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

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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 monoprotonated 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 hysrosis 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 hydrolysis 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
established that the first protonation takes place at N1,⁴,⁵ 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-α-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 10⁴ to 10⁶ times greater than those for ethyl aldo fururonosides 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, $\Delta S^\circ = (51 \pm 6)$ 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)benzimidazoles²⁰ 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

$$\frac{d[S\text{(tot.)}]}{dt} = \frac{k_1K_1[H^+] + k_2K_1K_2[H^+]^2}{1 + K_1[H^+] + K_2[H^+]^2} [S\text{(tot.)}]$$

(1)

canstants $k_1$, $k_2$, $K_1$ 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 10³.²⁴ 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

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

(2)
Table 1. First-order rate constants, $k(\text{H}^+)$, for the hydrolysis of 9-(1-ethoxyethyl)adenine in various acid and buffer solutions. The temperature is 313.2 K if not otherwise stated.

<table>
<thead>
<tr>
<th>HA</th>
<th>$[\text{HA}]$ [mol dm$^{-3}$]</th>
<th>$[\text{A}^-]$ [mol dm$^{-3}$]</th>
<th>$-\lg \left( \frac{[\text{H}^+]}{[\text{mol dm}^{-3}]} \right)$</th>
<th>$k(\text{H}^+)$ $10^{-3}$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HClO$_4$</td>
<td>0.0250</td>
<td>1.60</td>
<td>5.35(6)</td>
<td></td>
</tr>
<tr>
<td>HClO$_4$</td>
<td>0.0200</td>
<td>1.70</td>
<td>4.46(6)</td>
<td></td>
</tr>
<tr>
<td>HClO$_4$</td>
<td>0.0150</td>
<td>1.82</td>
<td>3.15(5)</td>
<td></td>
</tr>
<tr>
<td>HClO$_4$</td>
<td>0.0100</td>
<td>2.00</td>
<td>2.30(2)</td>
<td></td>
</tr>
<tr>
<td>HClO$_4$</td>
<td>0.0100</td>
<td>2.00</td>
<td>0.682(4)$^b$</td>
<td></td>
</tr>
<tr>
<td>DCIO$_4$</td>
<td>0.0100</td>
<td>2.00</td>
<td>7.82(4)$^c$</td>
<td></td>
</tr>
<tr>
<td>DCIO$_4$</td>
<td>0.00500</td>
<td>2.30</td>
<td>5.77(4)</td>
<td></td>
</tr>
<tr>
<td>CICH$_2$COOH</td>
<td>0.100</td>
<td>0.0500</td>
<td>2.45$^e$</td>
<td>1.087(12)</td>
</tr>
<tr>
<td>CICH$_2$COOH</td>
<td>0.200</td>
<td>0.100</td>
<td>2.45$^e$</td>
<td>0.794(7)</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.0800</td>
<td>0.0100</td>
<td>2.71$^f$</td>
<td>0.793(7)</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.112</td>
<td>0.0140</td>
<td>2.71$^f$</td>
<td>0.478(5)</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.160</td>
<td>0.0200</td>
<td>2.71$^f$</td>
<td>0.477(3)</td>
</tr>
<tr>
<td>CICH$_2$COOH</td>
<td>0.0200</td>
<td>0.0200</td>
<td>2.75$^e$</td>
<td>0.485(2)</td>
</tr>
<tr>
<td>CICH$_2$COOH</td>
<td>0.0200</td>
<td>0.0200</td>
<td>2.75$^e$</td>
<td>0.397(4)</td>
</tr>
<tr>
<td>CICH$_2$COOH</td>
<td>0.0200</td>
<td>0.0400</td>
<td>3.05$^e$</td>
<td>0.229(2)</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.0600</td>
<td>0.0200</td>
<td>3.14$^f$</td>
<td>0.1772(16)</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.0640</td>
<td>0.0200</td>
<td>3.14$^f$</td>
<td>0.1205(13)</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.0200</td>
<td>0.0200</td>
<td>3.61$^f$</td>
<td>0.0610(5)</td>
</tr>
<tr>
<td>CH$_3$COOH</td>
<td>0.0200</td>
<td>0.0200</td>
<td>4.61$^e$</td>
<td>0.00638(9)</td>
</tr>
</tbody>
</table>

$^a$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$_2$O. $^e$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.

