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

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First-order rate constants for the hydrolysis of 9-(1-ethoxyethyl)purine have been determined at different concentrations of oxonium ion in solutions of several metal ions. The rate-retardations caused by divalent metal ions have been accounted for by competitive attachment of protons and metal ions to the substrate. The stability constants for the complexes of various metal ions with the substrate have been calculated from the kinetic data and the spectrophotometrically determined equilibrium constants for the protonation of the substrate. The unexpected strong rate-retarding effect of silver(I) ion in acidic solutions and the slight rate-enhancing effect of mercury(II) chloride have been discussed.

Nucleosides and related compounds, containing a heterocyclic aromatic base moiety, form in aqueous solution reasonably stable complexes with several metal ions.[1] Owing to the great importance of nucleosides in biological systems, the structures of these complexes have been quite extensively studied both in solution[1-3] and in crystalline state.[2,4] Silver(I), mercury(II), palladium(II), platinum(II) and several first-row transition metal ions, for example, have been shown to coordinate in aqueous solution at N1 and N7 of purine nucleosides, the preferential binding site being dependent on the acidity of the solution.[3] In addition, heteroatoms at C6 may participate.[1,3] In pyrimidine nucleosides N3 constitutes the main binding site, though coordination to oxygen substituents must also be taken into account.[3] Obviously complexing with metal cations has an essential effect on the solvolytic reactions of nucleosides and their analogues. In a previous paper[5] we reported the results of our studies concerning the effects of metal ions on the acidic hydrolysis of 2-substituted 1-(1-ethoxyethyl)benzimidazoles, relatively simple nucleoside analogues containing only one possible binding site in the base moiety. The marked rate-retardations caused by silver(I) and copper(II) ions and mercury(II) chloride were accounted for by competitive attachment of protons and metal ions to N3 of the substrate. The aim of the present work is to extend the investigations to nucleoside analogues having several potential coordination sites in the base moiety. To accomplish this, the effects of various metal ions on the hydrolytic decomposition of 9-(1-ethoxyethyl)purine have been studied at different concentrations of oxonium ion.

RESULTS AND DISCUSSION

We have shown previously[6,7] that the acidic hydrolysis of 2-substituted 1-(1-ethoxyethyl)-benzimidazoles involves a rapid initial protonation of the benzimidazole ring and a subsequent rate-limiting cleavage of the carbon – nitrogen bond to form free benzimidazole and an oxocarbenium ion derived from the 1-ethoxyethyl group. The hydrolysis of 9-(1-ethoxyethyl)purine can well be compared to that of 1-(1-ethoxyethyl)benzimidazole having a strongly electronegative substituent at C2. Introduction of two electronegative nitrogen atoms in the benzene ring of benzimidazole lowers the electron density at the nitrogen atoms of the imidazole ring, as does the introduction of an electron-attracting group at C2. Consequently, the basicity of the substrate is reduced, but at the same

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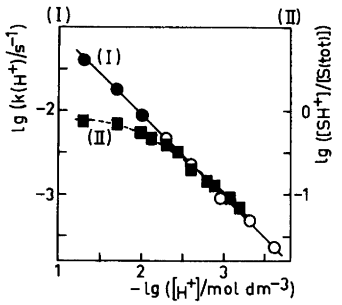
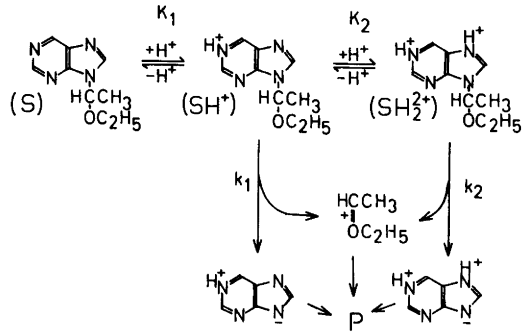


Fig. 1. Line I. The effect of the concentration of oxonium ion on the hydrolysis of 9-(1-ethoxyethyl)purine at 313.2 K. The ionic strength was adjusted to 0.20 mol dm^{-3} with sodium nitrate. The filled circles were obtained by extrapolation *via* the Arrhenius equation from the rate constants at 303.2 and 293.2 K.

