Solvolysis of Adenine Nucleosides. II. Effects of Sugars and Adenine Substituents on Alkaline Solvolyses

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Abstract: The alkaline solvolyses of adenine nucleosides proceed by two parallel routes. The one is cleavage of the glycosyl bond to a stable adenine and a sugar and these rates initially increase with alkali and then remain relatively constant at the higher alkalinities. Concomitantly, the imidazole ring is attacked and opened with subsequent cleavage of the ribosyl bond and loss of C-8 as formic acid to form compounds analogous to 4,5,6-triaminopyrimidine at rates that appear proportional to hydroxide ion concentrations even at the higher alkalinities. It follows that the latter route is favored at high alkalinities. The resultant 4,5,6-triaminopyrimidine degrades relatively rapidly to nonchromophoric compounds. The numbers of hydroxyl groups in the sugar moiety do not appear to have any highly significant effect on rates of alkaline hydrolyses. Adenosine and 2′-deoxyadenosine have equivalent rates. Electron-withdrawing substituents on the adenine moiety increase alkaline solvolysis rates whereas electron-donating substituents retard. This is consistent with the kinetically observed hydroxyl ion attack. The alkali-induced recyclization of 1-methyladenosine that occurs under room temperature and mildly alkaline conditions yields N^6-methyladenosine which subsequently solvolyzes at high alkalinities and temperatures, *e.g.*, 80°, in the manner previously cited for adenine nucleosides.

The effect of glycosidic structure and substituents in the adenine moiety on the acid-catalyzed solvolyses of adenine nucleosides to the respective adenines and sugars has been studied in detail in the previous paper of this series.[1]

In general, the nucleosides are more resistant to alkaline solvolysis. Although a reasonable number of systematic kinetic studies in the alkaline region have been conducted on pyrimidine nucleosides[2-4] and their corresponding substituted uracils,[5] the only systematic kinetic studies in the alkaline region of solvolyses of adenine nucleosides of which we are aware are those for psicofuranine (9-β-D-psicofuranosyladenine)[6] by analysis of the acid-liberated psicose from unreacted psicofuranine, and the spectrally monitored rearrangement of 1-methyladenosine to 6-methyladenosine.[7]

In addition to the solvolysis to the respective adenine and sugar certain purine nucleosides undergo alkaline rupture of the imidazole ring[8-13] with subsequent deformylation of the 5-amino group with loss of C-8 to result in appropriately substituted sugar-containing intermediates that are converted finally to the respective 4,5-diaminopyrimidines so that adenosine yielded 4,5,6-triaminopyrimidine.[12] If the initial step in the degradation is a nucleophilic attack by the hydroxide ion at C-8, the electron density at this carbon would depend on the substituents in the pyrimidine ring.[11b] Purine bases which existed as anions in alkaline solution did not show this alkaline instability.[12,14,15]

An alternate degradation of the purine nucleosides may involve ring opening of the pyrimidine section of the purine base.[1,16,17] This can occur readily if the nucleoside is "oxygenated" or alkylated at N-1 (*e.g.*, 1-methyladenosine) or alkylated at N-3 as in the cyclonucleosides. Many of these degradations are believed to involve an initial nucleophilic attack by hydroxide ion at C-2 to yield an intermediate formyl derivative as a result of ring cleavage.[17] Migration of the alkyl group from N-1 to the extranuclear amino group occurred on alkaline treatment of alkylated adenines by ring opening and reclosure.[7,16-20]

This paper considers the kinetics of alkaline solvolysis of variously substituted adenine nucleosides.

Experimental Section

The compounds used, their spectral characteristics, pK_a' values, and acknowledged sources have been given in the previous paper[1] of this series.

Monitoring of Alkaline Solvolyses by Paper Chromatography. The alkaline solvolysis of adenosine ($10^{-2}M$) in 1.0 *M* sodium hydroxide at 80.0° was monitored by paper chromatography. Samples of the reaction mixture were taken at intervals of time, equal volumes of 1.0 *M* hydrochloric acid were added, and the pH was adjusted to about 9–10. Ten microliters of these solutions were spotted on Whatman No. 1 paper along with 10 μl of 5.0×10^{-3} *M* solutions of adenosine, adenine, D-ribose, and 4,5,6-triaminopyrimidine sulfate as standards. Development was carried out with 5% disodium hydrogen orthophosphate solution in water saturated with isoamyl alcohol, and with butanol–ethanol–water (4:1:5), organic phase. The development was carried out until the solvent front reached about 15 cm. The paper was then taken out of the de-

