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Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues. V. The Effect of Metal Ions on the Acidic Hydrolysis of 9-(1-Ethoxyethyl)adenine

HARRI LÖNNBERG

Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku, Finland

First-order rate constants for the hydrolysis of 9-(1-ethoxyethyl)adenine have been determined at different oxonium ion concentrations in solutions of silver(I) and several first-row transition metal ions. The acid-catalyzed hydrolysis has been suggested to proceed under acidic conditions predominantly by a rapid initial formation of a dication of the substrate followed by rate-limiting heterolysis of this species to give an oxocarbenium ion from the 1-ethoxyethyl group. At low acidities the route *via* the monoprotonated substrate becomes kinetically important. The effect of the 3d transition metal ions on the hydrolysis rate has been explained by competitive attachment of protons and metal ions to the substrate. In contrast, using silver(I) ions as the complexing metal, a simultaneous proton and metal ion binding becomes significant. The rate-reductions observed at different oxonium ion concentrations have been quantitatively accounted for by a reaction scheme involving, besides mono- and diprotonated substrates, 1:1 silver complexes of the neutral and protonated substrates.

Interactions of metal ions with nucleic acid components, *i.e.* nucleosides, nucleotides, and their constituent bases, have received increasing interest after the finding that some platinum(II) complexes have antitumor activity.[1–3] Purine nucleosides, for example, have been shown to form reasonably stable complexes with several metal ions in neutral aqueous solutions.[4–7] This kind of complexing can be expected to influence the rates and possibly the mechanisms of the solvolytic reactions of purine nucleosides and related compounds. We have reported previously on the effects of metal ions on the acid-catalyzed hysrolysis of simple acyclic nucleoside analogues, 2-substituted 1-(1-ethoxyethyl)benzimidazoles[8] and 9-(1-ethoxyethyl)-purine.[9] The retardations caused by several metal ions in the rate of hydrolysis of the former compound have been accounted for by competitive attachment of protons and metal ions to the only potential binding site, N3, of the substrate. Similarly, the effects of first-row transition metal cations on the kinetics of the cleavage of 9-(1-ethoxyethyl)-purine can be explained on the basis of exclusive protonation and complexation of the substrate,[9] although in this case different atoms can be preferred in proton and metal binding.[7] In contrast, simultaneous attachment of protons and metal ions seems to become significant when applying the silver(I) ion as complexing agent.[9] To obtain more quantitative information of the interactions of metal ions with cationic nucleoside analogues the investigations are now extended to an acyclic adenosine analogue, 9-(1-ethoxyethyl)adenine, which is essentially completely protonated at fairly low concentrations of oxonium ions. In addition, the present study tends to elucidate the possibility of C6 $-NH_2$ to act as an additional binding site.

RESULTS AND DISCUSSION

Several lines of evidence suggest[10–16] that the acid-catalyzed hydolysis of purine nucleosides involves a rapid initial protonation of the purine ring, giving a mono- and dication, followed by a rate-limiting heterolysis of these species to a free nitrogen base and a cyclic oxocarbenium ion, as depicted in Scheme 1 for adenosine. It has been fairly well

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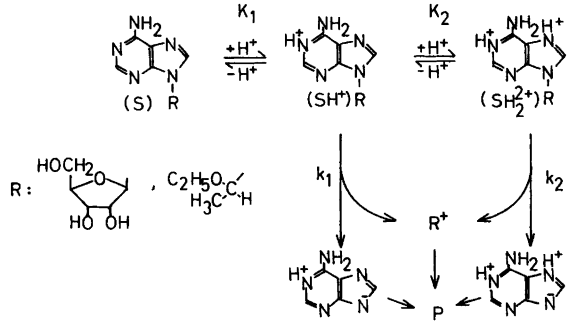


Scheme 1.

