxyadenosine were studied in detail as a function of acid concentration at temperatures 60.0–80.0° and 20.0–37.0°, respectively. Other compounds were studied for comparative purposes in 0.10 *M* hydrochloric acid at two–four temperatures. Solvolyses of adenosine and 2′-deoxyadenosine in acetic acid, acetate, and phosphate buffers were followed by the same general procedure described for their acid solvolysis. Buffer solutions of ionic strength 0.10 were used for all the kinetic experiments except those performed in hydrochloric acid solutions more concentrated than 0.10 *M*. Where necessary, potassium chloride was added for adjustment of the ionic strength to 0.10. Ionic strength corrections were applied to the ionization constants of the buffering acid or base when preparing the buffer.

The solvolysis of 2-deoxyadenosine was also studied in acetate buffers at pH 4.75 and ionic strength 0.10 at four different total buffer concentrations to check for any general acid–base catalysis. Effect of ionic strength was checked at ionic strengths 0.10 and 0.16. The thermodynamic parameters for the uncharged nucleoside were determined at pH 6.20 (phosphate buffer, $\mu = 0.10$).

Results

Spectrophotometric Determination of pK_a'. The spectral pK_a values of adenine, adenosine, 2′-deoxyadenosine, 3′-deoxyadenosine, 8-bromoadenosine, and 1-methyladenosine were determined and the data are included in Table I. The pK_a' was averaged from the calculated values at different buffer pH values in accordance with the expression

$$pK_a' = pH + \log \frac{A - A_1}{A_2 - A} \qquad (1)$$

where A is the absorbance of a given pH, A_1 is the absorbance of the neutral species, and A_2 is the absorbance of the protonated species.

Acid Solvolysis of Protonated Nucleosides. Paper chromatographic analyses with time of a solution of 2′-deoxyadenosine (10^{-2} *M*) in 0.50 *M* hydrochloric acid maintained at 24.0° showed that the spot due to the 2′-deoxyadenosine became progressively smaller and fainter with time, and finally disappeared completely. At the same time, a spot appeared with the R_f value of adenine (0.31) and became progressively larger and darker. No other spots were visible under ultraviolet lamp. The Dische reagent[14] spray revealed a spot (R_f 0.45) due to 2-deoxyribose whose growth paralleled that of adenine. This indicated that the acid solvolysis of 2′-deoxyadenosine involved splitting of the glycosyl bond only.

Ultraviolet spectrophotometric absorbance recording between 350 and 230 nm showed that the initial 5 (or 6) $\times 10^{-5}$ *M* 2′-deoxyadenosine in the acid changed progressively to that of adenine of the same molarity with an isosbestic point at 267 nm maintained throughout the reaction, which provided an independent and strong indication of a 1:1 transformation. However, this procedure of absorbance measurements in acid solution was not generally used because of two disadvantages: (a) the solvolysis of the nucleoside continued during the absorbance measurement, and (b) the spectral characteristics of the nucleosides and the corresponding adenine bases were similar (see Table I) and resulted in small differences between the initial and final absorbance values. Both of these disadvantages

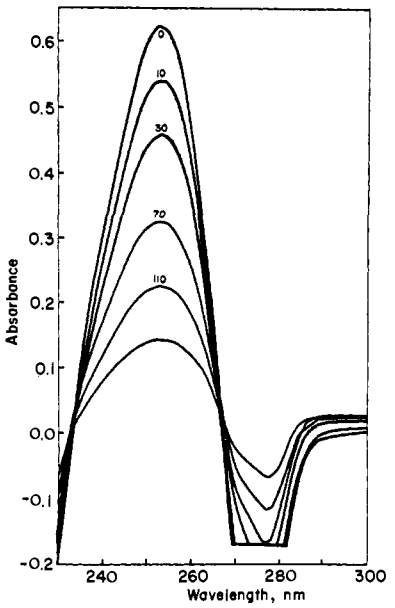


Figure 1. Typical differential spectrophotometric curves for the solvolysis of 2.0×10^{-4} M 2′-deoxyadenosine solution in 0.127 M hydrochloric acid at 24.6°. (Blank: 2.0×10^{-4} M adenine solution in 0.127 M hydrochloric acid.) The sample and blank were treated with equal volumes of 0.20 M sodium hydroxide. The curves are labeled as to the minutes of reaction.

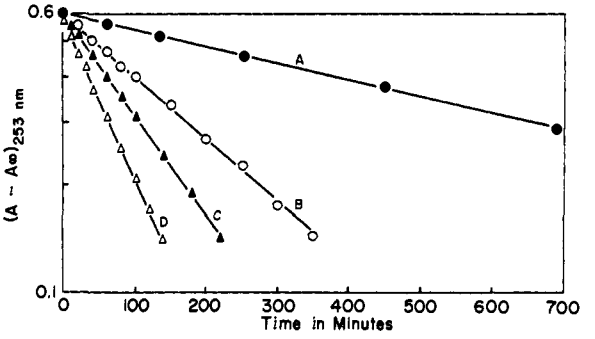


Figure 2. Apparent first-order plots for the solvolysis of 1.2×10^{-4} M adenosine in 0.10 M hydrochloric acid at (A) 80.0°; (B) 75.0°; (C) 70.0°; (D) 60.0°, as monitored by differential spectrophotometry after dilution with an equal volume of 0.20 M sodium hydroxide. (Blank: 1.2×10^{-4} M adenine in 0.10 M hydrochloric acid diluted with an equal volume of 0.20 M sodium hydroxide.)

