Reactions of Adenine Nucleosides with Aqueous Alkalies: Kinetics and Mechanism[1]

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Received January 6, 1986

The hydrolysis of adenosine in aqueous alkali has been studied by liquid chromatography (LC), NMR spectroscopy, and isotopic labeling techniques. The first step of the multistage reaction pathway has been shown to be a nucleophilic attack of hydroxide ion on the C8 atom with concomitant opening of the imidazole ring. The equilibrium mixture of 4-amino-5-formamido-6-(ribosylamino)pyrimidines obtained undergoes three competitive reactions, viz., intramolecular cyclization to adenine nucleosides and N^6-ribosyladenines and degradation to nonchromophoric products, most probably via intermediary formation of 4,5-diamino-6-(ribosylamino)pyrimidines. Isomeric N^6-ribosyladenines are further hydrolyzed to adenine and D-ribose. The rate constants for the different partial reactions have been determined at various concentrations of hydroxide ion. The mechanisms of individual steps are discussed. Comparative kinetic studies with 2′-deoxy-, 2′,3′-*O*-isopropylidene-, and 5′-*O*-methyladenosine and 9-β-D-arabinofuranosyladenine and its 5′-*O*-methyl derivative are interpreted to indicate that the glycon moiety hydroxyl groups do not play any important role in the alkaline cleavage of adenine nucleosides.

Detailed information of the reactions of nucleosides with aqueous alkalies is helpful in attempting to elucidate the degradation of oligonucleotides and nucleic acids in basic solutions. It has been repeatedly suggested that the decomposition of purine nucleosides[2-8] and 9-substituted purine bases[9] is initiated by a nucleophilic attack of hydroxide ion on the base moiety. The preferential site of attack is C2 in inosine[6,7] and C8 in adenosine,[4,5] 9-β-D-ribofuranosylpurine,[2,3,8] and 6-chloro-9-β-D-ribofuranosylpurine.[4] Cleavage of the glycosidic bond may compete with these reactions, as well as displacement of the C6 substituent by hydroxide ion.[4,5]

Little is known about the kinetics and mechanisms of the reactions that follow the initial attack of hydroxide ion. We have described previously[8] the kinetics for the multistage cleavage of 9-β-D-ribofuranosylpurine. With this compound the initial attack of hydroxide ion on the C8 atom, the deformylation of the resulting 5-formamido-4-(ribosylamino)pyrimidine, and the subsequent hydrolysis of the deformylated product to 4,5-diaminopyrimidine and D-ribose are all kinetically fairly well separated. In contrast, the corresponding intermediates have not been detected to accumulate during the alkaline decomposition of adenosine.[5] The aim of the present study is to give a kinetic description for the hydrolysis of adenosine and to compare the kinetics of different partial reactions to the data obtained with 9-β-D-ribofuranosylpurine. The role that the glycosyl hydroxyl functions play in the cleavage of adenine nucleosides has been elucidated by comparative kinetic studies with 2′-deoxy-, 2′,3′-*O*-isopropylidene-, and 5′-*O*-methyladenosine and 9-β-D-arabinofuranosyladenine and its 5′-*O*-methyl derivative.

Results and Discussion

LC analyses of the aliquots withdrawn at different intervals from the aqueous sodium hydroxide solutions of adenosine indicated that adenine is the main product of the alkaline decomposition of adenosine, as reported earlier by Garrett and Mehta.[5] In Figure 1 the mole fractions of adenine formed are plotted against the mole fractions of adenosine disappeared. The plot exhibits a marked upward curvature, suggesting that adenine is not released directly from the starting material but is formed via a reasonably stable intermediate. In other words, a nucleophilic displacement of an intact base moiety by either a hydroxide ion or an ionized hydroxyl group cannot constitute the main pathway for the formation of adenine. The data in Figure 1 also indicate that the release of the ^{14}C8 atom from [8-^{14}C]adenosine and [1-^{14}C]D-ribose from [1′-^{14}C]adenosine is preceded by formation of a detectably stable intermediate that still contains the C8 atom and the ribofuranosyl group.

In fact, detailed LC analyses verified the accumulation of three intermediates during the hydrolysis of adenosine, two of which were equilibrium mixtures of two major and two minor components. Moreover, a small amount of inosine was formed, as shown earlier.[4] Figure 2 shows the time-dependent product distribution in 0.50 mol dm^{-3} aqueous sodium hydroxide.