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 $\Delta A$ is the change in absorbance on going from a solution where S is totally deprotonated to a solution having an oxonium ion concentration $[\text{H}^+]$, and $\Delta A(\text{max})$ 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, $k(\text{H}^+)$, a straight line presented in Fig. 1 is obtained. The intercept and slope of this line yield the values of $(4.2 \pm 0.1) \times 10^{-3}$ s$^{-1}$ and $(0.24 \pm 0.01) \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ 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 $2 \times 10^{-4}$ 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.

<table>
<thead>
<tr>
<th>M(^{2+})</th>
<th>(k(M^{2+})/10^{-6}) s(^{-1})*</th>
<th>(k(H^+)/k(M^{2+})^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(^{2+})</td>
<td>7.22(5)</td>
<td>0.96</td>
</tr>
<tr>
<td>Co(^{2+})</td>
<td>7.13(8)</td>
<td>0.98</td>
</tr>
<tr>
<td>Ni(^{2+})</td>
<td>7.22(9)</td>
<td>0.96</td>
</tr>
<tr>
<td>Cu(^{2+})</td>
<td>6.24(5)</td>
<td>1.12</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>7.09(6)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* \(k(M^{2+})\) refers to 0.200 mol dm\(^{-3}\) solutions of Mg(ClO\(_4\))\(_2\). ** \(k(H^+)\) refers to a 0.200 mol dm\(^{-3}\) solution of Mg(ClO\(_4\))\(_2\).

Under 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 \(K_3\)

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

stands for the formation constant of the complex SM\(^{2+}\). Formation of species SH\(^+\)M\(^{2+}\) and SM\(_2^+\) 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 \(k(H^+)/k(Cu^{2+})\). 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

\[
\begin{align*}
S & \xrightleftharpoons[k_3]{k_1}{\text{+H}^+} \text{SH}^+ \quad \text{SH}_2^{2+} \\
\text{SA}^+ & \xrightleftharpoons[k_2]{k_1}{\text{+H}^+} \text{SH}^+\text{Ag}^+ \xrightarrow[k_1]{k_2}\text{P}
\end{align*}
\]

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.

<table>
<thead>
<tr>
<th>[H(^+)]/mol dm(^{-3})</th>
<th>[Ag(^+)]/mol dm(^{-3})</th>
<th>(k(Ag^+))*/10(^{-3}) s(^{-1})</th>
<th>(k(H^+))/k(Ag(^+))</th>
<th>(k(H^+, \text{calc.})^b)/k(Ag(^+))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0100</td>
<td>0.0200</td>
<td>1.974(24)</td>
<td>1.17</td>
<td>1.14</td>
</tr>
<tr>
<td>0.0100</td>
<td>0.0500</td>
<td>1.534(20)</td>
<td>1.50</td>
<td>1.33</td>
</tr>
<tr>
<td>0.0100</td>
<td>0.0100</td>
<td>1.280(19)</td>
<td>1.80</td>
<td>1.67</td>
</tr>
<tr>
<td>0.0100</td>
<td>0.0100</td>
<td>0.980(5)</td>
<td>2.35</td>
<td>2.24</td>
</tr>
<tr>
<td>0.0500</td>
<td>0.0100</td>
<td>0.722(14)</td>
<td>1.48</td>
<td>1.62</td>
</tr>
<tr>
<td>0.0200</td>
<td>0.0100</td>
<td>2.59(3)</td>
<td>1.72</td>
<td>1.63</td>
</tr>
<tr>
<td>0.0050</td>
<td>0.0100</td>
<td>0.573(9)</td>
<td>1.90</td>
<td>1.73</td>
</tr>
<tr>
<td>0.0030</td>
<td>0.0100</td>
<td>0.310(3)</td>
<td>2.10</td>
<td>1.82</td>
</tr>
<tr>
<td>0.0020</td>
<td>0.0100</td>
<td>0.224(4)</td>
<td>1.94</td>
<td>1.91</td>
</tr>
<tr>
<td>0.0010</td>
<td>0.0100</td>
<td>0.0942(9)</td>
<td>2.31</td>
<td>2.17</td>
</tr>
</tbody>
</table>

