

Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues. III. The Effect of Metal Ions on the Acidic Hydrolysis of 2-Substituted 1-(1-Ethoxyethyl)benzimidazoles

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First-order rate constants for the hydrolysis of a few 2-substituted 1-(1-ethoxyethyl)benzimidazoles have been measured in solutions of several metal ions at various concentrations of oxonium ion. The rate-retardations caused by some of the metal ions are accounted for by competitive attachment of protons and metal ions to N3 of the substrate. Equilibrium constants for the latter reactions are evaluated on the basis of the formal kinetics followed. The results obtained have been verified in the case of silver ion by direct spectrophotometric measurements. The effects of C2 substituents on the protonation and complexation of the adjacent nitrogen atom are discussed.

Interactions of metal ions with nucleic acids and their constituent bases, nucleosides and nucleotides have received attention^{1,2} after establishment of the dominant role of these compounds in biological systems. Nucleosides, for example, have been shown to form reasonably stable complexes in aqueous solutions with several metal ions, including silver,^{3,4} mercury,^{5,6} lead,⁷ cadmium⁸ and the first-row transition metal ions.⁷⁻⁹ Complexing with these cations undoubtedly affects the kinetics and the mechanisms for the solvolytic decompositions of nucleosides and related compounds to free nitrogen bases and carbonyl compounds. However, no investigations dealing with this subject have been reported in the literature.

The present paper, which is the first part of our studies concerning the effects of metal ions on the solvolytic decompositions of nucleosides, describes the kinetic data obtained for the acid-catalyzed hydrolysis of some 2-substituted 1-(1-ethoxyethyl)-

benzimidazoles in the presence of different metal ions. These relatively simple nucleoside analogues have been chosen as model compounds, since the uncertainty in the assignment of the attachment of protons and metal ions to various potential coordination sites in nucleoside molecules¹ would severely complicate the interpretation of the influences observed. In 1-(1-ethoxyethyl)benzimidazoles the only binding site available is N3 of the base moiety, corresponding to N7 of purine nucleosides, *i.e.* the atom playing a central role in the complexing of these compounds with metal ions.^{1,2} Moreover, the mechanism for the acidic hydrolysis of 2-substituted 1-(1-ethoxyethyl)benzimidazoles is fairly well known.^{10,11}

RESULTS AND DISCUSSION

In previous papers^{10,11} the hydrolysis of 2-substituted 1-(1-ethoxyethyl)benzimidazoles has been shown to proceed in acidic solutions by a rapid initial protonation of the benzimidazole moiety and a subsequent unimolecular heterolysis of the protonated substrate giving free benzimidazole and an oxocarbenium ion formed from the 1-ethoxyethyl group. In sufficiently acidic solutions, where the substrate is completely protonated, the observed first-order rate constant becomes independent of the acid concentration, being equal to the first-order rate constant for the heterolysis of the protonated substrate. In the case of the unsubstituted 1-(1-ethoxyethyl)benzimidazole this is the situation, as the concentration of oxonium ion exceeds 10^{-3} mol dm⁻³.¹⁰ Under such conditions,

Table 1. First-order rate constants for the hydrolysis of 1-(1-ethoxyethyl)benzimidazole in acidic salt solutions at 353.2 K.