Spectroscopic characterization of the first intermediate, separated by preparative LC, strongly suggests that it is an equilibrium mixture of anomeric furanoid and pyranoid isomers of 4-amino-5-formamido-6-(ribosylamino)pyrimidine (**2**αf, **2**βf, **2**αp, and **2**βp in Scheme I). Table I records the NMR data observed. The ^{13}C NMR shifts of the aromatic carbons resemble the shifts of 4,6-diamino-5-formamidopyrimidine, and the shifts of the ribosyl carbons are almost identical with those reported[11] for anomeric *N*-phenyl-D-ribopyranosylamines. The ^{1}H NMR spectra exhibit the anomeric proton doublets of the major components at δ 5.53 (**2**αp) and 5.46 (**2**βp) and two singlets in the aromatic proton region. The anomeric protons of the minor components resonate at a lower field (δ 5.75 and 5.98), as expected for furanoid isomers.[12] The UV spectra of the compounds closely resembled that of 4,6-diamino-5-formamidopyrimidine.[13] Accordingly, it seems most probable that adenosine is decomposed via a 5-formamidopyrimidine derivative, analogously to the unsubstituted purine riboside.[2,3,8]

(1) Part B of the series "Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues".
(2) Gordon, M. P.; Weliky, V. S.; Brown, G. B. *J. Am. Chem. Soc.* **1957**, *79*, 3245.
(3) Magrath, D. I.; Brown, G. B. *J. Am. Chem. Soc.* **1957**, *79*, 3252.
(4) Jones, A. S.; Mian, A. M.; Walker, R. T. *J. Chem. Soc. C* **1966**, 692.
(5) Garrett, E. R.; Mehta, P. J. *J. Am. Chem. Soc.* **1972**, *94*, 8542.
(6) Suzuki, Y. *Chem. Lett.* **1973**, 547.
(7) Suzuki, Y. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 898.
(8) Lönnberg, H.; Lehikoinen, P. *J. Org. Chem.* **1984**, *49*, 4964.
(9) Lönnberg, H.; Lukkari, J.; Lehikoinen, P. *Acta Chem. Scand., Ser. B* **1984**, *38*, 573.
(10) Breitmaier, E.; Jung, G.; Voelter, W. *Chimia* **1972**, *26*, 139.
(11) Chavis, C.; de Goyrcy, C.; Dumont, F.; Imbach, J.-L. *Carbohydr. Res.* **1983**, *113*, 1.
(12) Kotowycz, G.; Lemieux, R. U. *Chem. Rev.* **1973**, *73*, 669.
(13) Cavalieri, L. F.; Bendich, A. *J. Am. Chem. Soc.* **1950**, *72*, 2587.

0022-3263/86/1951-3819$01.50/0

Table I. ^1H and ^{13}C NMR Chemical Shifts[a] for the Intermediates of the Alkaline Hydrolysis of Adenosine

	^1H NMR		^{13}C NMR	
compd[b]	H1′	base moiety	ribosyl moiety	base moiety
1βp[c]	5.70 (d)	8.22 (s), 8.36 (s)	65.2, 66.6, 68.1, 71.1, 79.7	118.7, 139.8, 150.0, 152.6, 155.8
2αp[d]	5.53 (d)	8.13 (s), 8.46 (s)	63.0, 67.1, 68.5, 69.6, 77.9[e]	94.2, 156.5, 158.8, 159.3, 164.8[f]
2βp[d]	5.46 (d)	8.13 (s), 8.50 (s)	63.4, 66.4, 69.0, 70.6, 78.2[g]	94.1, 156.5, 158.8, 159.4, 165.0
3αp[h]	5.62 (d)	8.17 (s), 8.25 (s)	63.2, 67.2, 68.5, 70.2, 77.9	117.0, 141.1, 152.0 (2C), 152.7
3βp[h]	5.87 (d)	8.17 (s), 8.23 (s)	64.3, 67.4, 69.5, 71.1, 78.0	115.5, 141.7, 152.2 (2C), 152.8

[a] Taken as ppm from Me_4Si. [b] For the structures see Scheme I. [c] In Me_2SO-d_6, consistent with the data in D_2O.[10] [d] In D_2O. [e] For *N*-phenyl-α-D-ribopyranosylamine in Me_2SO-d_6: 62.1, 67.8, 69.9, 69.9, 81.3 ppm.[11] [f] For 4,6-diamino-5-formamidopyrimidine in Me_2SO-d_6: 93.8, 155.5, 159.4 (2 C), 160.9 ppm. [g] For *N*-phenyl-β-D-ribopyranosylamine in Me_2SO-d_6: 63.1, 67.4, 70.1, 70.4, 81.6 ppm.[11] [h] In Me_2SO-d_6, consistent with the data obtained with an authentic sample.