established that the first protonation takes place at N1,[4,7] whereas the assignment of the second proton attachment at N7 is tentative. Most probably the same mechanism can also be extended to the hydrolysis of 9-(1-ethoxyethyl)adenine, since formation of an acyclic oxocarbenium ion from the 1-ethoxyethyl group is a far more facile process than formation of a cyclic oxocarbenium ion from the β-D-ribofuranosyl group. In the latter case the electronegative hydroxyl group of the glycon ring lowers the electron density at the anomeric carbon atom and thus retard the developing of a positively charged oxocarbenium ion center at this site. For comparison, the second-order rate constant for the acid-catalyzed hydrolysis of diethyl acetal of acetaldehyde,[17] proceeding *via* the 1-ethoxyethyl oxocarbenium ion, is 10^4-10^6 times greater than those for ethyl aldofuranosides reacting *via* glycosyl ions.[18] The major part of this reactivity difference can be ascribed to the difference in the stabilities of the oxocarbenium ion intermediates, although the different basicities of the substrates also contribute.[19] Some further evidence of the suggested mechanism comes from the studies of the acidic hydrolysis of 2-substituted 1-(1-ethoxyethyl)-benzimidazoles. These compounds have been shown to react by rate-limiting formation of an oxocarbenium ion from the 1-ethoxyethyl group.[20,21] Since protonated adenine, as a leaving group, can well be compared to a protonated benzimidazole having a strongly electron-withdrawing substituent at C2, it is likely that the decomposition of 9-(1-ethoxyethyl)adenine occurs *via* the same intermediate.

Table 1 records the kinetic data for the acid-catalyzed hydrolysis of 9-(1-ethoxyethyl)adenine at 313.2 K. The observed first-order rate constants, $k(H^+)$, increase almost linearly with the concentration of the oxonium ion. No catalysis by the undissociated buffer acids was observed. The entropy of activation, $\Delta S^{\neq} = (51 \pm 6)$ J K^{-1} mol^{-1}, determined in 0.010 mol dm^{-3} perchloric acid at 313.2 K, is of the same magnitude as reported for 2-substituted 1-(1-ethoxyethyl)benzimidazoles[20] and consistent with the unimolecular nature of the rate-limiting step in the assumed mechanism.[22] The solvent deuterium isotope effect, $k(D^+,D_2O)/k(H^+,H_2O) = 2.5$, also agrees with the A-1 mechanism.[23]

The rate-law for the mechanism depicted in Scheme 1 can be expressed by eqn. (1), where the

$$-\frac{d[S(tot.)]}{dt} = \frac{k_1K_1[H^+]+k_2K_1K_2[H^+]^2}{1+K_1[H^+]+K_1K_2[H^+]^2}[S(tot.)] \quad (1)$$

constants k_1, k_2, K_1 and K_2 are as indicated in the scheme. Since attachment of a proton to one of the purine nitrogen atoms considerably lowers the electron density at the other potential binding sites and thus makes further protonation difficult, the value of K_2 can be expected to be small compared to K_1. For example, for free adenine the ratio of K_1/K_2 has been established to be larger than 10^3.[24] Consequently, the term $K_1K_2[H^+]$ can be neglected in the denominator of eqn. (1) as long as the concentration of the oxonium ion is of the order of $1/K_1$. Under such conditions the expression for the observed first-order rate constant, $k(H^+)$, can be transformed to eqn. (2). Observations of the changes

$$\frac{K_1[H^+]+1}{K_1[H^+]}k(H^+) = k_2K_2[H^+]+k_1 \quad (2)$$

Table 1. First-order rate constants, $k(H^+)$, for the hydrolysis of 9-(1-ethoxyethyl)adenine in various acid and buffer solutions.[a] The temperature is 313.2 K if not otherwise stated.