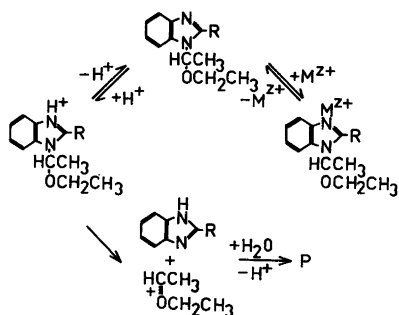
Salt	$[H^+]/10^{-3} \text{ mol dm}^{-3}$	$k(\text{obs})/10^{-3} \text{ s}^{-1}$
	100	2.11 ± 0.02^a
AgNO ₃ 0.25 mol dm ⁻³	12.5	1.83 ± 0.03
	5.0	1.52 0.02
	2.5	1.24 0.01
	1.3	0.91 0.02
Cu(NO ₃) ₂ 0.30 mol dm ⁻³	10.0	1.66 ± 0.01
	5.0	1.41 0.01
	2.6 ^b	1.20 0.01
	1.2 ^b	0.86 0.01
Ni(NO ₃) ₂ 0.30 mol dm ⁻³	10.0	2.20 ± 0.06
	5.0	2.12 0.07
	2.5	1.98 0.03
	1.0	1.69 0.03
Cd(NO ₃) ₂ 0.30 mol dm ⁻³	10.0	1.93 ± 0.03
	5.0	1.87 0.06
	2.5	1.71 0.04
	1.0	1.67 0.03
Zn(NO ₃) ₂ 0.30 mol dm ⁻³	10.0	2.00 ± 0.03
	5.0	1.85 0.06
	2.5	1.77 0.03
	1.0	1.68 0.03
Pb(NO ₃) ₂ 0.30 mol dm ⁻³	5.0	2.24 ± 0.04
NaCl 1.0 mol dm ⁻³	2.5	2.28 ± 0.04
HgCl ₂ 0.20 mol dm ⁻³	10.0	1.98 ± 0.04
	5.0	1.70 0.02
	2.5	1.15 0.02
	1.0	0.64 0.01

^a See Ref. 1. ^b Corrected with respect to the hydrolysis of copper(II) ion using the data in Ref. 12.

addition of silver(I) or copper(II) ions into the reaction mixture retards the hydrolysis considerably (Table 1). Mercury(II) chloride, which does not markedly hydrolyze in acidic solutions,¹³ exerts an even stronger effect, while zinc(II), nickel(II) and cadmium(II) ions are only slightly rate-retarding. Sodium or lead(II) ions have practically no influence on the observed rate constants. The latter finding clearly indicates that the rate-retardations caused by silver(I) and copper(II) ions cannot be accounted for by common salt effects. Evidently these cations are complexes with 1-(1-ethoxyethyl)benzimidazole, the most probable co-ordination site being N3 of

the benzimidazole moiety, *i.e.* the atom which is protonated in the pre-equilibrium stage of the hydrolysis reaction. This kind of complexing results in a decrease in the concentration of the protonated substrate and hence a decrease in the hydrolysis rate.

Taking the possible complexing with metal ions into account, the acidic hydrolysis of 1-(1-ethoxyethyl)benzimidazole can be depicted by Scheme 1. At oxonium ion concentrations greater than $10^{-3} \text{ mol dm}^{-3}$ the concentration of the unprotonated substrate is negligible compared to the sum of the concentrations of the protonated and complexed

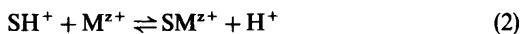


Scheme 1.

species. Under these conditions the rate-law of eqn. (1) is obeyed. Here $k(H^+)$ stands for the first-

$$-\frac{d[S(\text{tot})]}{dt} = \frac{k(H^+)[H^+]}{K[M^{z+}] + [H^+]} [S(\text{tot})] = k(\text{obs})[S(\text{tot})] \quad (1)$$

order rate constant for the decomposition of the protonated substrate and K is the equilibrium constant for reaction (2). The expression for the observed rate constant, $k(\text{obs})$, can also be trans-



formed into eqn. (3). Accordingly, plots of

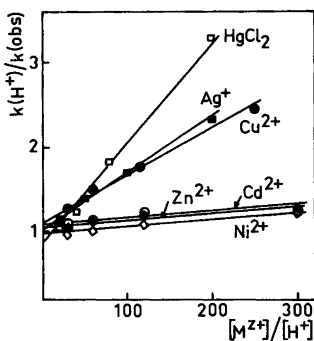


Fig. 1. The effect of metal ions on the acidic hydrolysis of 1-(1-ethoxyethyl)benzimidazole at 353.2 K. The ratio of the rate constants, $k(H^+)$ and $k(\text{obs})$, plotted against the ratio of the concentrations of the metal and oxonium ions. $k(H^+)$ stands for the first-order rate constant in conditions where the substrate is completely protonated, and $k(\text{obs})$ is the first-order rate constant observed in a given salt solution.

