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Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues. VII. The Acidic Hydrolysis of 9-(1-Ethoxyethyl)-purine and -adenine in Solutions of Mercury(II) Chloride

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First-order rate constants for the hydrolysis of 9-(1-ethoxyethyl)purine and -adenine have been determined in acidic solutions of mercury(II) chloride at 293.2 K. The kinetic results combined with the potentiometric data for protonation and complexation of the corresponding β-D-ribofuranosyl derivatives at various oxonium ion concentrations are interpreted to indicate that mercury(II) chloride accelerates the decomposition of the monoprotonated substrates, but exerts no effect on the cleavage of the diprotonated species. The possible appearance of mercury(II) chloride in the transition state of the former reaction is discussed.

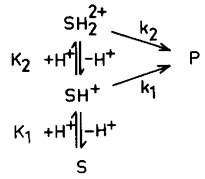
Interactions of mercury(II) salts and alkylmercurials with nucleic acids and their constituents, nucleosides and nucleotides, have been rather extensively studied in recent years,[1,2] the mutagenic nature of organomercurials being responsible for the great interest. Purine nucleosides, for example, have been shown to form relatively stable complexes with mercury(II) and methylmercury(II) ions in aqueous solution.[3–5] In adenosine N1 constitutes the primary binding site, while with guanosine and inosine complexing takes place either at N1 or N7, depending on the acidity of the medium.[5,6] Under alkaline conditions, binding at the primary amino groups, *viz.* C6–NH_2 in adenosine and C2–NH_2 in guanosine, with displacement of an amino proton becomes significant.[5,7] In addition, methylmercury (II) ion has been observed to bond in slightly basic solutions to C8 of inosine and guanosine.[8]

We have shown previously[9–11] that complexing with metal ions generally retards the acidic hydrolysis of acyclic analogues of purine nucleosides. The rate-decelerations that 3d transition metal ions exert on the hydrolysis of 9-(1-ethoxyethyl)-purine and -adenine at different oxonium ion concentrations have been quantitatively accounted for by a reaction scheme involving, besides mono- and diprotonated substrates, 1:1 metal complexes of the unprotonated substrates.[10,11] With silver(I) ion, complexing of the monocation of the reactant must also be taken into account.[11] In contrast, the effect of mercury(II) chloride on the hydrolysis of 9-(1-ethoxyethyl)purine has been observed to be rate-enhancing.[10]

The aim of the present study is to obtain a more detailed picture of the mechanism with which mercury(II) chloride influences the hydrolytic decomposition of purine nucleosides.

RESULTS AND DISCUSSION

As described earlier,[10–13] the acidic hydrolysis of 9-(1-ethoxyethyl)purines proceeds by a rapid initial protonation of the purine ring, giving a mono- and dication, followed by a rate-limiting heterolysis of these species to a purine base and an oxocarbenium ion derived from the 1-ethoxyethyl group (Scheme 1). The observed first-order rate



Scheme 1.

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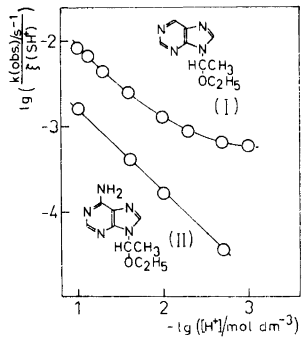


Fig. 1. The effect of the oxonium ion concentration on the hydrolysis of 9-(1-ethoxyethyl)purine (I) and -adenine (II) at the ionic strength of 0.20 mol dm^{-3} at 293.2 K. k(obs) stands for the observed first-order rate constant and $\xi(SH^+)$ the extent of protonation of the substrate. The values of $\xi(SH^+)$ refer to the protonation constants of 120 dm^3 mol^{-1} [10] and 6600 dm^3 mol^{-1} [11] for (I) and (II), respectively.

constant, k(obs), for this mechanism depends on $[H^+]$ according to eqn. (1) under conditions where the concentration of the dication SH_2^{2+}, is negligible compared to the sum of $[SH^+]+[S]$, *i.e.* unless the oxonium ion concentration is extremely high.[11] Consequently, k(obs) divided by the extent of protonation of the substrate, defined by eqn. (2), is proportional to $[H^+]$ when the reaction *via* the dication, SH_2^{2+}, prevails, and independent of $[H^+]$ when the decomposition of the monocation, SH^+, serves as the major reaction pathway (eqn. 3). Fig. 1 shows the graphs of the logarithm of the left-hand