(1) E. R. Garrett and P. J. Mehta, *J. Amer. Chem. Soc.*, 94, 8557 (1972).
(2) E. R. Garrett, P. B. Chemburkar, and T. Suzuki, *Chem. Pharm. Bull.*, 13, 1113 (1965).
(3) E. R. Garrett and G. J. Yakatan, *J. Pharm. Sci.*, 57, 1478 (1968).
(4) H. J. Nestler and E. R. Garrett, *ibid.*, 57, 1117 (1968).
(5) E. R. Garrett, H. J. Nestler, and A. Somodi, *J. Org. Chem.*, 33, 3460 (1968).
(6) E. R. Garrett, *J. Amer. Chem. Soc.*, 82, 827 (1960).
(7) J. B. Macon and R. Wolfenden, *Biochemistry*, 7, 3453 (1968).
(8) D. J. Brown, *J. Appl. Chem.*, 4, 72 (1954).
(9) M. P. Gordon, V. S. Weliky, and G. B. Brown, *J. Amer. Chem. Soc.*, 79, 3245 (1957).
(10) D. I. Magrath and G. B. Brown, *ibid.*, 79, 3252 (1957).
(11) G. B. Brown, M. P. Gordon, D. I. Magrath, and A. Hampton in "The Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1957: (a) p 192; (b) pp 195–196.
(12) A. S. Jones, A. M. Mian, and R. T. Walker, *J. Chem. Soc. C*, 692 (1966).
(13) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 36, 1962 (1971).
(14) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).
(15) A. M. Mian and R. T. Walker, *J. Chem. Soc. C*, 2577 (1968).
(16) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960).
(17) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, p 32.
(18) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, 85, 193 1963).
(19) E. C. Taylor and P. K. Loeffler, *ibid.*, 82, 3147 (1960).
(20) J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, 15, 182 (1972).

velopment chamber, dried in a current of warm air, and examined under a short wavelength ultraviolet (250 nm) lamp. The Bratton–Marshall color reaction[21] was used to locate the primary aromatic amino group.

The alkaline solvolysis of 2′-deoxyadenosine and of 8-bromoadenosine was monitored by paper chromatography in the same way.

Procedures for Kinetic Studies. The nucleoside (usually 0.10 mmol) was weighed, quantitatively transferred to a 100-ml volumetric flask, and sodium hydroxide (or potassium hydroxide) solution (100 ml) of the required molarity was added. The flask was then transferred to a constant-temperature bath. Temperatures in the range of 60.0–80.0° were used for all the nucleosides except 1-methyladenosine at 25.5 and 35.0°. Samples were withdrawn from the reaction mixture at appropriate time intervals and quickly cooled to room temperature. An aliquot (2 ml) was treated with an equal volume of formic acid solution of appropriate molarity so that the pH of the mixture was adjusted to 3.5 ± 0.1. The absorbance of this solution was recorded between 350 and 280 nm, using a reagent blank prepared in the same way. Another aliquot (3 ml) of the cooled sample was diluted to 50 ml with distilled water or a sodium hydroxide solution of appropriate molarity so that the pH of the resulting dilute solution was about 12–13. The absorbance of this solution was recorded between 350 and 280 nm, using an appropiate reagent blank. Similar studies were conducted on adenine and 8-bromoadenine at 80.0° in 1.0 *M* sodium hydroxide.

The possibility of spectral interference from the alkaline decomposition products of 2-deoxy-D-ribose[22,23] was checked by maintaining a 10^{-3} *M* solution of the sugar in 1.0 *M* sodium hydroxide at 80.0°, and taking samples at intervals of time. A part of each sample was treated with an equal volume of 1.77 *M* formic acid (resulting pH 3.5 ± 0.1), and another part was diluted (1:25) with distilled water. The spectrophotometric absorbance of each solution was recorded between 350 and 230 nm using an appropriate blank.

Solutions of ionic strength 0.10 were used except for those in sodium hydroxide more concentrated than 0.10 *M*.

The rearrangement of 1-methyladenosine to N^6-methyladenosine at alkaline pH values was followed spectrophotometrically at 25.0 and 35.0°. A 5–6 × 10^{-5} *M* solution of the 1-methyladenosine in the appropriate buffer solution in a cuvette was maintained in the thermostated cell compartment of the Cary 15 recording spectrophotometer and the ultraviolet absorbance of the sample was recorded as a function of time between 350 and 230 nm. The buffer solution was used as the blank.

Analog Computer and Curve Fitting. An analog computer (PACE TR-48, Electronic Associates, Inc.) and a Mosely Model 2D2 recorder were used to fit the model

$$\begin{array}{l} \text{adenine glycoside} \xrightarrow{k_2} \text{triaminopyrimidine} \xrightarrow{k_3} \\ \quad\quad \downarrow k_1 \qquad\qquad\qquad\qquad \text{nonchromophoric products} \\ \text{adenine} + \text{sugar} \end{array} \quad (1)$$

for the dual pathways of alkaline solvolysis to the absorbance data at 260 nm at pH 12–13 monitored as a function of time. The rate constants k_1 and k_2 of eq 1 could be obtained from this fitting when the apparent first-order rate constant $k_T = k_1 + k_2$, for the overall loss, and the rate constant k_3, for the solvolysis of 4,5,6-triaminopyrimidine under the same experimental conditions, were known. The appropriate rate setting potentiometers were adjusted so that $k_T = k_1 + k_2$ for a known k_3. The potentiometers for k_1 and k_2 were adjusted until a good fit was obtained with regard to both the absorbance, A_{260}, against time and the spectral yield of adenine.