*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).
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 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)adene 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, $\Delta A$, 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 $S$ is completely complexed. The value of $(63 \pm 3)$ dm³ mol⁻¹ obtained for $K_3$ by this method is in good agreement with that determined potentiometrically²⁹ 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)adene. 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

\[
\frac{d[S(tot.)]}{dr} = \frac{K_1[H^+](k_1 + k_2K_2[H^+] + k_4K_4[Ag^+])[S(tot.)]}{1 + K_1[H^+] + K_2[H^+]^2 + K_3[Ag^+] + K_4[H^+] [Ag^+]} \tag{5}
\]

\[
k(Ag^+) = \frac{K_1[H^+](k_1 + k_2K_2[H^+] + k_4K_4[Ag^+])}{1 + K_1[H^+] + K_3[Ag^+] + K_4[H^+] [Ag^+]} \tag{6}
\]

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

Fig. 2. The differential spectra for 9-(1-ethoxyethyl)adene (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 \((6 \pm 1)\)
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 \(\text{SAg}^+\) is negligible compared to
that of \(\text{SH}^+\). Consequently, it appears likely that the
first-order rate constants, \(k_t\) and \(k_4\), for the heterolyses of \(\text{SH}^+\) and \(\text{SH}^+\text{Ag}^+\) 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 \(k_tK_4[\text{Ag}^+]\)
in the nominator of eqn. (6) remains small compared to
the term \(k_2K_3[\text{H}^+]\).

The last column in Table 3 gives the ratios
\(k(\text{H}^+)/k(\text{Ag}^+)\) calculated \(\text{via}\) 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)
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 for the N1
coordinated methylmercury(II) complex of aden-
osine.\(^9\) 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 methylmer-
cury 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 synthe-
sized by treating adenine at room temperature in
DMF solution with a slight excess of 1-chloroethyl
ethyl ether described as prepared 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 \(1^H\)
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 corre-
sponding 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.** \(^1^H\) and \(^13^C\) NMR chemical shifts \(^a\) for 9-(1-
ethoxyethyl)adenine.

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta(1^H))</th>
<th>(\delta(13^C))</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>8.25(1H)</td>
<td>d 153.2 (152.5; 152.6)(^b)</td>
</tr>
<tr>
<td>b</td>
<td>7.95(1H)</td>
<td>d 137.8 (141.4; 140.2)</td>
</tr>
<tr>
<td>c</td>
<td>s 156.0 (156.0; 156.3)</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>s 149.9 (149.9; 149.3)</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>s 119.5 (118.7; 119.6)</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>q 5.90(1H)</td>
<td>d 80.7</td>
</tr>
<tr>
<td>g</td>
<td>q 3.40(2H)</td>
<td>t 64.7</td>
</tr>
<tr>
<td>h</td>
<td>d 1.53(3H)</td>
<td>q 22.6</td>
</tr>
<tr>
<td>i</td>
<td>t 1.16(3H)</td>
<td>q 14.8</td>
</tr>
<tr>
<td>br.s</td>
<td>6.85(2H)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)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. The initial substrate concentration was of the order of $2 \times 10^{-4}$ 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 $2 \times 10^{-4}$ 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 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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**REFERENCES**

2. Rosenberg, B. Naturwissenschaften 60 (1973) 399.

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