Line II. Spectrophotometrically determined extent of protonation of 9-(1-ethoxyethyl)purine plotted against the concentration of oxonium ion at 293.2 K. The ionic strength was adjusted to 0.20 mol dm^{-3} with sodium perchlorate.

time the heterolysis of the carbon – nitrogen bond is facilitated.[6,8] In other words, it seems clear that purine is a better leaving group than benzimidazole, just like, for example, 2-cyanomethylbenzimidazole.[6] Accordingly, the mechanism described for the acidic hydrolysis of 1-(1-ethoxyethyl)benzimidazole [6,7] can probably be extended to the hydrolytic decomposition of 9-(1-ethoxyethyl)purine, as long as the reaction *via* a monocation of the substrate is concerned. If this were the only pathway, the hydrolysis rate would level off to a constant value in solutions where the substrate is almost completely protonated, as does the rate for the hydrolysis of the corresponding benzimidazole derivatives.[6,7] Fig. 1, however, clearly indicates that this is not the case with 9-(1-ethoxyethyl)purine. The first-order rate constant increases linearly with the concentration of oxonium ion in solutions having pH values lower than the pK_a value of the protonated substrate. The latter finding strongly suggests that the reaction *via* the dication of the substrate must also be considered. Most probably this partial reaction proceeds by rate-limiting departure of purinium ion, giving the same oxocarbenium ion intermediate as the reaction *via* a monocation. Hence the mechanism for the acid-catalyzed hydrolysis of 9-(1-ethoxyethyl)purine, depicted in Scheme 1, seems to be analogous to that suggested by Zoltewicz *et al.*[9,10] for purine



Scheme 1.

nucleosides. In Scheme 1 N1 of the substrate has been ascribed as the preferential protonation site, in accord with the suggestion that protonation of purine occurs at this site.[11] The second attachment of proton has been tentatively presented to take place at N7. Spontaneous decomposition of the substrate can probably be neglected, since the observed first-order rate constant for the hydrolysis of 9-(1-ethoxyethyl)purine is linearly related to the concentration of oxonium ion even at pH values as

Table 1. The effect of some divalent metal ions on the hydrolysis of 9-(1-ethoxyethyl)purine at different concentrations of oxonium ion at 313.2 K.

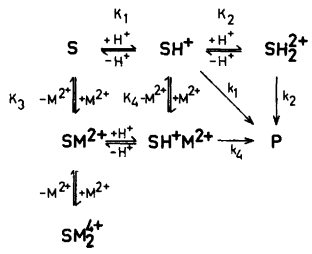
M^{2+}	$[H^+]$/ mol dm^{-3}	$k(H^+)/k(M^{2+})$ [a]	$lg(K_3/dm^3\ mol^{-1})$ [b]
Cu^{2+}	0.0025	2.18	0.92
	0.0100	1.79	1.03
	0.0150	1.55	0.99
	0.100	1.13 [c]	
Ni^{2+}	0.0025	2.10	0.89
	0.0100	1.76	1.01
	0.0150	1.44	0.89
	0.100	1.11 [c]	
Co^{2+}	0.0025	1.52	0.57
Cd^{2+}	0.0025	1.45	0.51
Zn^{2+}	0.0025	1.26	0.27
Mn^{2+}	0.0025	1.03	<0

[a] The first-order rate constants, $k(H^+)$ and $k(M^{2+})$, refer to 0.20 mol dm^{-3} solutions of $Mg(ClO_4)_2$ and $M(ClO_4)_2$, respectively. [b] Logarithmic formation constants for 1:1 complexes of metal ions with 9-(1-ethoxyethyl)purine. [c] Extrapolated from the measurements at lower temperatures.

high as 5, *i.e.* under conditions where the ratio of the concentrations of SH^+ and S is of the order of 10^{-3}. Catalysis by buffer acids was not observed.

Table 1 records the effects of some divalent metal ions on the hydrolysis of 9-(1-ethoxyethyl)purine at different concentrations of oxonium ion. The rate constants, $k(H^+)$, obtained in solutions of magnesium perchlorate have been used as reference values, since magnesium(II) ion has been shown not to interact markedly with the purine ring system.[1,2] Inspection of the data in Table 1 reveals that the ratios $k(H^+)/k(M^{2+})$, indicating the rate-retarding effects of various metal ions, exhibit their greatest values at low acidities, and approach to unity at high concentrations of oxonium ion. This kind of behaviour can be interpreted to reflect competitive attachment of protons and metal ions to the substrate, as indicated by the following treatment.