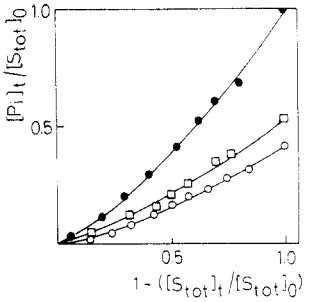


Figure 1. Hydrolysis of adenosine, [8-^{14}C]adenosine, and [1′-^{14}C]adenosine in 0.50 mol dm^{-3} aqueous sodium hydroxide at 363.2 K. The mole fraction of adenine (○), [^{14}C]formate ion (□), and [1-^{14}C]D-ribose (●) released plotted against the mole fraction of the starting material disappeared.

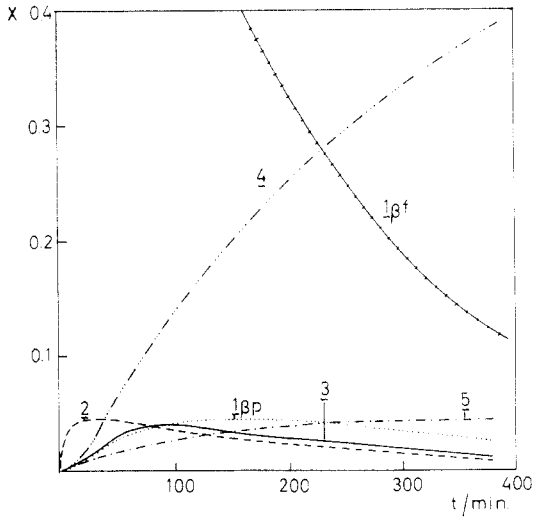
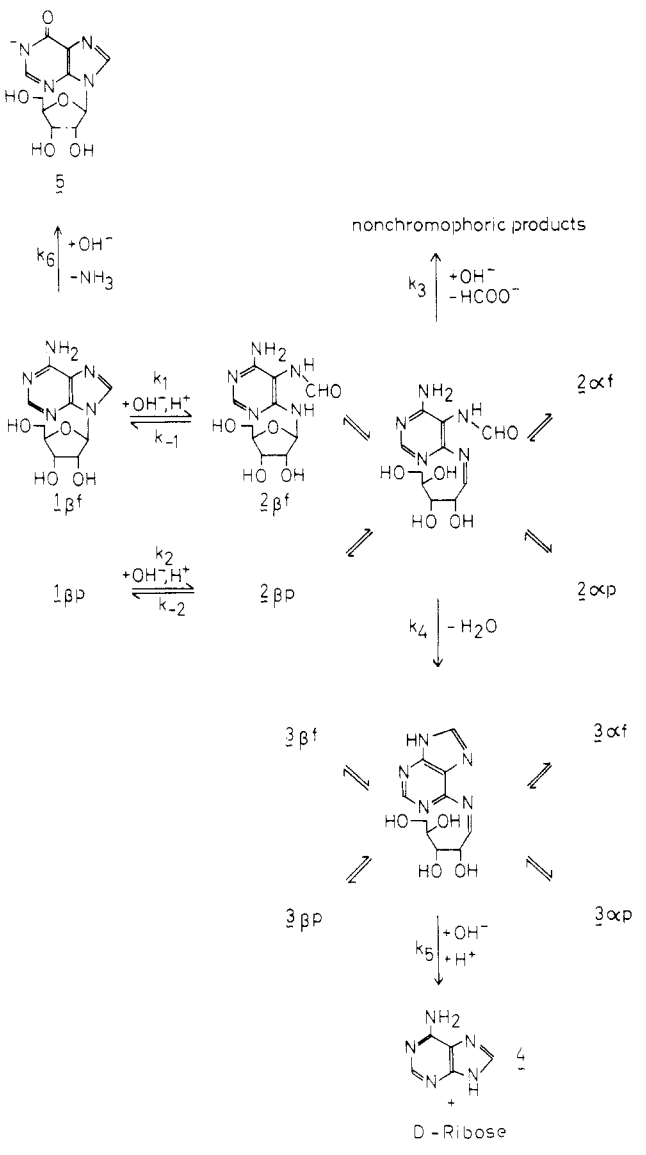


Figure 2. Time-dependent mole fractions for the intermediates and products of the hydrolysis of adenosine in 0.50 mol dm^{-3} aqueous sodium hydroxide at 363.2 K. The enumeration refers to Scheme I.