HA	[HA] / mol dm^{-3}	[A$^-$] / mol dm^{-3}	$-\lg\left(\frac{[H^+]}{\text{mol dm}^{-3}}\right)$	$k(H^+)$ / 10^{-3} s^{-1}
$HClO_4$	0.0250		1.60	5.35(6)
$HClO_4$	0.0200		1.70	4.46(6)
$HClO_4$	0.0150		1.82	3.15(5)
$HClO_4$	0.0100		2.00	2.30(2)
$HClO_4$	0.0100		2.00	0.682(4)[b]
$HClO_4$	0.0100		2.00	7.82(4)[c]
$DClO_4$[d]	0.0100		2.00	5.77(4)
$HClO_4$	0.00500		2.30	1.087(12)
$ClCH_2COOH$	0.100	0.0500	2.45[e]	0.794(7)
$ClCH_2COOH$	0.200	0.100	2.45[e]	0.793(7)
HCOOH	0.0800	0.0100	2.71[f]	0.478(5)
HCOOH	0.112	0.0140	2.71[f]	0.477(3)
HCOOH	0.160	0.0200	2.71[f]	0.485(2)
$ClCH_2COOH$	0.0200	0.0200	2.75[e]	0.397(4)
$ClCH_2COOH$	0.0200	0.0400	3.05[e]	0.229(2)
HCOOH	0.0600	0.0200	3.14[f]	0.1772(16)
HCOOH	0.0400	0.0200	3.31[f]	0.1205(13)
HCOOH	0.0200	0.0200	3.61[f]	0.0610(5)
CH_3COOH	0.0200	0.0200	4.61[g]	0.00638(9)

[a] The ionic strength adjusted to 0.20 mol dm^{-3} with $NaNO_3$. [b] At 303.2 K. [c] At 323.2 K. [d] In D_2O. [e] Estimated by the Debye-Hückel approximation from the data in Ref. 25. [f] Estimated by the Debye-Hückel approximation from the data in Ref. 26. [g] Estimated by the Debye-Hückel approximation from the data in Ref. 27.

in the UV-spectrum of the substrate at 280 nm as a function of oxonium ion concentration allow the determination of K_1 from the slope and intercept of eqn. (3). Here ΔA is the change in absorbance on

$$\frac{1}{\Delta A} = \frac{1}{\Delta A(\text{max}) K_1} \frac{1}{[H^+]} + \frac{1}{\Delta A(\text{max})} \qquad (3)$$

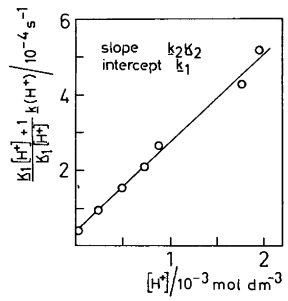


Fig. 1. The effect of oxonium ion concentration on the first-order rate constants, $k(H^+)$, for the acid-catalyzed hydrolysis of 9-(1-ethoxyethyl)adenine at 313.2 K. The partial rate and equilibrium constants, k_1, k_2, K_1 and K_2, are as indicated in Scheme 1.

going from a solution where S is totally deprotonated to a solution having an oxonium ion concentration $[H^+]$, and ΔA(max) is a parameter representing a change in A when S becomes completely protonated. When the value of $(6.6 \pm 0.7) \times 10^3$ dm^3 mol^{-1} obtained in this manner for K_1 is substituted into eqn. (2) together with the rate constants, $k(H^+)$, a straight line presented in Fig. 1 is obtained. The intercept and slope of this line yield the values of $(4.2 \pm 0.1) \times 10^{-5}$ s^{-1} and (0.24 ± 0.01) dm^3 mol^{-1} s^{-1} for k_1 and k_2K_2, respectively. Accordingly, the decomposition of 9-(1-ethoxyethyl)adenine proceeds mainly *via* a monocation of the substrate at oxonium concentrations smaller than 2×10^{-4} mol dm^{-3}. At higher concentrations of oxonium ion the route *via* a dication becomes predominant, although the equilibrium concentration of this species remains negligible.