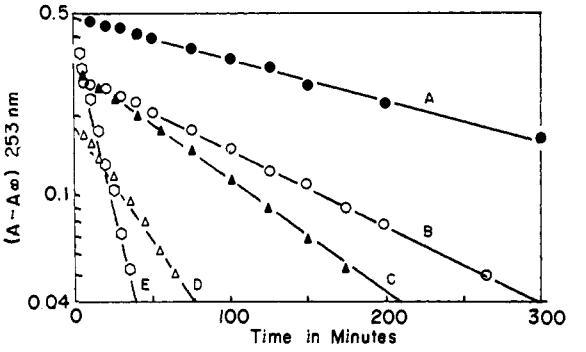


Figure 3. First-order rate plots for the solvolysis in 0.10 M hydrochloric acid at 80.0° of (A) 8-methoxyadenosine; (B) adenine xyloside; (C) 2-methyladenosine; (D) 8-bromoadenosine; (E) 3′-deoxyadenosine. The solvolysis of 8-methoxyadenosine and 2-methyladenosine was followed by measuring absorbance changes against a reagent blank. Differential spectrophotometry was used for the other compounds.

were overcome by adjusting the pH of the samples taken from the reaction mixture to pH 12–13 with a sodium hydroxide solution of appropriate molarity. The solvolysis of the nucleosides was negligible under the conditions of spectral assay for all practical purposes in neutral and alkaline medium. The greater advantage was the increased separation between the spectra of the nucleoside and the adenine anion (see Table I). This was due to a hyperchromic shift in the spectrum of adenine anion as compared to those of uncharged or protonated nucleoside or adenine. The yield of adenine, calculated from the molar absorptivities of the two compounds, was almost quantitative.

This almost quantitative transformation of the nucleoside into the corresponding adenine suggested use of differential spectrophotometry to follow the acid solvolysis. Nucleoside concentrations of up to 2.0×10^{-4} M were used. The absorbances of samples, adjusted to a pH of 12–13, were recorded between 350 and 230 nm. The blank was either a similarly treated 2.0×10^{-4} M adenine solution or samples from an identical nucleoside solvolytic experiment which was started at least 1 half-life earlier. These differential spectra showed an absorbance maximum at 253 nm at pH 12–13 with initial absorbance values of about 0.60, which approached the base line as the solvolysis neared completion (Figure 1). This procedure was used for the acid solvolysis of all the nucleosides other than 2-chloroadenosine, 2-methyladenosine, 8-methoxyadenosine, and 1-methyladenosine. A reagent blank was used in these cases.

No spectral interference from the acid degradation products of 2-deoxyribose was observed during the acid solvolysis of the adenine nucleosides. The apparent first-order rate constant for the production of the 261-nm chromophore from 2-deoxyribose in 0.25 M hydrochloric acid at 30.0° would be about 2.0×10^{-7} sec^{-1}.[15] Under similar conditions, the solvolytic rate constant for 2′-deoxyadenosine is greater than 2.0×10^{-4} sec^{-1} and no significant spectral interference from the 5-methyl-3(2H)-furanone, the acid degradation product of 2-deoxyribose,[16] was anticipated.

The apparent first-order rate constants were calculated from the slopes of the plots of the logarithms of the difference in absorbance, A, at any time, t, and the final absorbance, A_∞, at 253 nm for the samples made alkaline, against time according to

$$\log (A - A_\infty) = \log (A_0 - A_\infty) - \frac{kt}{2.303} \qquad (2)$$

where A_0 is the initial absorbance.

Typical first-order plots for the acid solvolysis of adenosine in 0.10 M hydrochloric acid at various temperatures are shown in Figure 2. Typical plots for the acid solvolysis of other nucleosides in 0.10 M hydrochloric acid at 80.0° are shown in Figure 3.

The conditions and the apparent first-order rate constants for the acid solvolysis of 2′-deoxyadenosine and adenosine are given in Tables II and III. Plots of the rate constants against the corresponding hydrochloric acid concentrations at each temperature of study were

(15) J. K. Seydel and E. R. Garrett, *Anal. Chem.*, 37, 271 (1965).
(16) J. K. Seydel, E. R. Garrett, W. Diller, and K. J. Schaper, *J. Pharm. Sci.*, 56, 858 (1967).

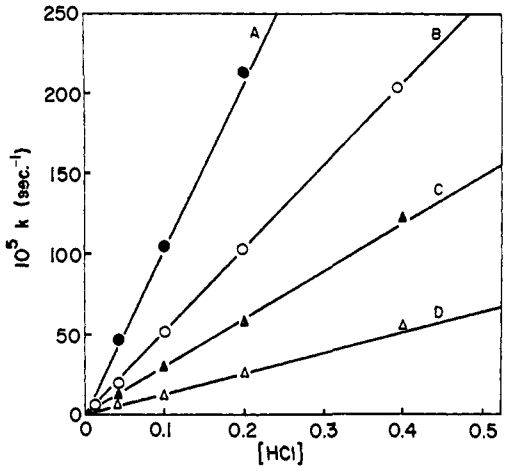


Figure 4. Dependence of the apparent first-order rate constants for the solvolysis of 2′-deoxyadenosine on hydrochloric acid concentration: (A) 37.0°; (B) 30.0°; (C) 25.0°; (D) 20.0°.

Table II. Apparent First-Order Rate Constants[a] (10^5k, sec^{-1}) for the Acid Solvolysis of Protonated 2′-Deoxyadenosine[b]

[HCl], *M*	37.0°	30.0°	25.0°	20.0°
0.013		6.30		
0.042	47.3	17.8	13.0	6.50
0.100	104	50.3	28.2	12.4
0.197	212	107	57.5	25.1
0.394		207	123	55.2
0.591		281		
10^5k_{HCl}[c]	1050	516	300	130

[a] Obtained by the differential spectrophotometric procedure. [b] Additional data were obtained at pH 2.20 in HCl–KCl buffer, μ = 0.1, and were 15.3×10^{-4} sec^{-1} at 47.0° and 3.94×10^{-5} sec^{-1} at 35.0°. [c] Obtained from the slopes of plots of k *vs.* [HCl] at the pertinent temperatures and in l. mol^{-1} sec^{-1}.