Results

Paper chromatography of samples taken at intervals of time from a 10^{-2} *M* solution of adenosine in 1.0 *M* sodium hydroxide maintained at 80.0° revealed that the spot due to the nucleoside became smaller, fainter with time, and disappeared in about 7.5–8.5 hr. Two other spots were visible under the short-wavelength ultraviolet lamp. One of these spots had the same R_f value as that of the adenine control (0.31). It reached its maximum size and intensity at the time the nucleoside spot disappeared and then changed very little, if at all. The other spot (R_f = 0.38) was suspected to be a product of imidazole ring opening. It gave a red color after treatment with nitrous acid followed by the Bratton–Marshall reagent[21] to show the presence of the primary aromatic amino group(s). It did not react in the silver nitrate test,[24] which suggested that the compound did not contain an attached ribose. Since it had been spectrally and chromatographically observed that purine riboside (in 0.04 *M* sodium hydroxide at 20.0°)[9] produced 4,5-diaminopyrimidine derivatives, and that adenosine had been chromatographically observed to form 4,5,6-triaminopyrimidine[11b] in 1.0 *M* sodium hydroxide at 100.0°, the presence of 4,5,6-triaminopyrimidine was suspected. The spot was eluted from several chromatograms with 0.10 *M* hydrochloric acid. The combined eluate showed the same absorbance maxima as 4,5,6-triaminopyrimidine in 1.8 *M* hydrochloric acid, in formate buffer pH 3.5, and in phosphate buffer pH 7.5 for the diprotonated, monoprotonated, and uncharged species, respectively.

The solvolysis of 2′-deoxyadenosine under the same conditions also produced adenine and 4,5,6-triaminopyrimidine. It was to be expected that changing the sugar moiety would not change the mechanism of attack on the adenine portion of the nucleosides. The solvolysis of 8-bromoadenosine in 1.0 *M* sodium hydroxide at 80.0° was followed by paper chromatography, and a spot for 8-bromoadenine plus another visible under the uv lamp was detected. No spectral changes were observed for 8-bromoadenine in 1.0 *M* sodium hydroxide at 80.0° for over 6 hr, which at least indicates that the ring is not opened under these conditions.

The course of alkaline solvolysis of 2′-deoxyadenosine (and the other nucleosides) was followed mainly by spectral measurements. A typical spectral monitoring is given in Figure 1 where the absorbance of 2′-deoxyadenosine in 1.0 *M* sodium hydroxide at 80.0° at the absorbance maximum (260 nm) decreased rapidly with an isosbestic point at 273 nm. About 4 hr after the start of the reaction, the isosbestic point was lost and the subsequent decrease in absorbance was accompanied by a small shift in the absorbance maximum to longer wavelengths. Another feature of the spectra was a small increase in absorbance in the 280–320-nm region for about the first 4 hr followed by a decrease in absorbance in that region. From a knowledge of the changes in the ultraviolet spectra of 2′-deoxyadenosine, adenine, and 4,5,6-triaminopyrimidine in their uncharged and protonated states (see Table I of ref 1) it was apparent that absorbance measurements in the region of 290–320 nm at pH 3.5 ± 0.1 permitted determination of 4,5,6-triaminopyrimidine independently of the 2′-deoxyadenosine or adenine in the reaction mixture.

Possible spectral interference by the alkaline decomposition products of 2-deoxy-D-ribose, formed during the degradation of 2′-deoxyadenosine, was checked out. Under the conditions used, the deoxyribose formed prod-

(21) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

(22) J. K. Seydel and E. R. Garrett, *Anal. Chem.*, 37, 271 (1965).

(23) J. K. Seydel, E. R. Garrett, W. Diller, and K. J. Schaper, *J. Pharm. Sci.*, **56**, 858 (1967).

(24) S. M. Partridge, *J. Chem. Soc.*, 1579 (1951).

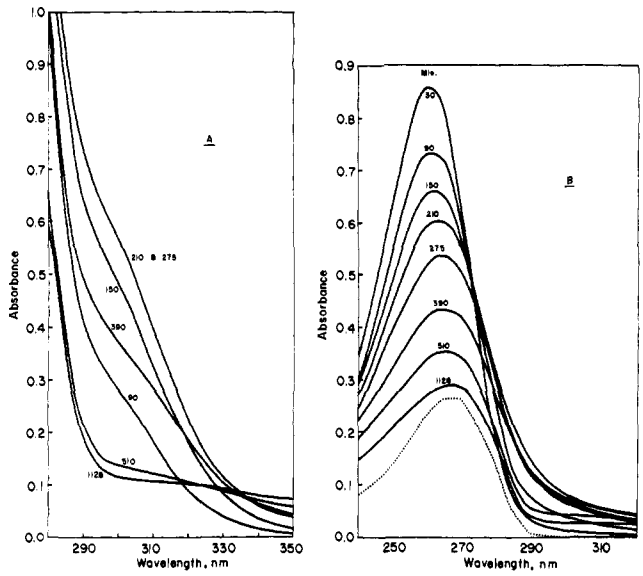


Figure 1. Typical spectral changes of adenine nucleosides allowed to react in alkali. The spectra, A, are of 2 ml of 10^{-3} M deoxyadenosine allowed to react in 1.0 M KOH at 80.0°, diluted 1:1 with formic acid buffer to a final pH of 3.5 and read against a similar formate buffer blank. The spectra, B, are of 3 ml, diluted to 50 ml with water and read against a water blank. The final spectral yield of adenine was 40.4%. A typical spectrum of 3×10^{-5} M adenine in 0.01 M NaOH is given as the dashed line in B. The curves are labeled with their respective times of reaction in minutes.