When treated with aqueous alkali, **2** was converted to the intermediates indicated by 1βp and **3** in Figure 2. Later on formation of adenine (**4**) was detected. However, the concentration of adenine was increased sigmoidally with time, suggesting that it is formed from **2** via another relatively stable intermediate. The major components of **3** were separated by preparative LC and characterized spectroscopically. 3αp and 3βp behaved chromatographically as anomeric N^6-ribopyranosyladenines, obtained by the method described previously.[14] The ^1H and ^{13}C NMR chemical shifts observed are listed in Table I. Most probably intermediate **3** is an equilibrium mixture of isomeric N^6-ribosyladenines (3αf, 3βf, 3αp, and 3βp) depicted in Scheme I. Compound 1βp was NMR spectroscopically identical with 9-β-D-ribopyranosyladenine.[10] When treated with aqueous alkali at 363.2 K, 3αp and 3βp were immediately converted to the equilibrium mixture of 3αf, 3βf, 3αp, and 3βp, whereas 1βp was not isomerized. Prolonged treatment with alkali resulted in the hydrolysis of **3** to adenine. No sign of formation of **2** was detected. In contrast, 1βp reacted via intermediary appearance of **2** and **3**.

Scheme I



However, adenosine is not quantitatively decomposed to adenine via **2** and **3**. As seen from Figure 1 the $^{14}C8$ atom is partially released during the alkaline cleavage of [8-^{14}C]adenosine, the proportion of the release being in-

(14) Fujishima, T.; Uchida, K.; Yoshino, H. Jpn Kokai Tokkyo Koho 75-34 040, 1975.

Table II. Pseudo-First-Order Rate Constants, k_1 (10^{-4} s^{-1}) for the Partial Reactions Involved in the Alkaline Decomposition of Adenosine at 363.2 K[a]

[OH^-], mol dm^{-3}	k_{d1}[b]	k_{d2}[c]	k_2	k_5
0.50	0.93 ± 0.02 (1:0.05)	15.9 ± 0.2 (1:1.22:0.65:0.35)	1.13 ± 0.02	3.14 ± 0.05
0.40	0.78 ± 0.01 (1:0.05)	15.6 ± 0.3 (1:0.92:0.65:0.35)		2.64 ± 0.07
0.30	0.65 ± 0.01 (1:0.04)	12.3 ± 0.1 (1:0.75:0.65:0.35)		2.22 ± 0.07
0.20	0.51 ± 0.01 (1:0.04)	10.4 ± 0.2 (1:0.59:0.65:0.35)		1.58 ± 0.04
0.10	0.31 ± 0.01 (1:0.03)	7.2 ± 0.1 (1:0.39:0.65:0.35)		0.98 ± 0.04

[a] The ionic strength adjusted to 0.50 mol dm^{-3} with sodium chloride. For the partial rate constants see Scheme I. [b] $k_{d1} = k_1 + k_6$. The ratio of k_1:k_6 given in the parentheses. [c] $k_{d2} = k_{-1} + k_{-2} + k_3 + k_4$. The ratio of k_4:k_3:k_{-2}:k_{-1} given in the parantheses.

creased with the increasing concentration of hydroxide ion. We have shown previously[15] that 4,6-diamino-5-formamidopyrimidine undergoes in aqueous alkali a base-catalyzed deformylation to 4,5,6-triaminopyrimidine, which competes with an intramolecular cyclization to adenine. The proportion of the former reaction is increased from 0.1 to 0.4 on going from [OH^-] = 0.10 mol dm^{-3} to [OH^-] = 0.50 mol dm^{-3} at 363.2 K. With all likelihood the situation is analogous with compound **2**. The N^5-formyl group may be attacked either intramolecularly by the 4-amino or 6-ribosylamino group or intermolecularly by a hydroxide ion. The latter reaction leads to a 4,5,6-triaminopyrimidine derivative, which may be expected to undergo a rapid fragmentation under the conditions employed.[5] This intermediate is probably the one assigned earlier[5] to 4,5,6-triaminopyrimidine on the bases of UV spectrometric studies. The fact that 4,6-diamino-5-formamidopyrimidine is not accumulated during the cleavage of **2** argues against deribosylation of **2** prior to deformylation. Since the rate of decomposition of **2** and 4,6-diamino-5-formamidopyrimidine are comparble,[15] the latter should appear as an intermediate if it were formed.