Table III. Apparent First-Order Rate Constants[a] (10^5k, sec^{-1}) for the Acid Solvolysis of Protonated Adenosine

[HCl], *M*	80.0°	75.0°	70.0°	60.0°
0.021	2.75			
0.050			2.05	
0.063	10.1			
0.10	16.5	10.7	6.50	2.10
0.21	34.5	21.9	12.3	3.38
0.31		32.9	20.8	6.24
0.42	75.1	46.5	30.1	8.08
10^5k_{HCl}[b]	164	106	64	20

[a] Obtained by the differential spectrophotometric procedure. [b] Obtained from the slopes of plots of k *vs.* [HCl] at the pertinent temperatures and in l. mol^{-1} sec^{-1}.

linear and passed through the origin for both of these nucleosides and indicated specific hydrogen-ion catalysis. A typical set of such plots is given for 2′-deoxyadenosine in Figure 4. The slopes of these plots were the apparent bimolecular rate constants, k_{HCl}, for the attack of hydrogen ion on the protonated species in accordance with the equation

$$k = k_{HCl}[HCl] \quad (3)$$

The acid solvolyses of the other adenine nucleosides were studied in 0.10 *M* hydrochloric acid as a function of temperature, and the apparent first-order rate constants obtained are listed in Table IV. The apparent bimolecular rate constants for the attack of hydrogen

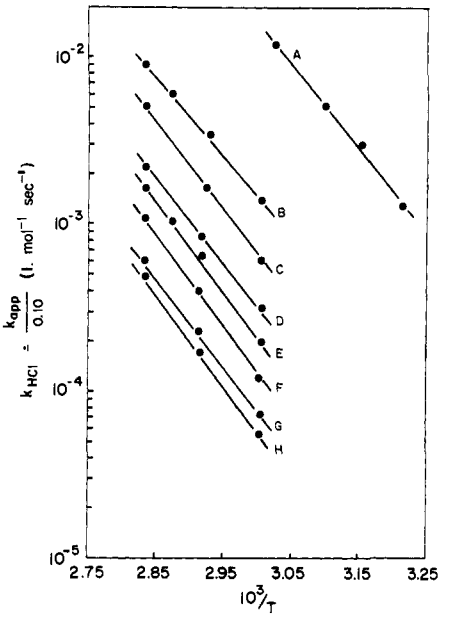


Figure 5. Arrhenius plots for the apparent bimolecular rate constants, $k_{HCl} = k/[HCl]$, for the acid-catalyzed solvolysis of protonated adenine nucleosides, where k is the apparent rate constant at [HCl]: (A) 2′-deoxyadenosine; (B) 3′-deoxyadenosine; (C) 8-bromoadenosine; (D) adenine arabinoside; (E) adenosine; (F) adenine xyloside; (G) 8-methoxyadenosine; (H) 2′-*C*-methyladenosine. The k_{HCl} for 2′-deoxyadenosine and adenosine were obtained from the slopes of the apparent first-order rate constants against [HCl]. The values for the other compounds were obtained at 0.10 *M* HCl. For the 2′-deoxyadenosine plot, add 0.2 unit to the abscissa.

ion on the protonated species were calculated from the k values at 0.10 *M* HCl.

The Arrhenius heat of activation, ΔH_a, for the various specific rate constants of the adenine nucleosides was derived from the slopes of the logarithms of the bimolecular rate constants against the reciprocals of the absolute temperature (Figure 5) in accordance with the logarithmic form of the Arrhenius equation

$$\log k_{HCl} = \frac{\Delta H_a}{2.303RT} + \log P \quad (4)$$

The Arrhenius heats of activation obtained are listed in Table IV. They are all in the range of about 22–25 kcal/mol.

The entropy of activation, $\Delta S^{\pm}$, was calculated using the absolute rate equation[17]

$$k_{HCl} = \frac{\bar{k}T}{\hbar} e^{-\Delta H^{\pm}/RT} e^{\Delta S^{\pm}/R} \quad (5)$$

where $\bar{k}$ is the Boltzmann constant 1.381×10^{-16} erg deg^{-1}, $\hbar$ is the Planck constant, 6.626×10^{-27} erg sec, and R is 1.987 cal deg^{-1} mol^{-1}. On taking logarithms and rearranging

$$\Delta S^{\pm} = 2.303R\left[\log k_{HCl} - \log \frac{\bar{k}T}{\hbar} + \frac{\Delta H^{\pm}}{2.303RT}\right] \quad (6)$$

where $\Delta H^{\pm} = \Delta H_a - RT$. The product RT is about 700 and 600 cal mol^{-1} at 80.0 and 25.0°, respectively. The calculated entropies of activation for the compounds studied are listed in Table IV, along with values

(17) K. J. Laidler, "Chemical Kinetics," 2nd ed, McGraw-Hill, New York, N. Y., 1965, p 89.

Table IV. Apparent First-Order Rate Constants (10^5k, sec^{-1}) and Thermodynamic Parameters for the Apparent Bimolecular Rate Constants (10^5k_{HCl}, l. mol^{-1} sec^{-1}) for the Solvolysis of Protonated Adenine Nucleosides in 0.10 *M* Hydrochloric Acid

Compound	10^5k, sec^{-1} 80.0°	75.0°	70.0°	60.0°	Parameters for k_{HCl}[a-c] $\Delta H^{\pm}$,[g] kcal mol^{-1}	$\Delta S^{\pm}$,[h] eu
Adenosine[a]	16.5	10.7	6.40	2.10	24.8	−3.8
					26.0[i]	+6.1[i]
3′-Deoxyadenosine[b]	91.6[j]	61.0	33.5	14.2	21.8	−8.3
Adenine arabinoside[b]	22.0		8.55	2.98	23.3	−7.1
Adenine xyloside[b]	11.0		3.95	1.30	24.7	−4.4
2′-*C*-Methyladenosine[b]	5.00		1.73	0.56	24.6	−5.9
1-Methyladenosine[b]	15.2[f]		5.38[f]		25.0	−1.6
2-Methyladenosine[b]	15.8					
2-Chloroadenosine[b]	19.0					
N^6-Methyladenosine[b]	18.5					
N^6-Dimethyladenosine[b]	13.6					
8-Bromoadenosine[b]	50.0		16.6	6.15	24.0	−3.4
8-Methoxyadenosine[b]	6.0		2.25	0.82	24.1	−5.9
2′,3′-Dideoxy-2′,3′-didehydroadenosine[b,c]	35,000		110 (25.0°)	60 (20.0°)	21.9	+0.8
2′-Deoxyadenosine[a,c]	12,500		104 (37.0°)	28.2 (25.0°)	23.2	+1.2
			68 (37.0°)[d]	11.7 (23.0°)[d]	22.2[i]	+3.2[i]
2′-Deoxy-8-bromoadenosine				49.7 (25.1°)[k]		
8-Mercapto-2′-deoxyadenosine				9.2 (25.0°)		
Psicofuranine[e]	6,070				22.5	+1.0