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

$$\xi(SH^+) = \frac{[SH^+]}{[S(\text{tot})]} = \frac{K_1[H^+]}{K_1[H^+]+1} \quad (2)$$

$$\frac{k(\text{obs})}{\xi(SH^+)} = k_2K_2[H^+]+k_1 \quad (3)$$

member of eqn. (3) as a function of $-\lg([H^+]/\text{mol dm}^{-3})$ for 9-(1-ethoxyethyl)purine and -adenine at 293.2 K. With the adenine derivative the ratio of k(obs)/$\xi(SH^+)$ is proportional to the oxonium ion concentration throughout the acidity range studied, indicating the predominance of the reaction *via* the dication. Our previous measurements[11] at lower acidities and higher temperatures suggest that this mechanism becomes the main pathway when the oxonium ion concentration exceeds 2×10^{-4} mol dm^{-3}. With the less basic purine derivative the ratio of k(obs)/$\xi(SH^+)$ initially decreases with the decreasing acid concentration, but levels off to a

Table 1. First-order rate constants for the hydrolysis of 9-(1-ethoxyethyl)purine (I), 9-(1-ethoxyethyl)adenine (II), and 6-dimethylamino-9-(1-ethoxyethyl)purine (III) in acidic solutions of mercury(II) chloride at 293.2 K.[a]

$[H^+]$/mol dm^{-3}	$[HgCl_2]$/mol dm^{-3}	k(obs)/10^{-4} s^{-1} I	II	III
0.10	–	77.9(9)	15.9(2)	15.9(2)
	–	82.2(8)[b]		
	0.10	83.0(9)	15.5(2)	
	0.20	89.4(9)	15.3(2)	15.3(2)
	0.20	86.3(11)[b]		
0.010	–	7.02(7)	1.59(2)	1.79(4)
	0.050	9.01(12)		
	0.10	10.6(2)	1.64(2)	
	0.20	13.0(3)	1.69(2)	1.88(2)
0.0020	–	1.26	0.320(4)	0.350(4)
	0.050	2.22(6)	0.342(4)	
	0.10	2.66(5)	0.351(3)	
	0.20	3.46(4)	0.388(3)	0.420(2)
	0.20	1.22(2)[b]		

[a] The ionic strength was adjusted to 0.20 mol dm^{-3} with sodium perchlorate if not otherwise stated. [b] Sodium chloride (0.20 mol dm^{-3}) was added.

constant value at low acidities. The values of 5.4×10^{-4} s^{-1} and 7.9×10^{-2} dm^3 mol^{-1} s^{-1} for k_1 and k_2K_2, respectively, give the best fit with the experimental points. In other words, the reactions *via* the mono- and dications are of equal importance at the oxonium ion concentration of 7×10^{-3} mol dm^{-3}.

As seen from Table 1, addition of mercury(II) chloride in the reaction mixture does not markedly affect the hydrolysis of 9-(1-ethoxyethyl)adenine, proceeding by heterolysis of the diprotonated substrate. In contrast, the decomposition of 9-(1-ethoxyethyl)purine is considerably accelerated by this salt, the relative increment in the hydrolysis rate being decreased with the increasing oxonium ion concentration, *i.e.* as the proportion of the reaction *via* the monocation is diminished. Accordingly, it appears probable that mercury(II) chloride affects only the rate of the partial reaction *via* the monoprotonated substrate. The fact that 6-dimethylamino-9-(1-ethoxyethyl)purine responds to the changes in the composition of the reaction mixture similarly as the corresponding adenine derivative excludes the possibility that introduction of an additional coordination site, C6−NH_2, in the purine ring could be the reason for the observed differences in the behavior of 9-(1-ethoxyethyl)-purine and -adenine.

The rate constants in Table 1 also indicate that sodium chloride cancels the rate-enhancing effect of mercury(II) chloride. At high concentrations of the chloride ion mercury(II) chloride is converted to the chloro complexes, $HgCl_3^-$ and $HgCl_4^{2-}$,[14] and hence its complexing with the reactants and intermediates of the hydrolysis reactions is prevented. The finding that the rate-accelerations are simultaneously cancelled suggests that mercury(II) chloride interacts with either the initial or the transition state (or both) of the hydrolysis reaction by complexing. If common salt or cosolute effects were responsible for the increased hydrolysis rate no such cancellation is expected.