Table 2. Kinetically determined equilibrium constants, K , for reaction (2) (see text) of 1-(1-ethoxyethyl)benzimidazole in aqueous solution at 353.2 K.

Complexing species	$K/10^{-3}$
Ag^+	6.5 ± 0.2^a
Cu^{2+}	$5.6 \quad 0.4^b$
Ni^{2+}	$0.9 \quad 0.2^b$
Zn^{2+}	$0.8 \quad 0.2^b$
Cd^{2+}	$0.8 \quad 0.3^b$
$HgCl_2$	$11.2 \quad 0.6^c$

^a At the ionic strength of 0.25 mol dm^{-3} . ^b At the ionic strength of 0.9 mol dm^{-3} . ^c In a 0.20 mol dm^{-3} solution of $HgCl_2$.

$$\frac{k(H^+)}{k(\text{obs})} = K \frac{[M^{z+}]}{[H^+]} + 1 \quad (3)$$

$k(H^+)/k(\text{obs})$ against $[M^{z+}]/[H^+]$ should be straight lines with slopes equal to the equilibrium constants, K . In Fig. 1 the data of Table 1 have been presented in terms of this equation. The values obtained for K are listed in Table 2.

The assumptions made above in connection with the derivation of eqn. (1) receive additional support from the fact that the kinetically determined value

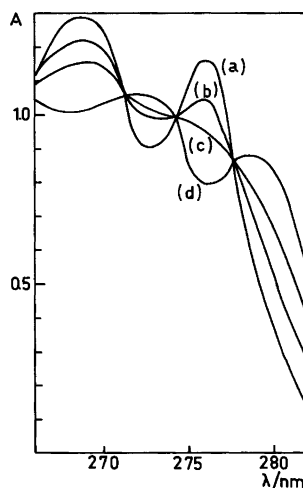


Fig. 2. The effect of silver ion on the UV-spectrum of 1-(1-ethoxyethyl)benzimidazole in acidic solutions at 298.2 K. Notation: (a) $[Ag^+] = 0$, (b) $[Ag^+] = 0.05 \text{ mol dm}^{-3}$, (c) $[Ag^+] = 0.10 \text{ mol dm}^{-3}$, and (d) $[Ag^+] = 0.20 \text{ mol dm}^{-3}$. In solutions (a)–(c) $[H^+] = 1 \times 10^{-3} \text{ mol dm}^{-3}$, in solution (d) $5 \times 10^{-4} \text{ mol dm}^{-3}$.

for the equilibrium constant, K , agrees, in the case of silver ion, fairly well with direct spectrophotometric observations. As seen from Fig. 2, addition of silver ions results in marked changes in the UV-spectrum of 1-(1-ethoxyethyl)benzimidazole recorded in acidic solutions. For example, the absorption maximum at 276 nm is shifted to 279 nm. Presumably the decrease in absorbance at the former wavelength, ΔA , is proportional to the ratio of the concentrations of the complexed and protonated forms of the substrate, as indicated by eqn. (4) where $\Delta A(\max)$ denotes the change in absorbance

$$\frac{\Delta A}{\Delta A(\max) - \Delta A} = \frac{[S \cdot Ag^+]}{[SH^+]} \quad (4)$$

caused by the transformation of the protonated substrate completely into the complexed species. Combining this equation with the expression for the equilibrium constant of reaction (2) gives eqn. (5). In Fig. 3 this equation has been applied to the

$$\frac{[Ag^+]}{\Delta A \cdot [H^+]} = \frac{1}{\Delta A(\max)} \cdot \frac{[Ag^+]}{[H^+]} + \frac{1}{K \cdot \Delta A(\max)} \quad (5)$$

spectrophotometric data obtained at the ionic strength of 0.25 mol dm^{-3} at 353.2 K. The slope of the line divided by the intercept yields the value of $(5.2 \pm 0.4) \times 10^{-3}$ for K in fairly good agreement with that determined kinetically.