[a] $k_{HCl} = k/[HCl]$, calculated from the slope of the plot of k *vs.* [HCl] for 80.0° [b] $k_{HCl} = k/0.10$ after the studies conducted only in 0.10 *M* HCl. [c] k_{HCl} estimated for 80.0° for purposes of comparison from the Arrhenius equation using data at lower temperature. [d] Data from ref 11. [e] Data from ref 9. [f] k_1 for the solvolysis to 1-methyladenine. [g] Calculated from the Arrhenius equation. $k = Pe^{-\Delta H_a/RT}$ and $\Delta H^{\pm} = \Delta H_a - RT$. [h] Calculated from the rearranged absolute rate expression: $\Delta S^{\pm} = 2.303R[\log k_{HCl} - \log(\bar{k}T/h) + \Delta H^{\pm}/2.303RT]$, where $\bar{k} = 1.3805 \times 10^{-16}$ erg deg^{-1} molecule^{-1}, $h = 6.6256 \times 10^{-27}$ erg sec, $R = 1.987$ cal deg^{-1} mol^{-1}, and $T = 353.2°$. [i] Recalculated using data from ref 11 and 12. [j] Some additional values for the solvolysis of 3′-deoxyadenosine were: 10^5k, [HCl]; 178, 0.20; 44.7, 0.05. [k] Actually studied in 0.2 *M* HCl with $10^5k = 99.4$.

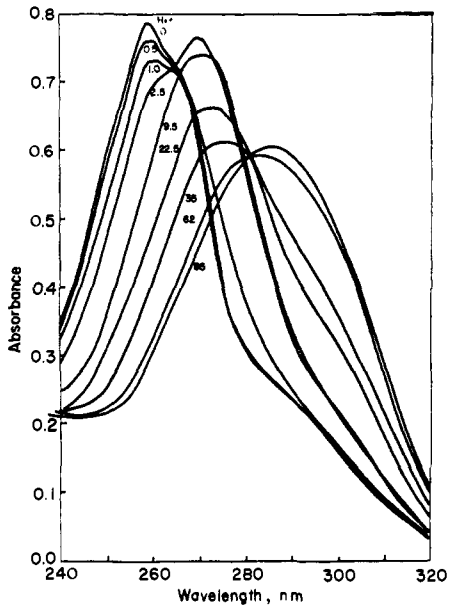


Figure 6. Typical curves of spectral changes of a 1.1×10^{-4} *M* solution of 1-methyladenosine in 0.10 *M* hydrochloric acid at 80.0°. Each curve is labeled as to the number of hours after the start of the degradation. Each sample was treated with an equal volume of 0.20 *M* sodium hydroxide and the absorbance of the mixture recorded *vs.* reagent blank.

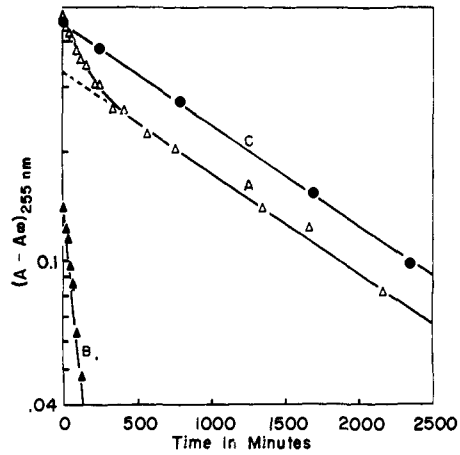


Figure 7. Analysis of the change in absorbance, *A*, of 1-methyladenosine (curve A) and 1-methyladenine (curve C) in 0.1 *M* HCl at 80.0° with time where A_∞ is the final absorbance of each respective reacting mixture. The parallel terminal slopes are valid evidence for the fact that the 1-methyladenine is a degrading intermediate in 1-methyladenosine solvolysis. Curve B is obtained for the logarithm of the differences between the antilogarithmic values of the extrapolated dashed line and the antilogarithms of the actual initial value of curve A. The constants k_1 and k_2 are obtained from the slopes of curves B and C (or A), respectively.

for $\Delta H^{\pm}$ and $\Delta S^{\pm}$ calculated from the data for the adenine nucleosides in the literature.[9,10]

Since all these acid solvolytic studies were conducted below the pK_a' of the nucleosides, the derived constants and parameters discussed refer to the acid-catalyzed solvolyses of the protonated nucleosides.

The acid solvolysis of 1-methyladenosine differed from the simple glycosyl bond splitting of all other adenine nucleosides studied, and was characterized by two isosbestic points formed sequentially, at 263 and 280 nm, respectively (Figure 6). This indicated an A → B → C type reaction. When the logarithm of the difference between the absorbance, *A*, at any time, *t*, and the final absorbance, A_∞, at 255 nm for the samples made alkaline was plotted against time according to eq 2, a biphasic curve was obtained (curve A, Figure 7). The linear portion of this curve was extrapolated to zero time and the apparent first-order rate constant for the

B → C reaction was calculated from this straight line according to eq 2. The apparent first-order rate constant for the A → B reaction was calculated as follows. For a given time, t, starting with time zero, the antilogarithms of the log $(A - A_\infty)$ values obtained from the linear extrapolated section were subtracted from the corresponding antilogarithms of the log $(A - A_\infty)$ values of the original data. The logarithms of these differences were plotted against time to yield a straight line (curve B, Figure 7), and the apparent first-order rate constant for the A → B reaction was calculated from the slope of this line. The rate constants, k_1 and k_2, for the two steps are given in Table V. The apparent first-order rate constant for solvolysis of 1-methyladenine under the same conditions was obtained from the plot of log $(A - A_\infty)$ against time (curve C, Figure 7), and is given in Table VI. This rate constant agreed with that obtained for the B → C step for 1-methyladenosine (curve A, Figure 7).