For a detailed analysis of the rate-enhancing effect of mercury(II) chloride, knowledge about the extent of protonation and complexation of the substrates under various conditions is required. However, determination of these quantities with 9-(1-ethoxyethyl)purines is extremely difficult owing to the large hydrolysis rates of these compounds and the small solubilities of their mercury(II) complexes. To avoid these complications we went on to investigate the corresponding equilibria with

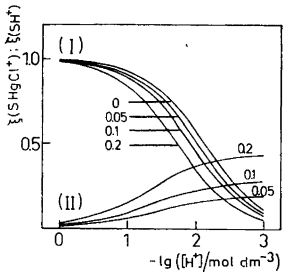


Fig. 2. The extent of protonation (I) and complexation (II) of 9-(β-D-ribofuranosyl)purine (10^{-3} mol dm^{-3}) in acidic solutions of mercury(II) chloride at 293.2 K. The ionic strength was adjusted to 0.2 mol dm^{-3} with sodium perchlorate. Each curve is labeled with the concentration of mercury(II) chloride (mol dm^{-3}) employed.

9-(β-D-ribofuranosyl)purines. The results obtained are presented in Fig. 2. Addition of 9-(β-D-ribofuranosyl)purine, for example, in solutions of mercury(II) chloride was observed to result in a release of chloride ions. Hence complexes of the form $SHgCl^+$ are presumably formed, analogously to complexing of mercury chloride with imidazole.[15] The values obtained for the extent of complexation by this method are compatible with the changes that mercury(II) chloride produces in the UV spectrum of 9-(β-D-ribofuranosyl)purine (Fig. 3). As seen from Fig. 2, the extent of complexation decreases with the increasing oxonium ion con-

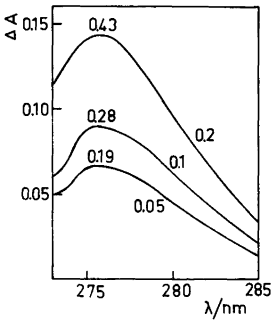


Fig. 3. The effect of mercury(II) chloride on the UV-spectrum of 9-(β-D-ribofuranosyl)purine (2×10^{-4} mol dm^{-3}) at the ionic strength of 0.2 mol dm^{-3} at 293.2 K. Each curve is labeled with the concentration of mercury(II) chloride (mol dm^{-3}) employed. The values at the top of the curves indicate the extent of protonation determined potentiometrically.

centration. Under highly acidic conditions, where the purine ring is completely protonated, no complex formation occurs. With adenosine no release of chloride ions was observed in the pH region 1 – 2. Accordingly, formation of the complexes of the type SH^+HgCl^+ may be neglected. Mild additional support for the suggestion that the protonated substrate does not markedly interact with mercury (II) chloride comes from the finding that the UV spectrum of the protonated 9-(β-D-ribofuranosyl)-purine remains unchanged in solutions of mercury-(II) chloride.

The values obtained potentiometrically for the extent of protonation of 9-(β-D-ribofuranosyl)purine at various concentrations of mercury(II) chloride are also included in Fig. 2. As expected, the competitive attachment of protons and metal ions to the purine ring reduces somewhat the extent of protonation. Since complexing of the protonated purine riboside can be neglected, as stated above, these values refer to the ratio of $[SH^+]/[S(tot)]$. Presumably the influence of mercury(II) chloride on the protonation of 9-(1-ethoxyethyl)purine is approximately the same as with the corresponding β-D-ribofuranosyl derivative. The data in Fig. 2 combined with the protonation constant (120 dm^3 mol^{-1} [10]) of 9-(1-ethoxyethyl)purine thus enable the estimation of the extent of protonation, $\xi(SH^+)$, for this compound under various conditions.

As stated in the foregoing, mercury(II) chloride does not markedly affect the hydrolysis rate of the dications of 9-(1-ethoxyethyl)purines. Accordingly, the portion of this partial reaction may be subtracted from the observed hydrolysis rate, when the extent of protonation of the substrate is known at different concentrations of mercury(II) chloride (eqn. 4). The remainder, k_1', represents the decom-

$$k_1' = \frac{k(\text{obs})}{\xi(SH^+)} - k_2K_2[H^+] \qquad (4)$$

position of the monoprotonated substrate in various solutions of mercury(II) chloride, since the hydrolysis of the complexed substrate, $SHgCl^+$, can with all likelihood be neglected.[9] The results for the hydrolysis of 9-(1-ethoxyethyl)purine are collected in Table 2. The values obtained for k_1' at different concentrations of mercury(II) chloride are approximately independent of $\xi(SH^+)$, as they should be if mercury(II) chloride really exerts its rate-enhancing effect on the cleavage of the monocation of the substrate. Since mercury(II) chloride does not markedly interact with protonated 9-(1-ethoxyethyl)purine, *i.e.* the initial state of the reaction, it seems likely that it appears in the transition state for the heterolysis of the monocation. Possibly mercury(II) chloride complexes with the incipient leaving group, the purine ring, and thus lowers the energy level of the transition state. This kind of mechanism operates in the solvolytic decompositions of alkyl halides, sulfides and disulfides.[16] In each case the rate-accelerating metal ion exhibits a great affinity for the leaving group. Since mercury(II) salts form stable complexes with aromatic nitrogen bases,[15,17] it does not seem impossible that similar interactions could promote the hy-