Table 2 summarizes the first-order rate constants, $k(M^{2+})$, for the hydrolysis of 9-(1-ethoxyethyl)-adenine in solutions of first-row transition metal perchlorates containing perchloric acid 0.00250 mol dm^{-3}. Of the cations investigated only copper(II) ion exhibits a noticeable rate-retarding effect under

Table 2. The effect of some 3d transition metal ions on the acid-catalyzed hydrolysis of 9-(1-ethoxyethyl)adenine in aqueous 0.00250 mol dm^{-3} perchloric acid at 313.2 K.

M^{2+}	$k(M^{2+})/10^{-4}$ s^{-1} [a]	$k(H^+)/k(M^{2+})$ [b]
Mn^{2+}	7.22(5)	0.96
Co^{2+}	7.13(8)	0.98
Ni^{2+}	7.22(9)	0.96
Cu^{2+}	6.24(5)	1.12
Zn^{2+}	7.09(6)	0.98

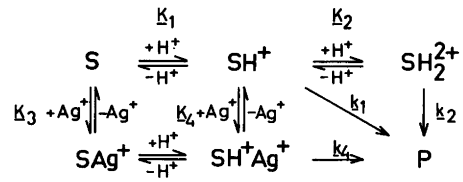
[a] $k(M^{2+})$ refers to 0.200 mol dm^{-3} solutions of $M(ClO_4)_2$. [b] $k(H^+)$ refers to a 0.200 mol dm^{-3} solution of $Mg(ClO_4)_2$.

the acidic conditions employed. We have previously shown [9] that for the hydrolytic decomposition of 9-(1-ethoxyethyl)purine the ratio of the rate constants, $k(H^+)/k(M^{2+})$, in the absence and in the presence of metal ions can be expressed by eqn. (4), where K_3

$$\frac{k(H^+)}{k(M^{2+})} = \frac{1+K_1[H^+]+K_3[M^{2+}]}{1+K_1[H^+]} \quad (4)$$

stands for the formation constant of the complex SM^{2+}. Formation of species SH^+M^{2+} and SM_2^{4+} can be neglected, since attachment of one positively charged particle at the purine ring retards further complexing. The influence of divalent cations on the protonation of adenosine and 9-(β-D-ribofuranosyl(purine lends additional support for this argument.[28] The validity of eqn. (4) in the hydrolysis of 9-(1-ethoxyethyl)adenine can be tested by substituting the values determined potentiometrically for K_1 nad K_3 of adenosine [28] in this equation. By this procedure a value of 1.09 is obtained for the ratio of $k(H^+)/k(Cu^{2+})$. Complexes with the other 3d metal ions are too weak to cause the ratio $k(H^+)/k(M^{2+})$ to deviate appreciably from unity. As seen from Table 2, these predictions are, within the limits of experimental error, consistent with the observed rate-retarding effects.

In contrast, the effects of the silver(I) ion on the rate of the acidic hydrolysis of 9-(1-ethoxyethyl)-adenine cannot be explained by eqn. (4). Substitution of the ratios $k(H^+)/k(Ag^+)$, listed in Table 3, into this equation yields values for the formation constant, K_3, that increase smoothly with increasing concentration of the oxonium ion. We have suggested previously [9] that complexing of the protonated substrate, SH^+, must also be taken into account when the rate-retarding effects of silver(I) ion are to be analyzed. In other words, the equations



Scheme 2.

Table 3. The effect of silver(I) ion on the hydrolysis of 9-(1-ethoxyethyl)adenine at various concentrations of oxonium ion at 313.2 K.