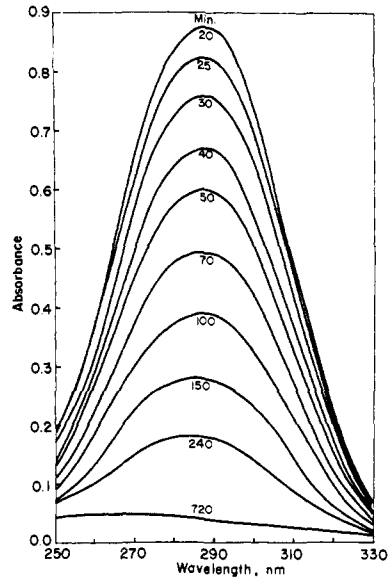


Figure 2. Typical spectral changes of 2.0×10^{-4} M 4,5,6-triaminopyrimidine in 1.0 M NaOH at 80.0° with each curve labeled with the number of minutes after the start of the degradation. Each sample was treated with an equal volume of 1.77 M formic acid to give a final pH of 3.5 ± 0.1 and was read against a similarly treated formate buffer blank.

ucts which had a very small absorbance (<0.02) at 260 nm and pH 12–13. However, an absorbance of about 0.25 at 310 nm and pH 3.5 was obtained 9 hr after the start of degradation and it remained unchanged for about 7 more hr. An absorbance value of 0.26 was obtained under these conditions. The absorbance values measured at 310 nm and pH 3.5 during the degradation of 2′-deoxyadenosine were corrected for these sugar decomposition products.

Estimation of Rate Constants for Parallel Reactions. The alkaline solvolysis of the adenine nucleosides resulted in the paper chromatographically identifiable corresponding adenines, *viz.*, adenine, 8-bromoadenine, N^6-methyladenine, and N^6,N^6-dimethyladenine, which were then stable in the alkaline medium. The pure adenine bases had shown no change spectrally in 1.0 M sodium hydroxide at 80.0° over a period of 36 hr. The 4,5,6-triaminopyrimidine, formed by a parallel reaction, disappeared quickly under the experimental conditions (Figure 2) so that even when a substantial fraction of the initial nucleoside concentration solvolyzed by that pathway, the 4,5,6-triaminopyrimidine made only a small contribution to the total absorbance of the reaction mixture. The model for the alkaline solvolysis of the adenine nucleosides consistent with all these facts is given in eq 1, where the apparent first-order rate constants k_1, k_2, and k_3 represent the rate-determining steps.

Rate Constants for the Solvolysis of 4,5,6-Triaminopyrimidine. The apparent first-order rate constants, k_3, of eq 1 were obtained from the loss of absorbance at 287 nm of 4,5,6-triaminopyrimidine under experimental conditions similar to those for the solvolysis studies of adenine nucleosides, after adjusting the reaction mixture to pH 3.5 ± 0.1.

The obtained first-order rate constants were functions of sodium hydroxide concentration in accordance with

$$k_3 = k_0 + k_{OH}[NaOH] \quad (2)$$

and the bimolecular rate constant for the attack of OH^- on the uncharged 4,5,6-triaminopyrimidine, k_{OH}, and the apparent first-order rate constant for the water attack on the uncharged species, k_0, were estimated from the plots of k_3 (Table I) against the sodium hydroxide concentrations.

Table I. Apparent First-Order Rate Constants, k_3 (sec^{-1}), for the Solvolysis of 4,5,6-Triaminopyrimidine to Nonchromophoric Products

[NaOH], M	10^5k_3 80.0°	70.0°	60.0°
1.00	24.5	14.4	8.03
0.80	18.3		
0.40	14.5		
0.10	9.40		
0.01	7.91	5.71	3.25
Phosphate buffer (pH 7.50)	3.96		
Acetate buffer (pH 5.30)	4.09		
10^5k_0,[a] sec^{-1}	7.6	5.0	2.9
10^5k_{OH},[a] l. mol^{-1} sec^{-1}	16.9	9.4	5.1

[a] These values were estimated from the slopes and intercepts of plots of k_3 against [NaOH] in accordance with $k_3 = k_0 + k_{OH}$·[NaOH]. Estimated thermodynamic values from k_{OH} were $\Delta H_a^{\ddagger}$ = 14.8 kcal mol^{-1} and $\Delta S^{\ddagger} = -7.9$ eu where $k_3 = Pe^{-\Delta H_a^{\ddagger}/RT}$, and $\Delta H^{\ddagger} = \Delta H_a^{\ddagger} - RT$ and $\Delta S^{\ddagger} = 2.303R[\log k_{OH} - \log (kT/h) + (\Delta H^{\ddagger}/2.303)RT]$.