Table 2. First-order rate constants for the hydrolytic decomposition of the monocation of 9-(1-ethoxyethyl)purine in acidic solutions of mercury(II) chloride at 293.2 K.[a]

$[H^+]$/mol dm^{-3}	$[HgCl_2]$/mol dm^{-3}	$\xi(SH^+)$[b]	$k_1'/10^{-4}$ s^{-1}[c]
0.10	–	0.92	5.7
	0.10	0.88	15
	0.20	0.82	30
0.010	–	0.55	4.9
	0.050	0.49	11
	0.10	0.43	17
	0.20	0.32	33
0.0020	–	0.19	5.1
	0.050	0.16	12
	0.10	0.13	19
	0.20	0.086	39

[a] The ionic strength was adjusted to 0.20 mol dm^{-3} with sodium perchlorate. [b] $\xi(SH^+) = [SH^+]/[S(tot)]$. [c] Calculated by eqn. (4) from the rate constants, k(obs), given in Table 1.

drolysis of the monoprotonated 9-(1-ethoxyethyl)-purine. In the decomposition of the diprotonated substance the leaving group is the protonated purine ring, with which mercury(II) chloride obviously interacts much more weakly.

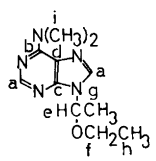
Mercury(II) salts also complex with purine nucleosides by displacing a proton at C8.[8] Under the acidic conditions employed in the present investigation the deprotonation of C8 is, however, so slow[18] that mercuration at this site can be neglected.

EXPERIMENTAL

Materials. The preparation of 9-(1-ethoxyethyl)-purine[10] and -adenine[11] has been described earlier. 6-Dimethylamino-9-(1-ethoxyethyl)purine was synthesized analogously. The product melted at 77–78 °C and exhibited the ^{1}H and ^{13}C NMR chemical signals reported in Table 3. The assignment of the compound as the N9 isomer can be made on the basis of the ^{13}C NMR data as described earlier.[11]

Adenosine and 9-(β-D-ribofuranosyl)purine were the products of Sigma Chemical Company, and they were used as received. The salts employed were of reagent grade.

Table 3. ^{1}H and ^{13}C NMR chemical shifts[a] for 6-dimethylamino-9-(1-ethoxyethyl)purine.



Position	δ(^{1}H)[b]	δ(^{13}C)[c]
a	s 8.04 (1H)	d 152.6 (153.2)[d]
	s 7.72 (1H)	d 135.6 (137.8)
b		s 155,0 (156,0)
c		s 150.5 (149.9)
d		s 119.9 (119.5)
e	q 5.90 (1H)	d 80.4 (80.7)
f	q 3.40 (2H)	t 64.5 (64.7)
g	d 1.72 (3H)	q 22.6 (22.6)
h	t 1.15 (3H)	q 14.9 (14.8)
i	s 3.48 (6H)	q 38.5

[a] Taken as ppm with respect to TMS. [b] In CCl_4. [c] In $CDCl_3$. [d] The values in parentheses refer to 9-(1-ethoxyethyl)adenine.[11]

Kinetic measurements. Kinetic measurements were performed as described earlier.[10]

Potentiometric measurements. The apparent protonation constants for 9-(β-D-ribofuranosyl)purine at different concentrations of mercury(II) chloride were determined by a modified potentiostatic technique described earlier.[19] The extent of protonation under various conditions were calculated from the results obtained. The extent of complexation of purine nucleosides with mercury(II) chloride was estimated by the following procedure. The appropriate salt solution (10 cm^3) was placed in a thermostated vessel equipped with an electrode sensitive to chloride ions (Orion 94–17A) and a calomel reference electrode connected through a salt bridge. The solution was agitated under nitrogen and a known amount of nucleoside (2×10^{-5} mol) was added. The amount of the chloride ions released was estimated by comparing the change in the meter reading to those produced by additions of known amounts of sodium chloride in the same solution in the absence of the nucleoside.

UV-spectrometric measurements. The UV spectra of 9-(β-D-ribofuranosyl)purine in solutions of mercury(II) chloride were recorded on a Cary 17D spectrophotometer.

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