$[H^+]$ / mol dm^{-3}	$[Ag^+]$ / mol dm^{-3}	$k(Ag^+)$ [a] / 10^{-3} s^{-1}	$k(H^+)/k(Ag^+)$	$k(H^+, calc.)/k(Ag^+, calc.)$ [b]
0.0100	0.0200	1.974(24)	1.17	1.14
0.0100	0.0500	1.534(20)	1.50	1.33
0.0100	0.0100	1.280(19)	1.80	1.67
0.0100	0.0190	0.980(5)	2.35	2.24
0.0500	0.0100	7.22(14)	1.48	1.62
0.0200	0.0100	2.59(3)	1.72	1.63
0.00500	0.0100	0.573(9)	1.90	1.73
0.00300	0.0100	0.310(3)	2.10	1.82
0.00200	0.0100	0.224(4)	1.94	1.91
0.00100	0.0100	0.0942(9)	2.31	2.17

[a] The observed rate constants in solutions in which the ionic strength was adjusted to 0.20 mol dm^{-3} with $NaNO_3$. [b] Calculated by eqn. (6).

$$-\frac{d[S(tot.)]}{dt} = \frac{K_1[H^+](k_1 + k_2K_2[H^+] + k_4K_4[Ag^+])[S(tot.)]}{1 + K_1[H^+] + K_1K_2[H^+]^2 + K_3[Ag^+] + K_1K_4[H^+][Ag^+]} \quad (5)$$

for the formal kinetics must be based on the reaction pattern depicted in Scheme 2. Eqn. (5) describes the rate-law for this reaction system. The assumption that the hydrolysis of the complex SAg^+ can be neglected receives support from the previous studies concerning the decomposition of 1-(1-ethoxyethyl)-benzimidazoles.[8] As stated above, the term $K_1K_2[H^+]^2$ can only be expected to become significant at high acid concentrations. Consequently, the observed first-order rate constant, $k(Ag^+)$, can be approximated by eqn. (6) under the experimental conditions employed. A

$$k(Ag^+) = \frac{K_1[H^+](k_1 + k_2K_2[H^+] + k_4K_4[Ag^+])}{1 + K_1[H^+] + K_3[Ag^+] + K_1K_4[H^+][Ag^+]} \quad (6)$$

possible way to test the validity of eqn. (6) is to calculate $k(Ag^+)$ from the partial rate and equilibrium constants and compare the result with the experimental value. Determination of constants k_1 and K_1, and the product k_2K_2 have been described above. The formation constant, K_3, for the complex SAg^+ can be estimated from the changes in the UV-spectrum of 9-(1-ethoxyethyl)adenine caused by silver(I) ions. As seen from Fig. 2, the absorbance at 274 nm increases with increasing concentration of the silver(I) ion and finally levels off to a constant value. If it is assumed that the increments, ΔA, are proportional to the concentration of the complexed substrate, K_3 can be calculated from the slope and intercept of the line (7), where $\Delta A(max)$ is the change in A observed when S

$$\frac{1}{\Delta A} = \frac{1}{\Delta A(max)K_3}\frac{1}{[Ag^+]} + \frac{1}{\Delta A(max)} \quad (7)$$

is completely complexed. The value of (63 ± 3) dm^3 mol^{-1} obtained for K_3 by this method is in good agreement with that determined potentiometrically[29] for the corresponding adenosine complex at a markedly lower ionic strength. The formation constant, K_4, for the complex of the protonated substrate with silver(I) ion cannot be measured in a similar way, since the UV-spectra of the protonated and complexed substrates do not differ sufficiently. Titrimetric methods, in turn, cannot be applied in acidic solutions owing to the high hydrolysis rate of 9-(1-ethoxyethyl)adenine. For this reason K_4 was estimated by studying potentiometrically the complexing of adenosine with silver(I) ion under conditions where the ligand exists essentially in a monocationic form. As seen from Fig. 3, addition of adenosine in acidic solutions of silver nitrate