In summary, the pathway for the alkaline hydrolysis of adenosine may be depicted by Scheme I. Initially hydroxide ion attacks the C8 atom of adenosine with concomitant opening of the imidazole ring. The deamination of adenosine to inosine (**5**) competes with the formation of **2**, but the proportion of this side reaction remains low. 4-Amino-5-formamido-6-(β-D-ribofuranosylamino)pyrimidine (**2**βf) is rapidly anomerized to **2**αf, **2**αp, and **2**βp, probably via an acyclic Schiff base. Adenosine does not undergo such anomerization, since the N9 atom is part of an aromatic system, and hence the mesomeric electron release, needed to stabilize the Schiff base, is impeded. The N^5-formyl group of **2** is susceptible to three competitive reactions. First, nucleophilic attack of the 6-ribosylamino group results in formation of 1βp and to a lesser extent 1βf. Second, nucleophilic attack of the 4-amino group gives isomeric N^6-ribosyladenines. Third, attack of hydroxide ion on the carbonyl carbon leads to a departure of the N^5-formyl group as formate ion, and the resulting 4,5-diamino-6-(ribosylamino)pyrimidine is rapidly degraded to nonchromophoric compounds. Intermediate **3** is finally hydrolyzed to adenine and D-ribose.

The data presented do not strictly exclude the possibility that adenine would be partially released via the rupture of the C1′–N9 bond. However, this reaction must be of minor importance. Moreover, the fact that the unsubstituted purine riboside does not yield purine make the direct release of adenine a less attractie alternative.[8]

Table II summarizes the pseudo-first-order rate constants for the partial reactions indicated in Scheme I. The rate constants, k_1, for the disappearance of adenosine depend curvilinearly on [OH^-] at low base concentrations, but at [OH^-] ≥ 0.20 mol dm^{-3} the dependence turns linear

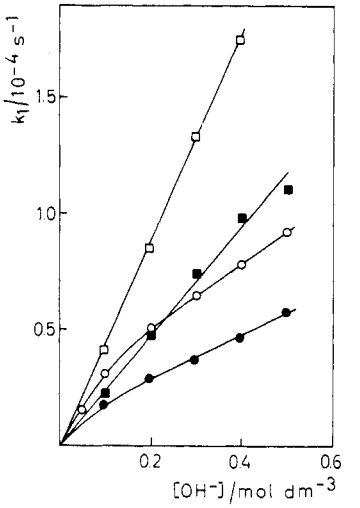


Figure 3. First-order rates constants, k_1, for the hydrolysis of adenosine (○), 5′-*O*-methyladenosine (●), 2′,3′-*O*-isopropylideneadenosine (□), and 2′-deoxyadenosine (■) in aqueous sodium hydroxide at 363.2 K. The ionic strength adjusted to 0.50 mol dm^{-3} with sodium chloride.

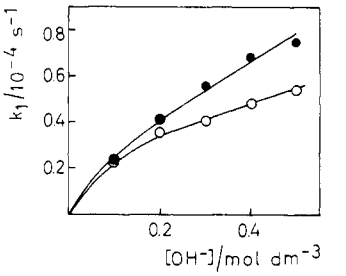


Figure 4. First-order rate constants, k_1, for the hydrolysis of 9-β-D-arabinofuranosyladenine (○) and 9-(5′-*O*-methyl-β-D-arabinofuranosyl)adenine (●) in aqueous sodium hydroxide at 363.2 K. The ionic strength adjusted to 0.50 mol dm^{-3} with sodium chloride.

(Figure 3). The situation is similar with 5′-*O*-methyladenosine and 9-β-D-arabinofuranosyladenine and its 5′-*O*-methyl derivative (Figures 3 and 4). In contrast, with 2′-deoxy- and 2′,3′-*O*-isopropylideneadenosine k_1 is proportional to the concentration of hydroxide ion over the whole basicity range studied (Figure 3). Probably the ionization of the 2′-hydroxyl function, which is the most acidic hydroxyl group in nucleosides,[16] retards the nucleophilic attack of hydroxide ion on the C8 atom and is thus responsible for the downward curvature of the plot of k_1 vs. [OH^-].

We have shown previously[8] that k_1 may be expressed by eq 1, when the ionization is taken into account. Here

$$k_1 = \frac{k_1(SH)[OH^-] + k_1(S^-)K[OH^-]^2}{K[OH^-] + 1} \quad (1)$$

(15) Lehikoinen, P.; Lönnberg, H. *Chem. Scr.* **1986**, *26*, 103.

(16) Izatt, R. M.; Rytting, J. H.; Hansen. L. D.; Christensen, J. J. *J. Am. Chem. Soc.* **1966**, *88*, 2641.