Kinetic Analysis of Sequential and Parallel Adenine Nucleoside Solvolysis. An initial estimate of $k_T = k_1 + k_2$ values for eq 1 was obtained from the apparent first-order loss of absorbance, A, of the adenine nucleoside to a final value which was assigned to the adenine produced, A_∞, as measured at 260 nm at pH 12–13. The adenine yield, as determined in this manner, was *ca.* 90% or better for adenosine and 2′-deoxyadenosine in 0.01, 0.05, and 0.10 M sodium hydroxide at 80°. The adenine yield, $[Ad]_\infty$, decreased signifi-

cantly, and thus the amount of purine ring fractured increased, with increasing alkali concentration (Table II). These values were obtained from the 260-nm absorbance at the end of reaction (Figure 1) when all of the nucleoside had disappeared. The plots of log $(A - A_\infty)$ against time were appropriately linear and thus demonstrated that the interference from the absorbance of the transient degrading 4,5,6-triaminopyrimidine with the apparent first-order plot was negligible. Since in this case, adenine, Ad, is a product of one of the parallel reactions[25]

$$\frac{k_1}{k_T} = \frac{k_1}{k_1 + k_2} = \frac{[Ad]_\infty}{[AR]_0} \quad (3)$$

where $[AR]_0$ is the initial concentration of adenine nucleoside. Since k_T, $[Ad]_\infty$, and $[AR]_0$ are known, k_1 and k_2 values were estimated from eq 3. Such values for various nucleosides are listed in Table II.

Table II. Estimates of Rate Constants and Adenine Yields for the Alkaline Solvolysis of Adenine Nucleosides

T, °C	[NaOH], M	10^5k	% yield of adenine[a]	10^5k_1	10^5k_2	10^5k_3
		Adenosine				
80.0	1.00[b]	6.26	36	2.25	4.01	26
	0.60[b]	3.97	42	1.67	2.30	18
	0.20	1.13	66	0.75	0.38	11
	0.10	0.757	77	0.58	0.19	
	0.10	0.669	82	0.55	0.12	
	0.05	0.404	85	0.34	0.06	
75.0	1.00		26			
	0.20		63			
	0.10	0.522	82	0.43	0.10	
	0.05	0.454	90	0.41	0.04	
70.0	1.00	2.82	23	0.64	2.19	
60.0	1.00	0.92	18	0.17	0.75	
	0.05		90			
		2′-Deoxyadenosine				
80.0	1.00[b,c]	6.00	37	2.22	3.78	
	0.80	4.62	48	2.22	2.40	
	0.40	3.05	67	2.06	1.00	
	0.20	1.76	79	1.39	0.37	
	0.10	1.20	88	1.09	0.15	
	0.10	1.20	88	1.05	0.15	
	0.05	0.346	92	0.32	0.03	
71.0	0.20	0.640	71	0.46	0.19	
		6-Dimethylaminopurine Ribonucleoside				
80.0	1.00	0.39	~100			
60.0	1.00	0.24				
		6-Methylaminopurine Ribonucleoside				
80.0	1.00	1.60				
60.0	1.00	0.45				
		Isopropylideneadenosine				
80.0	1.00	12	28	3.3	8.7	
75.0	0.100	0.740				
		Adenine Arabinoside				
80.0	1.00	2.92	31	0.92	2.00	
		8-Bromoadenosine				
80.0	1.00	105				

[a] Calculated from % adenine $= 100\epsilon_A A_\infty/\epsilon_{Ad}A_0$, where A_0 and A_∞ are the initial and final absorbances at 260 nm with ϵ_A and ϵ_{Ad} the molar absorptivities of the adenine nucleoside and adenine, respectively. [b] Absorbance data fitted by analog computer to the parallel reaction pathway model to adenine and triaminopyrimidine were consistent with the derived rate constants and the known k_3 value (Table I) for the alkaline degradation of triaminopyrimidine. [c] Per cent yields of adenine at 75.0, 70.0, and 60.0° were 37.2, 30.4, and 25.2, respectively.

(25) E. R. Garrett, *J. Amer. Chem. Soc.*, **80**, 4049 (1958).

The absorbance, A_{260}, is a linear function of the absorbances of the adenine nucleoside (AR), adenine (Ad) and 4,5,6-triaminopyrimidine (TAP) at any given time, t, during the alkaline degradation of the adenine nucleoside

$$A_{260} = \epsilon_{260}^{AR}[AR]_t + \epsilon_{260}^{Ad}[Ad]_t + \epsilon_{260}^{TAP}[TAP]_t \quad (4)$$

where the ϵ values are the molar absorptivities for the compounds at the pH of measurement. Typical values for adenosine are: $\epsilon_{260}^{AR} = 15{,}290$, $\epsilon_{260}^{Ad} = 12{,}310$, and $\epsilon_{260}^{TAP} = 5600$.