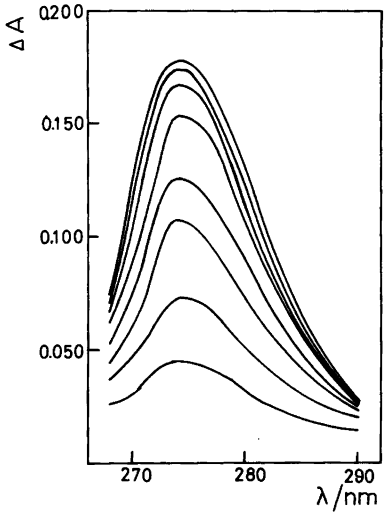


Fig. 2. The differential spectra for 9-(1-ethoxyethyl)adenine (2×10^{-4} mol dm^{-3}) at various concentrations of silver nitrate. $[Ag^+] = 0.20$ (upper curve), 0.15, 0.10, 0.070, 0.040, 0.020, 0.010, and 0.0050 mol dm^{-3} (lower curve).

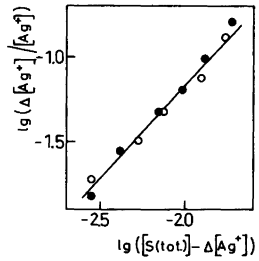


Fig. 3. The binding of silver(I) ions to adenosine under acidic conditions. Open circles refer to $[H^+] = 0.10$ mol dm^{-3} and filled circles to $[H^+] = 0.030$ mol dm^{-3}. $\Delta[Ag^+]$ is the necessary change in the total concentration of the silver(I) ion to return concentration of free silver(I) ions to its initial value.

reduces the concentration of free silver ion, the decrease being, within the limits of experimental error, linearly related to the concentration of adenosine. Accordingly, it seems reasonable to base the determination of the formation constant on the rough approximation that only formation of a 1:1 complex is significant under the conditions employed. This assumption leads to a value of (6 ± 1) $dm^3\ mol^{-1}$ for K_4. The fact that the values obtained are independent of the oxonium ion concentration as long as the ligand remains completely protonated, suggests that K_4 really refers to the reaction of the silver(I) ion with monoprotonated adenosine. Evaluation of the rate constant, k_4, is the most difficult problem. Earlier studies with 1-(1-ethoxyethyl)benzimidazoles[8] indicate that the hydrolysis rate of SAg^+ is negligible compared to that of SH^+. Consequently, it appears likely that the first-order rate constants, k_1 and k_4, for the heterolyses of SH^+ and SH^+Ag^+ are of the same order of magnitude. This approximation can be made with considerable confidence, since, owing to the relatively low value of K_4, the term $k_4K_4[Ag^+]$ in the nominator of eqn. (6) remains small compared to the term $k_2K_3[H^+]$.

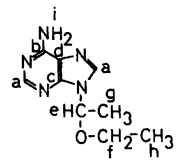
The last column in Table 3 gives the ratios $k(H^+)/k(Ag^+)$ calculated *via* eqn. (6) using the values given above for the partial rate and equilibrium constants. The calculated values agree fairly well with the experimental ones, suggesting that Scheme 2 describes satisfactorily the influence of the silver(I) ion on the acidic hydrolysis of 9-(1-ethoxyethyl)-adenine. Although the sites of coordination of the silver(I) ion cannot be definitely derived from the present data, the following observations suggest that nitrogen atoms N1 and N7 are involved. First, the difference spectra in Fig. 2 exhibit absorption maxima at 274 nm, as does that for the N1 coordinated methylmercury(II) complex of adenosine.[30] When binding of the methylmercury(II) ion occurs at C6–NH_2, the absorption maximum is shifted to somewhat higher wavelengths.[30] Secondly, 9-(1-ethoxyethyl)purine, having no primary amino group, appears to form silver complexes nearly as stable as does 9-(1-ethoxyethyl)adenine.[9] Thirdly, the complexing of protonated adenosine with the silver(I) ion seems to be pH-independent. Accordingly, this reaction cannot occur with displacement of a proton from the C6 amino group. It is possible that binding of the silver(I) ion takes place at N1 of the neutral substrate, analogous to complexing of methylmercury ion with adenosine, and at N7 of the protonated substrate. Under more basic conditions the primary amino group may constitute the most favorable binding site.[31]