Table III. Kinetic Parameters for the Initial Step of the Alkaline Decomposition of Adenine Nucleosides at 363.2 K[a]

compd	k_1 (S⁻),[b] 10^{-4} dm³ mol⁻¹ s⁻¹	k_1 (SH),[c] 10^{-4} dm³ mol⁻¹ s⁻¹	k_1 (SH)/K,[d] 10^{-4} s⁻¹
adenosine	1.29 ± 0.03	~5[e]	0.23 ± 0.01
2′-deoxyadenosine		2.25 ± 0.16	
2′,3′-*O*-isopropylidene-adenosine		4.63 ± 0.10	
5′-*O*-methyladenosine	0.97 ± 0.04		0.09 ± 0.02
9-β-D-arabinofuranosyl-adenine	0.53 ± 0.08		0.26 ± 0.02
9-(5′-*O*-methyl-β-D-arabinofuranosyl)-adenine	1.18 ± 0.10		0.19 ± 0.04

[a] See Scheme I and eq 1. The data refer to the ionic strength of 0.50 mol dm⁻³ adjusted with sodium chloride. [b] For the disappearance of the monoanion of the substrate. [c] For the disappearance of the neutral substrate. [d] K defined by eq 2. [e] Estimated with the aid of the thermodynamic data reported for the ionization of adenosine.[16]

k_1(HS) and k_1(S⁻) are the second-order rate constants for the disappearance of neutral adenine nucleoside, SH, and its 2′-oxyanion, respectively. The ionization constant, K, is defined by eq 2. In highly alkaline solutions, i.e., when

$$K = \frac{[S^-]}{[SH][OH^-]} \quad (2)$$

$K[OH^-] \gg 1$, eq 1 is reduced to eq 3. Table III records the values obtained for $k_1(S^-)$ and $k_1(SH)/K$. The data

$$k_1 = k_1(S^-)[OH^-] + \frac{k_1(SH)}{K} \quad (3)$$

reveal that 2′-deoxy- and 2′,3′-*O*-isopropylideneadenosine, containing no ionizable group at C2′, are attacked by hydroxide ion 2–5 times more readily than the 2′-oxyanion of adenosine and its 5′-*O*-methyl derivative. Evidently the negative charge retards the approach of the anionic nucleophile. The lower reactivity of 2′-deoxyadenosine compared to that of 2′,3′-*O*-isopropylideneadenosine may, in turn, be attributed to the weaker electron-attracting ability of the glycon moiety of the former compound. Methylation of the 5′-hydroxyl function of adenosine and changing the configuration at C2′ exert only a modest effect on the hydrolysis rate. Accordingly, the glycosyl hydroxyl functions do not appear to participate as intramolecular nucleophiles in the alkaline decomposition of adenine nucleosides.

Figure 5 shows the first-order rate constants for the deformylation and intramolecular cyclizations of intermediate **2** at various concentrations of hydroxide ion. The rate of deformylation is proportional to [OH⁻] over the whole basicity range studied, while the rate of cyclization levels off to a constant value at high alkalinities. These findings can be accounted for by the mechanisms proposed[15] for the corresponding reactions of 4,6-diamino-5-formamidopyrimidine. The deformylation of **2** may thus be assumed to involve a rapid initial attack of hydroxide ion on the N^5-formyl group of the neutral substrate and a base-catalyzed rate-limiting breakdown of the tetrahedral intermediate to formate ion and 4,5-diamino-6-(ribosylamino)pyrimidine. The N⁵-monoanion of the starting material is unreactive. Accordingly, the reaction order with respect to hydroxide ion is decreased from 2 to 1, when the N⁵-monoanion becomes the predominant species.[15] Evidently this is the case under the conditions of the present study. For comparison, the pK_a value of 4,6-diamino-5-formamidopyrimidine is less than 13 at 298.15 K,[15]

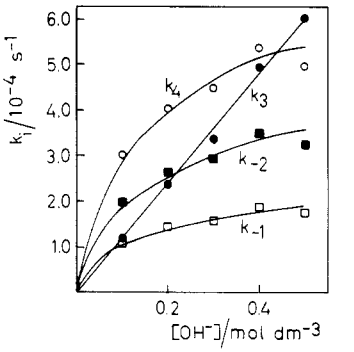


Figure 5. First-order rate constants for the parallel reactions involved in the decomposition of intermediate **2** of the alkaline hydrolysis of adenosine. For the individual rate constants see Scheme I. The ionic strength adjusted to 0.50 mol dm⁻³ with sodium chloride.

and the electronegative N^6-ribosyl group may be expected to increase the acidity. The intramolecular cyclization probably proceeds by a base-catalyzed nucleophilic attack of the neighboring amino or ribosylamino group on the unionized N^5-formyl group. Again the N⁵-monoanion is unreactive. The reaction order with respect to hydroxide ion is thus changed from 1 to 0 at alkalinities where the N^5-formyl group is ionized.[15]