Since the products of the alkaline solvolysis of the adenine nucleosides that contribute to the absorbance are only adenine and 4,5,6-triaminopyrimidine (eq 1), the concentration of the latter can be calculated from

$$[TAP]_{calcd} = \frac{k_2[AR]_0}{k_3 - k_T}(e^{-k_T t} - e^{-k_3 t}) \quad (5)$$

where the rate constant k_3 was obtained from studies on the degradation of 4,5,6-triaminopyrimidine (Table I) and k_2 was calculated from the rearranged eq 3

$$k_2 = k_1\left\{\frac{[AR]_0}{[Ad]_\infty} - 1\right\} \quad (6)$$

where k_1 was estimated from the known k_T values and the fractional yield of adenosine that is adenine (Table II) as per eq 3. The actual concentration of the 4,5,6-triaminopyrimidine, [TAP], was calculated at any time, t, from the measurement of the 310-nm absorbance at pH 3.5 and its molar absorptivity, $\epsilon_{310}^{pH\,3.5} = 5700$. The total absorbance, A_{260}, at pH 12–13 was calculated from eq 4 using the known absorptivities and the relations

$$[AR]_t = [AR]_0 e^{-k_T t} \quad (7)$$

$$[Ad]_t = \frac{k_1[AR]_0}{k_T}(1 - e^{-k_T t}) \quad (8)$$

$$[TAP]_t = \frac{A_{310}^{pH\,3.5}}{\epsilon_{310}^{pH\,3.5}} \quad (9)$$

where the values of k_T (eq 3) and k_2 (eq 6) were estimated as detailed previously. The agreement between the measured and calculated absorbances (eq 4) at 260 nm was excellent (see Figure 3) and indicated that the model of eq 1 for the routes of alkaline solvolysis was essentially correct. The maximum differences between the calculated and measured absorbances for the data of Figure 3 were −4% at 3.5 hr and +2.5% at 1.5 hr.

The apparent first-order rate constants at 80.0°, k_T for the overall loss of adenosine and 2′-deoxyadenosine, k_1 for their solvolyses to adenine, and k_2 for their solvolyses to triaminopyrimidine are plotted against the sodium hydroxide concentration in Figure 4. The rate constant for the production of adenine appears to be initially a function of alkaline concentration and then tends to become pH independent at high alkali concentrations. In contrast, the rate of increase of the rate constant for the production of triaminopyrimidine tends to increase at the lower alkali concentrations until it appears to become proportional at the higher hydroxide ion concentrations. The most rational explanation for these phenomena is that the adenine nucleosides undergo either the dissociation of a proton or the acceptance of an hydroxyl ion with an apparent

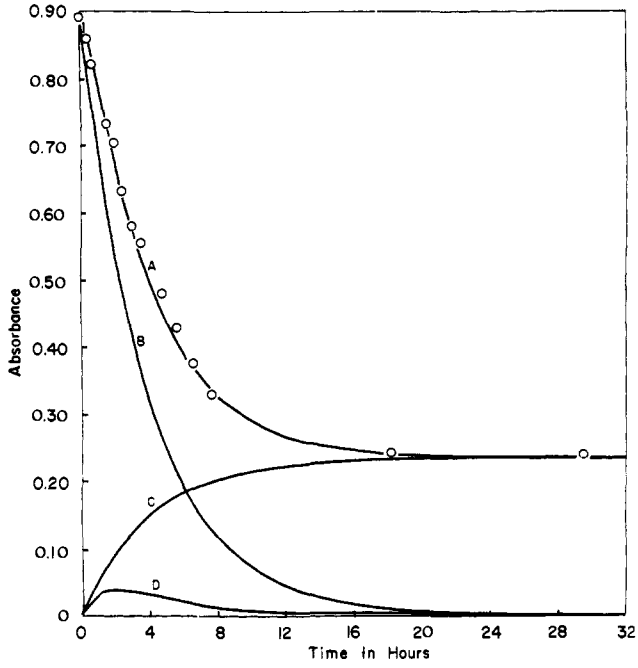


Figure 3. Typical analog computer fit of the total absorbance at 260 nm *vs.* time data (curve A) for the alkaline solvolysis of 9.4×10^{-4} *M* 2′-deoxyadenosine (absorbance values are for the solution diluted to 5.6×10^{-5} *M*) in 1.0 *M* NaOH at 80.0°. Curve B is the time course for the absorbance attributable to 2′-deoxyadenosine (AR) where curves C and D are for the absorbances of the products adenine (Ad) and 4,5,6-triaminopyrimidine (TAP), respectively. The program for the analog computer was based on Ad $\rightarrow$ (k_1) AR $\rightarrow$ (k_2) TAP $\rightarrow$ (k_3) nonchromophoric products. The fit of the computer drawn curve A to the experimental absorbance data is well demonstrated.