Table 3 records the rate constants for the

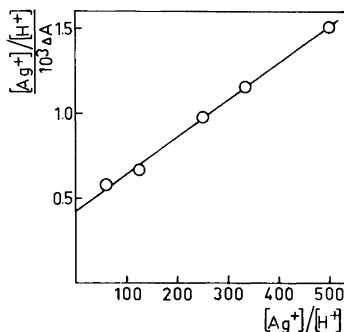


Fig. 3. The effect of silver ion on the UV-spectrum of 1-(1-ethoxyethyl)benzimidazole in acidic solutions at 353.2 K. ΔA is the change observed in absorbance at 276 nm as the silver ion concentration is increased from zero to a given value of $[Ag^+]$, while the oxonium ion concentration remains unchanged.

hydrolysis of a few 2-substituted derivatives of 1-(1-ethoxyethyl)benzimidazole in acidic solutions of copper(II) ion. The equilibrium constants, K , calculated by eqn. (3) for the reaction (2) of these compounds are listed in Table 4. To facilitate the comparison of the effects that C2 substituents exert on the protonation and complexation of the substrate, the formation constants of the protonated and complexed species, $K(H^+)$ and $K(Cu^{2+})$, are included in the same table. The latter constants are, however, approximate in the sense that the values employed in their calculations for $K(H^+)$ refer to

Table 3. First-order rate constants for the hydrolysis of some 2-substituted 1-(1-ethoxyethyl)benzimidazoles at various concentrations of oxonium ion in a 0.30 mol dm^{-3} solution of copper(II) nitrate.

Substituent at C2	T/K	$[H^+]/10^{-3} \text{ mol dm}^{-3}$	$k/10^{-3} \text{ s}^{-1}$
CH ₃	353.2	10.0	1.07 ± 0.02
		5.0	1.07 0.02
		2.6 ^a	0.94 0.03
		1.2 ^a	0.89 0.02
CH ₂ OH	343.2	10.0	1.80 ± 0.03
		5.0	1.37 0.02
		2.6 ^a	0.84 0.01
CH ₂ Cl	313.2	10.0	0.48 0.01
		5.0	0.42 0.01
		3.5	0.37 0.01
		2.5	0.34 0.01
		1.5	0.28 0.01

^a See footnote b in Table 1.

Table 4. Equilibrium constants, K , for reaction (2) of some 2-substituted 1-(1-ethoxyethyl)benzimidazoles with copper(II) ion (see text), and the logarithms of the formation constants, $K(\text{H}^+)$ and $K(\text{Cu}^{2+})$, for the protonated and complexed forms of the substrate, respectively.

Substituent at C2	$\frac{K^a}{10^{-3}}$	$\lg \left(\frac{K(\text{H}^+)^b}{\text{dm}^3 \text{ mol}^{-1}} \right)$	$\lg \left(\frac{K(\text{Cu}^{2+})}{\text{dm}^3 \text{ mol}^{-1}} \right)$
CH_3^c	1.0+0.3	5.02 ± 0.16	2.0 ± 0.2
H^c	5.6 0.4	4.43 0.07	2.2 0.1
CH_2Cl^d	5.5 0.3	3.46 0.14	1.2 0.1
CH_2OH^e	17 2	4.34 0.10	2.6 0.1

^a Calculated by eqn. (3) (see text). The values of $(1.09 \pm 0.02) \times 10^{-3} \text{ s}^{-1}$, $(2.44 \pm 0.05) \times 10^{-3} \text{ s}^{-1}$ and $(0.59 \pm 0.01) \times 10^{-3} \text{ s}^{-1}$ were used for $k(\text{H}^+)$ in the case of methyl, hydroxymethyl and chloromethyl derivatives, respectively.¹⁰ ^b From Ref. 10. ^c At 353.2 K. ^d At 313.2 K. ^e At 343.2 K.

the ionic strength of 0.10 mol dm^{-3} ,¹⁰ while the values for the equilibrium constants, K , are obtained at markedly higher electrolyte concentrations.