Table V. Apparent First-Order Rate Constants[a] for the Sequential Acid Solvolysis of 1-Methyladenosine

Temp, °C	[HCl], M	10^5k_1, sec^{-1}	10^5k_2, sec^{-1}
80.0	0.40	54.2	4.80
	0.20	26.0	2.35
	0.10	15.2	1.07[b]
80.0	(pH 2.20)[c]	2.15	0.17
70.0	0.10	5.38	0.36

[a] Obtained from the slopes of plots of log $(A - A_\infty)$ at 255 nm (of the samples made alkaline to pH 12–13) *vs.* time as described under the Results where 1-methyladenosine → (k_1) 1-methyladenine → (k_2) 5-aminoimidazole-4-*N'*-methylcarboxamidine. [b] The apparent first-order rate constant for the solvolysis of 1-methyladenine to 5-aminoimidazole-4-*N'*-methylcarboxamidine in 0.10 M HCl at 80.0° was 1.05×10^{-5} sec^{-1}. [c] HCl–KCl buffer of ionic strength 0.10.

Acid Solvolysis of Unchanged Adenine Nucleosides. The solvolysis of 2′-deoxyadenosine and adenosine at pH values higher than 3.0 was followed by the same procedure as used for the solvolysis of these compounds at the lower pH values. When the absorbance of the samples of the solvolyzing nucleoside solution made alkaline (pH 12–13) was recorded *vs.* a reagent blank, the isosbestic point at 267 nm was maintained throughout the reaction. The spectral changes were similar to those for 2′-deoxyadenosine at high acidities. This was interpreted as the nucleoside solvolyzing to form adenine and sugar only. The apparent first-order rate constants (Table VI) for these solvolyses were obtained in the same manner as described for the acid solvolysis of the protonated nucleosides.

The solvolysis of 2′-deoxyadenosine in acetate buffer, pH 4.75, was followed at four different buffer concentrations and ionic strength 0.10 (Table VI). No significant change in the apparent first-order rate constants was observed. There was thus no detectable general acid–base catalysis by acetic acid or the acetate ion. This is consistent with the failure to observe general acid–base catalysis by acetate buffers in the solvolysis of psicofuranine[9] and of 5-iodo-2′-deoxyuridine.[18]

The solvolysis of adenine nucleosides can be assigned to the hydrogen ion catalyzed solvolysis of both the protonated, ARH^+, and nonprotonated species, AR, *i.e.*

$$k[AR]_T = k_1 = k_1[H^+][ARH^+] + k_2[H^+][AR] \quad (7)$$

whence[1]

$$k = \{k_1/(1 + K_a/[H^+]) + k_2/(1 + [H^+]/K_a)\}[H^+] \quad (8)$$

Agreement between the experimental and calculated values of the apparent first-order rate constants was obtained when the values were taken as $k_1 = 1.13$ l. mol^{-1}, sec^{-1}, $k_2 = 3.16$ l. mol^{-1} sec^{-1}, and $K_a = 2.50 \times 10^{-4}$ for the protonated 2′-deoxyadenosine. When these values were taken as $k_1 = 0.00164$ l. mol^{-1} sec^{-1}, $k_2 = 0.00353$ l. mol^{-1} sec^{-1}, and $K_a = 4.0 \times 10^{-4}$ for the protonated adenosine, agreement between the experimental and calculated apparent first-order rate constants was obtained. Experimental and selected calculated values are given in Table VI.

Table VI. Conditions and Apparent First-Order Rate Constants[a] for the Solvolysis of 2′-Deoxyadenosine (A2dR) and Adenosine (AR) in Acetate and Phosphate Buffers

Temp, °C	pH	Buffer composition, M [CH_3COOH]	[CH_3COO^-]	[KCl]	10^5k, sec^{-1} A2dR	AR
80.0	3.20	0.018		0.100		0.165[h]
	3.80	0.346	0.050	0.050	39.9[b]	0.055[i]
	4.20	0.275	0.100		20.0[c]	
	4.75	0.078	0.100		6.35[d]	5.8×10^{-3} [j,l]
	4.75	0.0624	0.080	0.020	6.47	
	4.77	0.0312	0.040	0.060	6.25	
	4.75	0.0156	0.020	0.080	6.40	
80.0	5.20	0.0280 [$H_2PO_4^-$]	0.100 [HPO_4^{2-}]		1.82[e]	
	6.20	0.060	0.013		0.159[f,m]	1.25×10^{-4} [k,l]
80.0	7.50	0.007	0.031		0.014[g]	
70.0	6.20	0.060	0.013		0.063[m]	
60.0	6.20	0.060	0.013		0.025[m]	

[a] Calculated 10^5k values for 80.0° by $k = \{k_1/(1 + K_a/[H^+]) + k_2/(1 + [H^+]/K_a)\}[H^+]$ are: [b] 37.8, [c] 17.3, [d] 5.93, [e] 1.98, [f] 0.107, [g] 0.011, [h] 0.177, [i] 0.051, [j] 6.3×10^{-3}, [k] 2.3×10^{-4}, where the microscopic rate constants were taken in l. mol^{-1} sec^{-1} as $k_1 = 1.13$, $k_2 = 3.16$, and $K_a = 2.50 \times 10^{-4}$ for the protonated 2′-deoxyadenosine and as $k_1 = 0.00164$, $k_2 = 0.00353$, and $K_a = 4.0 \times 10^{-4}$ for the protonated adenosine. [l] Estimated by calculating the end absorbance value on the presumption that the solvolysis gave a quantitative yield of adenine. [m] The apparent ΔH_a value for these pH 6.2 data for 2′-deoxyadenosine solvolysis is 21.5 kcal in accordance with $\log k = \log P - (\Delta H_a/2.303R)\cdot(1/T)$ where $\log P$ is 12.52.