EXPERIMENTAL

Materials. 9-(1-Ethoxyethyl)adenine was synthesized by treating adenine at room temperature in DMF solution with a slight excess of 1-chloroethyl ethyl ether prepared as described earlier.[32] Triethylamine was added to the reaction mixture to neutralize the hydrogen chloride liberated. The filtrated solution was concentrated under reduced pressure and the product was crystallized from acetone. 9-(1-Ethoxyethyl)adenine prepared in this manner melted at 154 – 155 °C and exhibited the ^1H and ^{13}C NMR chemical shifts collected in Table 4. The ^{13}C NMR data show that the 1-ethoxyethyl group is bound to N9 of the purine ring. The shifts obtained closely resemble those observed for adenosine and 9-methyladenine.[33] In the corresponding N7 derivatives the signals for C4 and C5 would appear at 160 and 110 ppm from TMS, respectively.

Adenosine (Sigma Chemical Company) was used without further purification. The salts employed were of reagent grade.

Table 4. ^1H and ^{13}C NMR chemical shifts[a] for 9-(1-ethoxyethyl)adenine.



Position		$\delta(^1H)$		$\delta(^{13}C)$	
a	s	8.25(1H)	d	153.2	(152.5; 152.6)[b]
	s	7.95(1H)	d	137.8	(141.4; 140.2)
b			s	156.0	(156.0; 156.3)
c			s	149.9	(149.9; 149.3)
d			s	119.5	(118.7; 119.6)
e	q	5.90(1H)	d	80.7	
f	q	3.40(2H)	t	64.7	
g	d	1.53(3H)	q	22.6	
h	t	1.16(3H)	q	14.8	
i	br.s	6.85(2H)			

[a] In $CDCl_3$ taken with respect to TMS. [b] The values in parentheses refer to 9-methyladenine and adenosine, respectively.

Kinetic measurements. The kinetic measurements were performed as described earlier.[9] The initial substrate concentration was of the order of 2×10^{-4} mol dm^{-3}.

Determination of the equilibrium constants. The protonation constant, K_1, for 9-(1-ethoxyethyl)-adenine was determined spectrophotometrically by measuring the absorbances of the substrate in formic acid buffers of various oxonium ion concentrations at 280 nm. The buffer solutions (3 cm^3) were thermostated at 313.2 K, and exactly 0.1 cm^3 of aqueous stock solution of the substrate was added, giving a concentration of 2×10^{-4} mol dm^{-3}. The absorbances were recorded by taking 20 readings at 1 s intervals. To eliminate the effect of the hydrolysis of the substrate the readings were extrapolated to the zero time. In the reference cell distilled water was added instead of substrate solution. The protonation constant, K_1, was calculated from eqn. (3).

An analogous method was applied to the determination of the formation constant, K_3, for the silver complex of 9-(1-ethoxyethyl)adenine. Substitution of the absorbance increments observed at 274 nm in eqn. (7) gives K_3.

Determination of the formation constant, K_4, for the silver complex of protonated adenosine was carried out potentiometrically using a solid silver electrode. The reference electrode was an Ag|AgCl(s) electrode, which was connected to the reaction solution *via* a KNO_3 salt bridge. To a thermostated vessel containing 10 cm^3 of an acidic solution of silver nitrate, the concentration of which was 10^{-3} mol dm^{-3}, a known amount of adenosine was added. After complete dissolution of the ligand, which took place within one minute, 0.1 mol dm^{-3} silver nitrate was added from an agla micrometer syringe until the potential reached its initial value. All manipulations were performed under nitrogen. The results obtained are presented in Fig. 3.

All determinations of the equilibrium and rate constants were performed at the ionic strength of 0.2 mol dm^{-3} adjusted with sodium nitrate.

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