Obviously the structure of the glycon moiety has only a minor effect on the competition between the deformylation and cyclization of **2**, since the yield of adenine was observed to be roughly the same in the hydrolysis of all the adenine nucleosides studied. The latter finding is consistent with the data of Garrett and Mehta.[5]

Comparison with previous data[8] indicates that unsubstituted 9-β-D-ribofuranosylpurine is attacked by hydroxide ion about 200 times more readily than adenosine, whereas the resulting 5-formamido-4-(ribosylamino)pyrimidine is deformylated only 20 times faster than intermediate **2**. This difference can be explained as follows. Introduction of an amino group at C6 increases the electron density at the C8 atom of adenosine and hence retards the attack of hydroxide ion. Similarly the 4-amino group increases the electron density at the N^5-formyl group of **2**, but at the same time it reduces the acidity of the substrate and thus the concentration of the unreactive anionic species. These two influences partially cancel each other. Consequently, the mole fraction of **2** remains low during the hydrolysis of adenosine compared to the mole fraction of the corresponding intermediate in the cleavage of purine riboside.

As seen from Table II, the first-order rate constants for the hydrolysis of intermediate **3** are almost linearly related to the concentration of hydroxide ion. The reason for the slight downward curvature remains obscure. It may be tentatively assumed that **3** is hydrolyzed by a mechanism analogous to that suggested for the Schiff bases of aromatic amines.[17] Accordingly, the rate-limiting attack of hydroxide ion on the anomeric carbon of the acyclic Schiff base form would lead to formation of a carbinolamine intermediate, which is rapidly heterolyzed to adenine and D-ribose. Possibly the ionization of the ribosyl hydroxyl functions retard slightly the attack of hydroxide ion.

In summary, adenosine is decomposed in aqueous alkali via intermediary formation of 4-amino-5-formamido-6-(ribosylamino)pyrimidine, i.e., analogously to 9-β-D-ribofuranosylpurine. The complete reaction pathway is, however, more complicated than with purine riboside, since the 4-amino group participates as an intramolecular nu-

(17) Jencks, W. P. *Progr. Phys. Org. Chem.* **1964**, *2*, 63.

cleophile in the subsequent reactions of the 5-formamido intermediate.

Experimental Section

Materials. Adenosin, 2'-deoxyadenosine, 2',3'-*O*-isopropylideneadenosine, 9-β-D-arabinofuranosyladenine, adenine, inosine, and 4,6-diamino-5-formamidopyrimidine were commercial products of Sigma Chemical Co. They were used as received, after checking their purity by LC. [8-^{14}C]Adenosine was a product of NEN, which was prior to use mixed with unlabeled adenosine and crystallized from water. [1'-^{14}C]Adenosine was prepared from adenine and tetra-*O*-acetyl[1-^{14}C]ribofuranose by the method of Vorbrüggen et al.[18] The labeled ribofuranose tetraacetate was synthesized from [1-^{14}C]D-ribose (NEN) according to Guthrie and Smith.[19] The anomeric mixture of N^6-ribosyladenine was obtained by fusing adenine and D-ribose.[14] 5'-*O*-Methyladenosine and 9-(5'-*O*-methyl-β-D-arabinofuranosyl)adenine were gifts from the group of Prof. D. Shugar (University of Warsaw). Their preparation has been described elsewhere.[20]

Preparative Separation of the Intermediates. Intermediates 1βp, 2αp, 2βp, 3αp, and 3βp were separated preparatively on a Spherisorb RP-18 column (250 mm × 8 mm, particle size 5 μm) by using an acetic acid buffer (0.02 mol dm^{-3}, pH 4.3) containing 6% (v/v) of acetonitrile as eluant. The fractions obtained were lyophilized, and the buffer constituents were removed by passing the compounds through the column mentioned above with a mixture of water and acetonitrile as eluant. Finally the separated products were crystallized from a minimum volume of water.

Spectroscopic Measurements. The UV spectra were recorded on a Unicam SP 8100 spectrophotometer and the NMR spectra on a Jeol GX-400 spectrometer.

Isotopic Labeling Studies. The release of the ^{14}C8 atom from [8-^{14}C]adenosine and the [1-^{14}C]ribosyl group from [1'-^{14}C]-adenosine was followed as described earlier.[8]

Kinetic Studies by LC. The progress of the reactions of adenine nucleosides and compounds 1βp, 2αp, 2βp, 3αp, and 3βp with aqueous alkalies was followed by the LC technique described earlier.[8] The peak heights were transformed to concentrations with the aid of calibration solutions of known concentrations. The rate constants were calculated via the integrated first-order rate equation by using the data obtained during two half-lives. During this period the proportions of the possible reverse reactions were negligible and the first-order kinetics were strictly obeyed.