(18) E. R. Garrett, P. B. Chemburkar, and T. Suzuki, *Chem. Pharm. Bull.*, 13, 1113 (1965).

Discussion

The adenine nucleosides investigated were solvolyzed in acid to the respective adenines and sugars. The solvolytic rates varied with the nature of the sugar moiety and with the nature and position of the adenine ring substituents (Tables II–IV). The opening of the pyridimine ring was observed only with 1-methyladenine subsequent to the acid solvolysis of 1-methyladenosine.

The facts that the rate constants for the acid solvolyses of adenosine and 2′-deoxyadenosine (Tables II, III, and VI) do not show the bell-shaped log k–pH profiles in the low pH region and that the general acid catalysis by acetate buffers that were observed for the Schiff bases[19] is not observed for 2′-deoxyadenosine (Table VI) are supporting evidence for the detailed arguments cited in the literature[5,7,8] for other specific nucleosides against the mechanism proposed on the supposed analogy to the solvolysis of the simpler glycosylamines[1] for some or all nucleosides.

Effect of Sugar Moiety on Acid Solvolysis Rates. The increased stability of the nucleosides to acid solvolysis with increasing numbers of hydroxyl groups in the sugar has been observed now in the adenine (Tables II–IV) series as well as in the quanidine[7] and pyrimidine[2] series and as among the glycosides.[20] In particular, the 2′-hydroxyl group which is on the carbon adjacent to the 1′-carbon substituted with the group to be solvolyzed appears to have a very strong stabilizing effect on the rate of acid solvolysis.[21] For example, the apparent first-order rate constant for the acid solvolysis of 2′-deoxyadenosine is about 1000 times that of adenosine under the same conditions (Table IV). This effect is independent of the nature of the aglycone,[2,11,20] and of whether the glycosyl moiety is furanosyl[2,11] or pyranosyl.[20]

Replacement of the 2′-hydroxy group by a more polar group appears to result in increased stability toward acid solvolysis. For example, methyl 2-*O*-toluene-*p*-sulfonyl-α-glucoside was found unchanged after heating with 0.33 *N* sulfuric acid for 8 hr at 70.0°,[22] and 2′-*O*-*p*-nitrobenzenesulfonyladenosine was stated to be stable under the conditions in which adenosine hydrolyzed.[4] This stabilization may be partly due to the steric effects of these large groups.

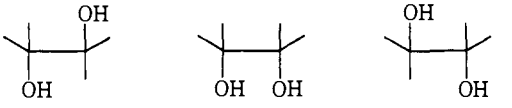
When the 3′-hydroxyl group of adenosine is replaced by a hydrogen atom, the stability of the resulting 3′-deoxyadenosine to acid solvolysis is decreased. However, this decrease in stability is apparently modified by the larger stabilizing influence of the retained 2′-hydroxyl group. Thus, the apparent first-order rate constant for the acid solvolysis of protonated 3′-deoxyadenosine is only about five–seven times that of adenosine under the same conditions (Table IV). When the 2′- and 3′-hydroxyl groups are both replaced by hydrogen atoms, the stabilizing effects of both these hydroxyl groups are removed. For example, 2′,3′-dideoxy-2′,3′-didehydroadenosine solvolyzed three–four times faster than 2′-deoxyadenosine under the same conditions (Table IV). Thus, 2′,3′,5′-trideoxyadenosine may be more unstable in acid than the corresponding dideoxy compound if this pattern holds.

Similar additive effects have been demonstrated with *O*-glycosides. A gradual increase in stability toward acid solvolysis occurs when increasing numbers of hydroxyl groups are substituted for hydrogens at the 3, 4, 5, and 6 positions of tetrahydro-2-methoxypyran.[20]

It has been speculated that the hydroxyl groups may exert induced electron withdrawing effects at the site of the protonation necessary to lead to reaction,[21,23] or they may compete as proton acceptor sites for the approaching hydrogen ion and by coulombic effects repel the attack at the reaction site and thus decrease the concentration of the molecules in the activated state necessary for solvolysis.

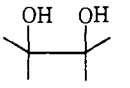
However, the effects of sugar moieties on the cleavage rate of nucleosides have been attributed[7,24] solely to the inductive effects introduced by hydroxyl group substituents such as those that decrease hydrolysis rates in acetals and glycosides where, in the latter case, the glycosidic ring remains intact during the reaction. The stabilization of nucleoside solvolysis by hydroxyl group substitution has been ascribed[7] to the inductive effect that inhibits the slow C–N bond cleavage of the protonated nucleoside that results in a C-1′ carbonium ion.

The rates of solvolysis of protonated adenine riboside, arabinoside, and xyloside were similar in magnitude (Table IV). This suggests that the configurations of the hydroxyl groups on the 2′- and 3′-carbon atoms of the fully hydroxylated sugars have only a minor influence (*i.e.*, in the range of twofold) on the acid solvolysis of these protonated compounds. However, it may be worthwhile to consider these minor effects. The observed decreasing order of reactivities (Table IV)



arabinoside riboside xyloside

may be attributed to the 2′-hydroxyl group in the "down" position and the 3′-hydroxyl group in the "up" position decreasing solvolysis rates and the fact that the position of the 2′-hydroxyl exerts a greater influence than the 3′-hydroxyl group. It is interesting to speculate that the lyxoside



should have the greatest reactivity on these premises.