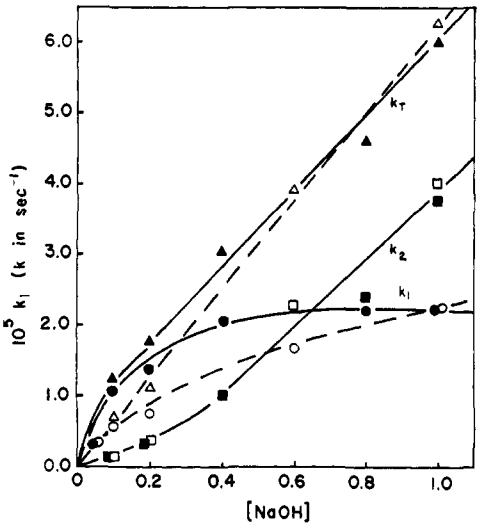


Figure 4. Apparent first-order rate constants at 80.0° for the solvolyses of adenosine (open symbols, dashed lines) and 2′-deoxyadenosine (solid symbols, solid lines) as a function of sodium hydroxide concentrations in accordance with the model

$$\text{adenine} \xleftarrow{k_1} \text{adenosine} \xrightarrow{k_2} \text{4,5,6-triaminopyrimidine}$$

where $k_T = k_1 + k_2$.

pK_a' corresponding to the pH conditions in 0.1 *N* NaOH. The plots of Figure 4 can then be rationalized by proposing a preferred mechanism for adenine production by hydroxyl ion attack on the neutral nucleoside whereas triaminopyrimidine production would be favored by hydroxide ion attack on a negatively charged species.

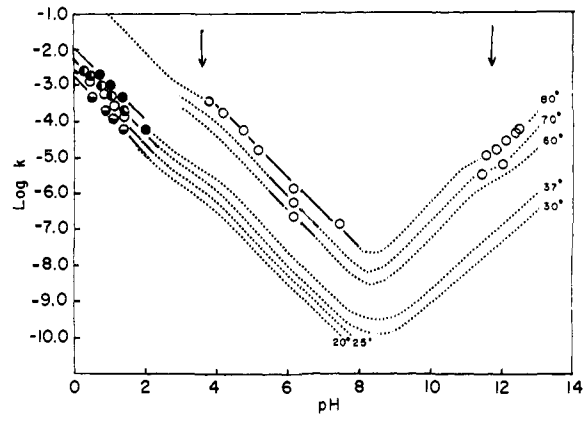


Figure 5. The pH profiles of the logarithm of the apparent first-order rate constants, k, in sec^{-1}, for the solvolysis of 2′-deoxyadenosine. The dashed lines are estimated portions of the curves. The arrows indicate apparent kinetic pK_a values.

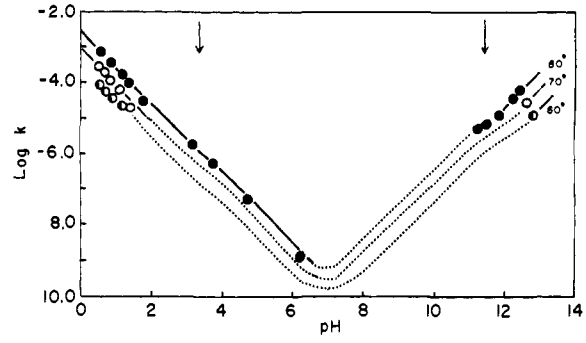


Figure 6. The pH profiles of the logarithm of the apparent first-order rate constants, k, in sec^{-1}, for the solvolysis of adenosine. The dashed lines are estimated portions of the curves. The arrows indicate apparent kinetic pK_a values.

The sum of the rate constants, k_T, for these two parallel reactions then appears fortuitously to be a linear function of alkali concentration.

Log *k*–pH Profiles for 2′-Deoxyadenosine and Adenosine. The overall rate of solvolysis of 2′-deoxyadenosine and adenosine decreased with increasing pH to a minimum and then increased with pH (Figures 5 and 6) and the increasing concentration of sodium hydroxide. The conditions and apparent first-order rate constants for acid solvolysis have been given previously,[1] whereas the constants for alkaline solvolysis are listed in Table II.

The pH values of the alkaline solutions were calculated from

$$\text{pH} = \text{p}K_w - \text{pOH} = \text{p}K_w - \log f[\text{NaOH}] \quad (10)$$

and those of the acid solutions from

$$\text{pH} = -\log f[\text{HCl}] \quad (11)$$

where the pK_w values and the mean activity coefficients for the experimental sodium hydroxide and HCl concentrations were obtained or extrapolated from data in the literature.[26]

(26) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold, New York, N. Y., p 657.

The Kinetics of the 1-Methyladenosine Rearrangement to N^6-Methyladenosine. In contrast to the sequential solvolysis of 1-methyladenosine to 1-methyladenine and then to 5-aminoimidazole-4-*N'*-carboxamidine[1] 1-methyladenosine showed another type of reaction in the neutral and alkaline regions.[7,16-20] The ultraviolet absorption spectrum of 1-methyladenosine at low temperatures and even in mildly alkaline solutions changed with time to the spectrum of N^6-methyladenosine (see spectral data in Table I of ref 1) with isosbestic points at 290 and 257 nm.