Acknowledgment. We thank Prof. D. Shugar and Dr. E. Darzynkiewicz for the generous gift of 5'-*O*-methyladenosine and 9-(5'-*O*-methyl-β-D-arabinofuranosyl)-adenine. Financial aid from the Academy of Finland, Research Council for the Natural Sciences, is gratefully acknowledged.

Registry No. 1βp, 10563-76-5; 2αp, 103960-07-2; 2βp, 103960-08-3; 3αp, 103960-09-4; 3βp, 103960-10-7; 4, 73-24-5; [8-^{14}C]adenosine, 3257-92-9; [1'-^{14}C]adenosine, 103960-06-1; adenosine, 58-61-7; 2'-deoxyadenosine, 958-09-8; 2',3'-*O*-isopropylideneadenosine, 362-75-4; 5'-*O*-methyladenosine, 20649-45-0; 9-(β-D-arabinofuranosyl)adenine, 5536-17-4; 9-(5'-*O*-methyl-β-D-arabinofuranosyl)adenine, 60738-17-2; D-ribose, 50-69-1.

(18) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
(19) Guthrie, R. D.; Smith, S. C. *Chem. Ind. (London)* **1968**, 547.
(20) Kazimierczuk, Z.; Darzynkiewicz, E.; Shugar, D. *Biochemistry* **1976**, *15*, 2735.

Direct Observation of the Reverse 1,5-Hydride Shift: The Mechanism of Acid-Catalyzed Isomerization at C-25 of Spirostanols

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Received April 5, 1986

(25*R*)-3β-Acetoxy-5α-[5,6,22-$^2H_3$]furostan-26-al (**9**) has been prepared by hydrogenation of diosgenin acetate under hydrogen gas in deuterated ethanol–ethyl acetate, containing a trace amount of deuterated perchloric acid, followed by oxidation. The deuterium atom at C-22 of the aldehyde **9** shifted to C-26 upon refluxing with hydrochloric acid in methanol giving 26α- and 26β-deuterated tigogenin in the ratio of 1:2 and 26α- and 26β-deuterated neotigogenin in the ratio of 1:4. The deuterium atom at C-26 of tigogenin acetate (**10b** and **10c**) shifted back to C-22 upon treatment with boron trifluoride in ethandithiol, giving (25*R*)-3β-acetoxy-5α-[5,6,22 or -26-$^2H_3$]furostan 26-dithioacetal (**13** and **14**). Evidence for the 1,5-reverse hydride shift in the overall acid isomerization reaction of a spirostanol came from the observation that the ratio of the deuterium atom at 26α to that at 26β changes from 1:4 for neotigogenin to 1:1 for the acid isomerization products tigogenin and neotigogenin.

In 1939, Marker et al.[1] reported the acid-catalyzed isomerization of spirostanols ("iso reaction"), when isomers were still considered to differ in the configuration at C-22. In 1953, Scheer et al.[2] showed that the "normal" and "iso" spirostanols differ in configuration at C-25 and this was confirmed by Callow et al.[3] using the deuterium–hydrogen exchange reaction at C-25 under the acid isomerization reaction condition. A mechanism via furostan-26-al (**3**) was proposed by Woodward et al.,[4] who synthesized 3β-hydroxy-5α-furostan-26-al (**3**) which was transformed to tigogenin (3β-hydroxy-5α-spirostan) by acid treatment, suggesting that the hydrogen at C-22 of the aldehyde **3** migrates to C-26 to form a spirostanol. Soon after that, Djerassi et al.[5] trapped the aldehyde **3** as a dithioacetal from a spirostanol and suggested that the hydrogen at C-26 of tigogenin migrates to C-22. Although the 1,6-hydrogen

(1) Marker, R. E.; Rohrmann, E. *J. Am. Chem. Soc.* **1939**, *61*, 846.
(2) Scheer, I.; Kostic, R. B.; Mosettig, E. *J. Am. Chem. Soc.* **1953**, *75*, 4871.
(3) Callow, R. K.; Massay-Beresford, P. N. *J. Chem. Soc.* **1958**, 2645.
(4) Woodward, R. B.; Sondheimer, F.; Mazur, Y. *J. Am. Chem. Soc.* **1958**, *80*, 6693.
(5) Djerassi, C.; Halpern, O.; Pettit, G. R.; Thomas, G. H. *J. Org. Chem.* **1959**, *24*, 1.

0022-3263/86/1951-3823$01.50/0