It has been suggested that intramolecular hydrogen bonding exists between the 3-nitrogen of adenine and the 2′-hydroxyl group of the sugar in adenosine on the basis of nmr[25] and ir[26] data, but such bonding was not indicated with the 2′-hydroxyl group of 3′-deoxyadenosine. Such phenomena could modify the induc-

(19) E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, **85**, 2843 (1963).
(20) E. Dyer, C. P. J. Glaudemans, M. J. Koch, and R. H. Marchessault, *J.Chem. Soc.*, 3361 (1962).
(21) A. Streitweiser, Jr., *Chem. Rev.*, **56**, 571 (1956).
(22) D. M. Brown, G. D. Fasman, D. I. Magrath, and A. R. Todd, *J. Chem. Soc.*, 1448 (1954).
(23) S. Winstein and E. Grunwald, *J. Amer. Chem. Soc.*, 70, 828 (1948).
(24) A. Streitweiser, Jr., "Solvolytic Displacement Reactions," Wiley, New York, N. Y., 1962, pp 112–113.
(25) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Amer. Chem. Soc.*, **90**, 1042 (1968).
(26) J. Pitha, S. Chladek, and J. Smrt, *Collect. Czech. Chem. Commun.*, **38**, 1622 (1963).

tive effects of hydroxyls by the introduction of steric and conformational factors which may perturb C–N cleavage and may be the basis upon which these minor effects of configuration of the hydroxyl group on the 2′ and 3′ on solvolysis can be rationalized.

The substitution of a methyl group for a hydrogen on C-2′ on the sugar moiety of adenosine as exemplified by 2′-*C*-methyladenosine decreased the solvolytic rate constant to about one-third the value for adenosine under the same conditions (Table IV). The methyl group, as a better electron donor, should have increased solvolysis if the inductive effects assigned[7] to a hydroxyl in a similar position were to be accepted. At least no decrease in solvolytic rate would have been anticipated by this substitution. However, methyl group substitution for hydrogens on hydroxylated carbons adjacent to carbons with hydrolyzable groups did not give the significant or consistent changes in solvolytic reactivities that were observed for some alkyl halides.[21] This possible anomaly may be rationalized by postulating that the 2′-methyl sterically hinders solvation of the developing carbonium ion in the transition state. An alternative rationalization is that the 2′-hydroxyl binding to N-3 of the adenine moiety lessens the inductive effect of the 2′-hydroxyl, a binding which does not occur with a 2′-methyl.

Effects of Purine Ring Substituents on Acid Solvolysis Rates. The effects of substituents on the adenine ring on hydrogen ion catalyzed solvolysis of the corresponding monoprotonated nucleosides are small compared with those of the sugar substituents. For example, the substitution of methyl group(s) for hydrogen(s) on the exocyclic nitrogen (N^6-methyl- and N^6,N^6-dimethyladenosine), on N-1 (1-methyladenosine), or on C-2 (2-methyladenosine) brings about little or no change in the reactivity of these compounds toward acid solvolysis compared with that of the unsubstituted adenosine (Table IV). The fact that 1-methyladenosine cation solvolyzes in acid at about the same rate as the adenosine cation strongly suggests that it is the 1-protonated form of the latter that reacts with a second proton to result in a solvolyzing dication.

The substitution of methyl groups brings about a greater change in the pK_a' of the nucleosides than that brought about by changes in the sugar hydroxyls (Table I). Although the methyl groups do change the electron density in the adenine ring, the small change in the reactivity toward acid solvolysis can only be interpreted to mean that the reactive site for the second proton is somewhat removed from these positions in the pyrimidine portion of the adenine and resides in the imidazole portion.

The 2-chloro substituent (in 2-chloroadenosine) also brought about very little change in the reactivity (Table IV). This could be rationalized on the same basis as that offered for the methyl substituents in the pyrimidine portion of adenosine.

Substituents Br and OCH_3 in the 8 position of the adenine moiety demonstrated the following reactivity for the acid solvolysis: 8-bromoadenosine > adenosine > 8-methoxyadenosine (Table IV), which is the appropriate order for inductive effects aiding the second protonation which results in the solvolyzing dication. These substituents are on the imidazole ring and are closer to the affected C–N bond cleavage and would be expected to exert a greater influence on the solvolysis than the substituents in the pyrimidine ring if the imidazole ring as the site of the second protonation is accepted.

The acid solvolysis of the phenyl α- and β-D-glucopyranosides has been presumed to proceed by an A-1 mechanism[27] since it has been argued that an electron-withdrawing group in the phenyl ring results in a composite effect, both enhancing and retarding the solvolysis.[27] Such a group would tend to decrease the solvolyzing protonated nucleosides in the solution, but enhance the rate of their decomposition. These counteracting effects should tend to minimize the changes in reactivity. It has been suggested[6,8] that the contrary order of reactivity observed[2] for the acid solvolysis of uracil nucleosides, *viz.*, 5-bromodeoxyuridine > thymidine > deoxyuridine, may be explained on the basis of the A-1 mechanism, which order was stated[2] to be contrary to the effects expected if the solvolysis proceeded *via* the Schiff base mechanism.[1-4]

Support for the A-1 mechanism is given by the entropies of activation $\Delta S^{\pm}$, obtained in these studies (Table IV) and in others in the literature,[7,8,27,28] similar glycosidic solvolyses. The entropies of activation, calculated using the bimolecular rate constant for the attack of a hydrogen ion on the protonated nucleosides, are small and negative except for the 2′-deoxyadenosine and 2′,3′-dideoxy-2′,3′-didehydroadenosine. The $\Delta S^{\pm}$ values are small and positive for these compounds and for the acid solvolysis of nonprotonated nucleosides (Table VI). It has been proposed[28] that all acid-catalyzed solvolysis reactions of the unimolecular, A-1, type could be expected to have small $\Delta S^{\pm}$ values of either positive or negative sign and those of the bimolecular A-2 type (such as water attack on a proton activated species) to have large negative $\Delta S^{\pm}$ values. The acid solvolysis of the adenine nucleosides may be considered to be of the A-1 type on the basis of this suggestion. This is also consistent with the entropies of activation calculated for the acid solvolysis of protonated 5-substituted 2′-deoxyuridines[2,5] and for the pH-independent solvolysis of some of the same compounds.[6]

The most basic position in the adenine derivatives is N-1 and it may be the first to be protonated.[13] This has been shown on the basis of ultraviolet and infrared spectra in acidic aqueous and deuterium oxide solutions.[13,29,30] During the acid-catalyzed solvolysis of the protonated adenine nucleosides, a second proton has to attack the molecule to result in a solvolyzing dication.