The data in Table 4 reveal that replacing the C2 hydrogen by a more electropositive methyl group increases the basicity of the adjacent nitrogen atom, whereas its complexing ability with copper(II) ion remains almost unchanged. In contrast, substitution of the C2 hydrogen by a more electronegative chloromethyl group opposes both the protonation and the complexation of N3. These two findings suggest that, besides electronic effects, steric hindrances play an important role in the complexing of the benzimidazole moiety with copper(II) ion. The fact that no increment in the complexing ability is observed for the methyl derivative suggests that the steric hindrance caused by the methyl group cancels its electronic influence. In the case of the 2-chloromethyl derivative, the steric and electronic effects of the 2-chloromethyl group enforce each other, resulting in a marked decrease in the complexing power of N3. Insertion of a hydroxymethyl group at C2 of 1-(1-ethoxyethyl)benzimidazole exerts only a slight effect on the basicity of the substrate, but increases markedly the complexing ability. Possibly the oxygen atom of the hydroxymethyl group affords an additional point of attachment for copper(II) ion and thus stabilizes the complex formed.

The order of the complexing efficiencies of various metal ions with 1-(1-ethoxyethyl)benzimidazole correlates roughly with the interactions of these ions with adenine nucleosides, as would be expected assuming N7 of the latter compounds to be the site of complexing. Evidently the preceding discussion can, to a certain extent, be applied to the hydrolysis of adenine nucleosides, as long as the partial

reaction *via* the monocation of the substrate is in question. It should be noted, however, that the prediction of the rate-retardations on the bases of the known stability constants for the metal ion – nucleoside complexes is not quite straightforward, since the protonation can, in these compounds, take place at sites other than the co-ordination of metal ions.¹ Work dealing with this subject is in progress.

EXPERIMENTAL

Materials. Preparation of 2-substituted 1-(1-ethoxyethyl)benzimidazoles has been described earlier.¹⁰ The salts employed, *viz.* AgNO_3 , $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, HgCl_2 and NaCl , were of reagent grade and they were used without further purification. The oxonium ion concentrations of the reaction solutions were adjusted with nitric acid.

Kinetic measurements. Hydrolyses were carried out in stoppered bottles immersed in a bath, the temperature of which was kept constant within 0.1 K. Reactions were initiated by adding the substrates as 10% solutions in methanol into the prethermostated reaction solutions. The initial substrate concentration was about $2 \times 10^{-4} \text{ mol dm}^{-3}$. Samples of 2 cm^3 were withdrawn at suitable intervals and mixed with 1 cm^3 of 1 mol dm^{-3} aqueous sodium hydroxide in separatory funnels equipped with Teflon stopcocks. The unreacted substrate was extracted into 1 cm^3 of dichloromethane, and its concentration was determined spectrophotometrically at 250 nm. Benzimidazole liberated could not be detected in the organic phase. No decomposition of the substrate took place in the alkaline conditions used to stop the reaction.

Spectrophotometric measurements. The equilib-

rium constant for reaction (2) (see text) of 1-(1-ethoxyethyl)benzimidazole with silver(I) ion was determined by measuring the absorbances of the substrate solutions of known hydrogen and silver ion concentrations at 276 nm. Into 3 cm³ of the acidic salt solution, thermostated at 353.2 K, exactly 0.1 cm³ stock solution of the substrate was added giving a substrate concentration of about 2×10^{-4} mol dm⁻³. The absorbance was recorded immediately by taking 20 readings at 1 s intervals. During this time no marked change in the absorbance occurred. In the reference cell distilled water was added instead of the substrate solution.

The equilibrium constant was calculated as described above in connection with Fig. 3.

Acknowledgements. Financial aid from the Finnish Academy, Division of Sciences, and from the Foundation of University of Turku is gratefully acknowledged.

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Received November 2, 1979.