The apparent first-order rate constants at various pH values were for 35.0° (10^4k, pH) 0.211, 9.11; 0.528, 10.95; 0.790, 11.56 (in 0.010 *N* NaOH, calcd[26] pH 11.75), 3.20 (in 0.100 *N* NaOH, calcd[26] pH 12.57); and for 25.5° 0.0950, 9.08; 0.285, 10.95; 0.510 (in 0.010 *N* NaOH, calcd[26] pH 12.0); 1.09 (in 0.100 *N* NaOH, calcd[26] pH 12.9). The data at 25.5° are similar to those reported by Macon and Wolfenden.[7]

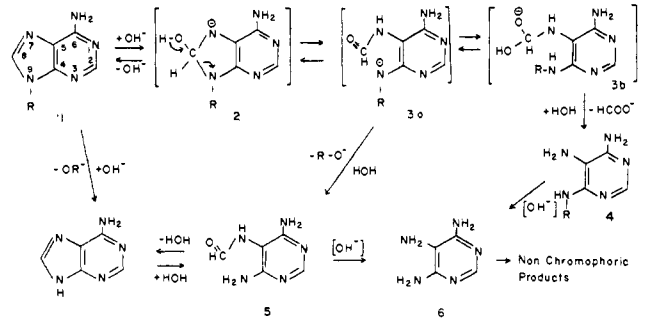
The ΔH_a is 9.74 kcal/mol based on the rate constants at 0.100 *M* for the two temperatures and the predicted $k_{NaOH} = k/0.1 = 6.62 \times 10^{-2}$ l. mol^{-1} sec^{-1} at 80.0°. An estimated $\Delta S^{\pm}$ is −8.4 eu.

The hydroxyl ion catalyzed solvolysis of purine nucleosides (*ca.* 10^{-5} sec^{-1} in 0.1 *M* NaOH at 80°, Table II) is apparently a much slower process than the hydroxyl ion attack that results in the rearrangement of 1-methyladenosine to N^6-methyladenosine (*ca.* 10^{-5} sec^{-1} at pH 9 at 25°).

Discussion

The studied adenine nucleosides undergo alkaline solvolysis by two parallel reactions (Scheme I and eq 1)

Scheme I



that appear to be simple cases of SN2 mechanisms. Relatively strenuous conditions of temperature (60–80°) are necessary to obtain reasonable rates (Table II). The cleavage of the glycosyl bond (the alkyl group of 7- or 9-alkylpurines) to a stable adenosine and sugar (or alcohol)[14] moiety is effected under such conditions. Such rates of solvolyses of adenosine and 2'-deoxyadenosine appear to initially increase with alkali and then remain relatively constant at the higher alkalinity (Figure 4 and Table II). Ionization of the attacked sugar moiety at higher alkalinities may inhibit hydroxide ion attack so that the rate of solvolysis to adenine is assignable to hydroxide ion attack on uncharged species only. The concomitant opening of the imidazole ring followed by cleavage of the ribosyl bond and loss of C-8 as formic acid to form compounds analogous to 4,5,6-triaminopyrimidine (Scheme I) appear to be proportional to hydroxide ion concentration even at the high alkalinities (Figure 4 and Table II). This route may be preferred when the sugar moiety is charged since hydroxide ion attack at a center further removed from the sugar anion is still possible.

The resultant 4,5,6-triaminopyrimidine degrades at faster or equivalent rates (Table I) with ultimate destruction of the pyrimidine ring (eq 2). Substitution of a group at position 7 and/or 9 apparently abets this route of degradation by ring opening. A hydrogen on the unsubstituted imidazole position of the purine ring undoubtedly dissociates under these alkaline conditions (when R = H in **1**) and the resultant negatively charged ion undoubtedly is resistant to solvolytic attack which may be mediated by hydroxyl ion attack on the same imidazole function.

A reasonable model for ring splitting is hydroxide ion attack on the C-8[11] of the 7 or 9 substituted adenine ring, **1**, to form a carbinolamine type intermediate which may equilibrate with the opened forms **3a** or **3b**. This latter structure may deformylate to irreversibly give the substituted 4,5,6-triaminopyrimidine (**4**) or **3a** may solvolyze to remove the glycoside. Subsequently, the intermediate **5** may deformylate or the intermediate **4** may cleave its glycosyl bond to give the 4,5,6-triaminopyrimidine (**6**). The chromatographic evidence and the good computer fits of the absorbance data with time to eq 1 demonstrate an accumulation of the relatively rapidly degradable **6** which implies that the steps **5** → **6** or **4** → **6** are not rate determining.

The enhancement of rates of alkaline solvolysis in 1.00 *M* NaOH with the electron-withdrawing 8-bromo derivative and the diminution of rates with the electron-donating 6-methylamino and 6-dimethylamino derivatives (Table II) are consistent with the proposed mechanisms of Scheme I.

The numbers of hydroxyl groups in the sugar moiety of the adenine nucleosides do not appear to have any highly significant effect on the rate of alkaline hydrolysis, as is demonstrated by comparison of the rates of alkaline solvolysis of adenosine and 2'-deoxyadenosine.

Acknowledgment. This work was supported in part by Grants GM-09864 and CA-10738 from the National Institutes of Health, U.S. Public Health Service, Bethesda, Md. It is based in part on work prepared for a Ph.D. Thesis to be submitted by P. J. Mehta to the University of Florida.