It has been argued that this attack may occur at N-7[31] since 8-aminoadenosine (**1**) was completely solvolyzed in refluxing 1 *M* hydrochloric acid in less than 15 min, whereas 6-amino-9-β-D-ribofuranosyl-8-purinone (8-hydroxyadenosine) (**2**) was stated to be essentially unchanged after 2 hr under the same conditions.[31]

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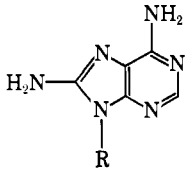
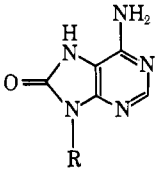
Table VII. Spectral and Paper Chromatographic Characteristics of 1-Methyladenosine and the Products of Its Acid Solvolysis

Compound	λ_{max}, nm 0.01 *M* HCl	λ_{max}, nm 0.01 *M* NaOH	R_f[c]	R_f (rel to adenine)
1-Methyladenosine	256 (256.5)[a]	257 (257)[a] 260 – 265 (s)	0.50	1.62
1-Methyladenine	259 (259)[b]	270 (270)[b]	0.48	1.55 (1.6)[b]
End of product of acid solvolysis	281	290	0.54	1.75
5-Aminoimidazole-4-*N'*-methylcarboxamidine	(281)[b]	(290)[b]		(1.8)[b]

[a] From ref 13. [b] From ref 34. [c] Developing system: isopropyl alcohol–5% ammonium sulfate (1:19) from ref 34.



1

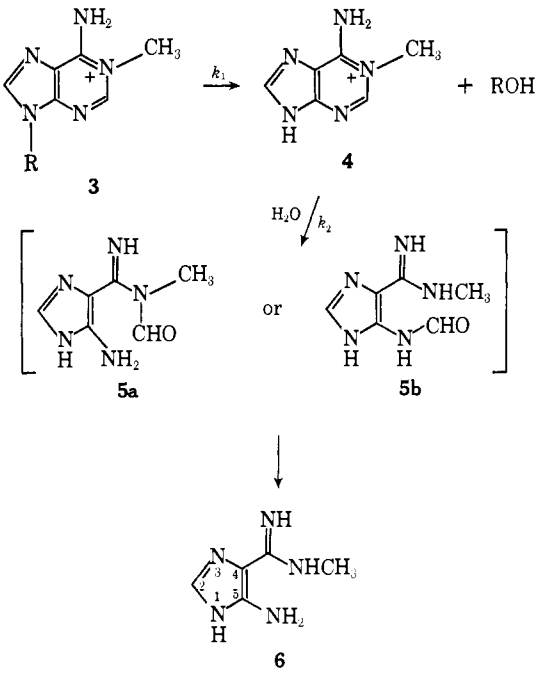


2

The possible explanation for this stability was that the purinone **2** existed in the keto form in position 8, and the hydrogen at N-7 in the uncharged molecule made protonation of the imidazole ring difficult.[31] It was further argued that in 8-aminoadenosine, the N-7 position should be more readily protonated than in adenosine to account for the rapid solvolysis of 8-aminoadenosine. The same behavior was noted for the corresponding guanosine derivatives.[31] This has been interpreted as lending support to the proposal[32] that protonation at N-7 is an important step in the acid solvolysis of all purine nucleosides. This is also consistent with the reported stability of 7-deazadenosine to acid solvolysis,[33] where there is a CH function instead of N at the N-7 position. At the very least this tends to be consistent with the presumption that hydrogen ion attack leading to solvolysis of a protonated nucleoside is on the imidazole portion of adenosine rather than on the ethereal oxygen.

Acid Solvolysis of 1-Methyladenosine. The acid solvolysis of **3** involved an initial, relatively rapid cleavage of the glycosyl bond with the formation of 1-methyladenine (**4**) which was then slowly transformed in the acid medium to a compound identified as 5-aminoimidazole-4-*N'*-methylcarboxamidine (**6**). The latter compound of the sequential reaction was formed by the opening of the pyrimidine ring and loss of C-2 of the reactive intermediate, **5a** or **5b**, from 1-methyladenine (**4**) (Scheme I). The assignments were made on the basis of spectral and paper chromatographic comparisons with similar data in the literature[17,34] (Table VII). The 5-aminoimidazole-4-*N'*-methylcarboxamidine had been obtained from the products of methylation of adenosine with dimethyl sulfate which had been refluxed with 1 *M* hydrochloric acid for 1 hr.[34] The same product had been obtained by refluxing 1-methyladenine with 6 *N* hydrochloric acid.[34] The apparent first-order rate constant for this transformation (k_2 = 1.05 × 10^{-5} sec^{-1} in 0.10 *M* hydrochloric acid at 80.0°) was determined by following the acid degradation of 1-methyladenine under the same conditions as used for 1-methyladenosine, and it was found that k_2 thus obtained was in excellent agreement with that calculated for the second step of the sequential reaction of 1-methyladenosine (Table V).

Scheme I



5-aminoimidazole-4-*N'*-methylcarboxamidine

Both the catalyses of 1-methyladenosine to 1-methyladenine and that of the latter to 5-aminoimidazole-4-*N'*-methylcarboxamidine were specific acid catalyzed and the respective specific hydrogen ion catalytic constants at 80° (Table V) were $(k_1)_{H^+}$ = 13.5 × 10^{-4} l. mol^{-1} sec^{-1} and $(k_2)_{H^+}$ = 1.19 × 10^{-4} l. mol^{-1} sec^{-1}.

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