When the possible complexing with metal ions is taken into account, the acid-catalyzed hydrolysis of 9-(1-ethoxyethyl)purine can be described by Scheme 2.



Scheme 2.

The rate-law obeyed can probably be written in the form (1), because the previous studies [5] have shown that the hydrolysis rate for the complexed species, SM^{2+}, is negligible compared to that of SH^+. Since attachment of one positively charged particle on the

$$-\frac{d[S(tot.)]}{dt} = k_1[SH^+] + k_2[SH_2^{2+}] + k_4[SH^+M^{2+}] \quad (1)$$

purine ring lowers the electron density at other potential binding sites, the further protonation or complexation of the substrate is difficult and thus formation of species SH_2^{2+}, SM_2^{4+}, and SH^+M^{2+} is expected to become quantitatively significant only at extremely high concentrations of oxonium and metal ions. Furthermore, it appears to be reasonable to assume that the rate constants, k_1 and k_4, would be of the same order of magnitude, and much less than k_2. The first part of the assumption receives support from the finding that metal ions attached to the substrate are catalytically inactive compared to protons,[5] and the latter part is evident on the basis of the data in Fig. 1. Accordingly, the term $k_4[SM^+M^{2+}]$ is probably negligible compared to $k_1[SH^+]$ or $k_2[SH_2^{2+}]$, and eqn. (1) can be transformed to eqn. (2). The meaning of the equilibrium constants, K_1 and K_2, is indicated in Scheme 1. The concentration of free substrate, S, can

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

be approximated by eqn. (3) at the concentrations of metal and oxonium ions employed in kinetic measurements. Substitution of [S] from this equation into eqn. (2) gives eqn. (4). When the first-order rate constant, $k(H^+)$, determined in the absence of metal

$$[S] = \frac{[S(tot.)]}{1+K_1[H^+]+K_3[M^{2+}]} \quad (3)$$

$$-\frac{d[S(tot.)]}{dt} = \frac{k_1K_1[H^+]+k_2K_1K_2[H^+]^2}{1+K_1[H^+]+K_3[M^{2+}]} \times [S(tot.)] = k(M^{2+})[S(tot.)] \quad (4)$$

ions is divided by the rate constant, $k(M^{2+})$, measured at the same oxonium ion concentration in the presence of metal ions, expression (5) is obtained.

$$\frac{k(H^+)}{k(M^{2+})} = \frac{1+K_1[H^+]+K_3[M^{2+}]}{1+K_1[H^+]} \quad (5)$$

The stability constant, K_3, for the complex between the substrate and metal ion, can thus be calculated from the values of $k(H^+)$ and $k(M^{2+})$ at any given concentration of oxonium ion (eqn. 6). In the

$$K_3 = \frac{1}{[M^{2+}]}\left(\frac{k(H^+)}{k(M^{2+})} - 1\right)(1+K_1[H^+]) \quad (6)$$

preceding derivation, formation of complexes containing more than one ligand molecule has been neglected, because the concentration of the metal ions is under the experimental conditions about a thousand times greater than that of the ligand.

Table 1 summarizes the results obtained for stability constant, K_3, from eqn. (6). The value of 170

Table 2. The effect of silver(I) ion on the hydrolysis of 9-(1-ethoxyethyl)purine at different concentrations of oxonium ion at 313.2 K.

$[H^+]$/ mol dm^{-3}	$[Ag^+]$/ mol dm^{-3}	$k(H^+)$/ $k(Ag^+)$[a]
0.0025	0.020	1.48
0.0025	0.050	2.07
0.0025	0.10	3.49
0.0025	0.20	5.61
0.0050	0.20	5.60
0.0100	0.20	5.28
0.0200	0.20	4.73
0.0400	0.20	4.38
0.100	0.20	4.02[b]

[a] The first-order rate constants, $k(H^+)$ and $k(Ag^+)$, refer to a 0.20 mol dm^{-3} solution of $NaNO_3$ and a solution where the sum of $[AgNO_3]$ and $[NaNO_3]$ is 0.20 mol dm^{-3}. [b] Extrapolated from the results at lower temperatures.

$dm^3\ mol^{-1}$ employed for K_1 in calculations has been estimated from the spectrophotometric measurement as described in the experimental. The fact that application of eqn. (6) at different concentrations of oxonium ion yields constant values for K_3 suggests that the approximations made in the foregoing are justified. Of the metal ions investigated copper(II) and nickel(II) ions are complexed most efficiently followed by cobalt(II), cadmium(II) and zinc(II) ions. Complexing with manganese(II) ion is too weak to be detected. In other words, the Irving-Williams order seems to be obeyed. The complexing efficiencies of various metal ions with 9-(1-ethoxyethyl)purine roughly correlate with their abilities to interact with adenosine,[12] the stability constants for the former complexes being somewhat greater than those for the latter. Consequently, the present data do not give any support for participation of the 6-amino substituent in the purine ring system in metal bonding. If some interaction with this substituent occurs, it does not result in any marked stabilization of the metal ion – nucleoside complex.

Table 2 records the kinetic data for the acid-catalyzed hydrolysis of 9-(1-ethoxyethyl)purine in solutions of silver nitrate. The retardations observed in the hydrolysis rate are more marked than those caused by divalent metal ions, suggesting efficient complexing with silver(I) ion. However, when the value of 83 $dm^3\ mol^{-1}$, obtained as described in the experimental, is used for K_1, the values eqn. (6) gives for K_3 increase smoothly with the increasing concentration of oxonium ion. In other words, the formal kinetics of eqn. (4) is not followed. Evidently the approximations made in the derivation of this equation are too rough where complexing with silver(I) ion is concerned. For example, formation of dicharged species, SH^+Ag^+ and SAg_2^{2+}, should possibly be taken into account when expression (4) is derived. Inclusion of such additional equilibria would, however, make the expressions for the observed rate constants so complicated that reliable values could not be obtained for the individual constants. Qualitatively a possibility that a dicharged species, SH^+Ag^+, is formed at high concentrations of silver(I) and oxonium ions, lowering the concentration of the extremely reactive species, SH_2^{2+}, seems attractive. The value of 1.4 obtained from eqn. (6) for $\lg K_3$ at the lowest concentration of oxonium ion employed is of the order that could be expected on the basis of previous results.[5,13] The extremely low solubility of the complex of 9-(1-ethoxyethyl)purine with silver(I) ion makes the investigation of the problem quite difficult.

It has been shown earlier[5] that mercury(II) chloride complexes efficiently with 1-(1-ethoxyethyl)benzimidazole, preventing the protonation and hence the hydrolytic decomposition of the substrate. In the hydrolysis of the corresponding purine derivative the situation is, however, the opposite. Addition of mercury(II) chloride in the acidic reaction mixture results in a slight increase in the observed first-order rate constant, the increment being reduced with the increasing concentration of oxonium ion (Table 3). This kind of difference in the effect of mercury(II) chloride can be understood, if it is assumed that protonation and complexation can

Table 3. The effect of mercury(II) chloride on the hydrolysis of 9-(1-ethoxyethyl)purine at different concentrations of oxonium ion at 313.2 K.

$[H^+]$/ mol dm^{-3}	$[HgCl_2]$/ mol dm^{-3}	$k(H^+)$/ $k(HgCl_2)$[a]
0.0025	0.050	0.57
0.0025	0.10	0.45
0.0025	0.15	0.40
0.100	0.050	0.89[b]
0.100	0.20	0.82[b]

[a] The first-order rate constants, $k(H^+)$ and $k(HgCl_2)$, refer to solutions the ionic strength of which was adjusted to 0.20 mol dm^{-3} with sodium nitrate. [b] Extrapolated from the results at lower temperatures.

occur in the purine derivative at different nitrogen atoms, whereas in 1-(1-ethoxyethyl)benzimidazole they take place at the same site. As mentioned above, the preferential protonation site in purine ring is N1,[11] whereas complexing of mercury(II) chloride most probably occurs both at N1 and N7. At least this is the case with 9-substituted adenine derivatives.[3,14] If mercury(II) chloride, which does not markedly hydrolyze in acidic solutions,[15] retains its chloro ligands during coordination to the purine ring, the proton attached at N1 can be assumed to exert only a slight effect on metal binding to N7. Similarly the substrate complexed at N1 can be protonated at N7, the basicity of which is probably comparable to that of N1.[11] Consequently, besides SH^+, considerable amounts of species $SH^+.HgCl_2$ can be formed. If the latter species is hydrolyzed somewhat more readily than the former, a rate-enhancement is observed. At high concentrations of oxonium ion the acceleration is decreased, because formation of SH^+HgCl_2 lowers the concentration of the highly reactive dication, SH_2^{2+}, of the substrate.

The discussion presented above can probably be extended to the hydrolysis of 9-(β-D-ribofuranosyl)-purine, called nebularine, since the metal ions considered in the preceding interact only weakly with the carbohydrate moiety of the molecule.[16]

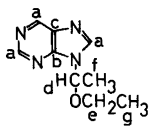
EXPERIMENTAL

Materials. 9-(1-Ethoxyethyl)purine was obtained by treating purine at room temperature in DMF solution with a slight excess of 1-chloroethyl ethyl ether prepared as described earlier.[17] Triethylamine was added to the reaction mixture to neutralize the hydrogen chloride liberated in the condensation reaction. The filtrated solution was concentrated to a thick syrup under reduced pressure. The product was extracted in diethyl ether. 9-(1-Ethoxyethyl)purine, crystallized from carbon tetrachloride, melted at 55 −56 °C. Table 4 records the ^1H and ^{13}C NMR spectrometric data for the compound. ^{13}C NMR chemical shifts clearly indicate that the 1-ethoxyethyl group is bound to N9 of the purine ring. As seen from Table 4, the shifts closely resemble those determined for the corresponding 9-(β-D-ribofuranosyl) derivative.[18] In the N7 isomer the signal for C4 would appear at about 160 ppm from TMS and that for C5 at about 10 ppm higher field than observed.

The salts employed were commercial products of reagent grade and they were used without further purification.

Kinetic measurements. Kinetic measurements were performed as described in Ref. 6 when the reaction solution did not contain ions of heavy metals. Otherwise the method described in Ref. 5 was employed. However, alkaline solution of EDTA was used to stop the reaction and the progress of the reaction was followed at 273 nm in the former method and at 260 nm in the latter.

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



Position	δ(^1H)[b]		δ(^{13}C)[c]	
a	s	8.97(1H)	d	152.8(152.2)[d]
	s	8.75(1H)	d	148.8(148.3)
	s	8.20(1H)	d	142.8(145.5)
b		–	s	151.3(151.1)
c		–	s	134.1(134.3)
d	q	6.02(1H)	d	80.9
e	q	3.42(2H)	t	64.9
f	d	1.80(3H)	q	22.5
g	t	1.16(3H)	q	14.8

[a]Taken as ppm with respect to TMS. [b]In CCl_4. [c]In $CDCl_3$. [d]The values in parentheses refer to 9-(β-D-ribofuranosyl)purine.[18]

Determination of equilibrium constants. The equilibrium constant for the protonation of 9-(1-ethoxyethyl)purine was determined spectrophotometrically by measuring the absorbances of the substrate solutions of known concentrations at various acid and buffer solutions at 282 nm. The solution (3 cm^3) of known concentration of oxonium ion was thermostated to 293.2 K. Exactly 0.1 cm^3 of aqueous stock solution of the substrate was added, giving concentration of about 2×10^{-4} mol dm^{-3}, and the absorbance was recorded by taking 20 readings at 1 s intervals. To eliminate the effect of the hydrolysis of substrate, the readings were extrapolated to the time zero. In the reference cell distilled water was added instead of the substrate solution. The equilibrium constants, K_1, were calculated from the slopes of the lines of eqn. (7). Here

$$\frac{1}{\Delta A} = \frac{1}{\Delta A(\max)K_1} \times \frac{1}{[H^+]} + \frac{1}{\Delta A(\max)} \qquad (7)$$

ΔA is the change in absorbance on going from a solution where S is totally deprotonated to a solution of a fixed concentration of oxonium ion. ΔA(max) is

an adjustable parameter representing the maximal change in A. By this method values of 120 $dm^3 mol^{-1}$ and 240 $dm^3 mol^{-1}$ were obtained for K_1 in 0.2 mol dm^{-3} solutions of sodium nitrate and magnesium perchlorate, respectively. Owing to the relatively rapid hydrolysis of 9-(1-ethoxyethyl)purine in acidic solution, the measurements could not be performed at the temperature where the kinetic runs were carried out, *viz.* 313.2 K. Extrapolation of the values of K_1 to this temperature was based on the assumption that the dependence of K_1 on temperature is with 9-(1-ethoxyethyl)purine the same as with adenosine, *i.e.* division by a factor of 1.4 would yield the values at 313.2 K.[1]

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