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Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues. XI. * Competitive Pathways for the Acidic Hydrolysis of 9-(β-D-Ribofuranosyl)purine

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Kinetics and product distributions of the hydrolysis of 9-(β-D-ribofuranosyl)purine have been examined over a wide acidity range. At high oxonium ion concentrations rate-limiting departure of the mono- and diprotonated purinyl group with concomitant formation of a glycosyl oxocarbenium ion constitutes the major reaction pathway, analogous to the hydrolysis of 6-substituted purine nucleosides. In slightly acidic solutions opening of the imidazole ring of the base moiety prevails, and 4-amino-5-formamidopyrimidine, formed as a relatively stable intermediate, undergoes further hydrolysis to 4,5-diaminopyrimidine or cyclization to purine, the product composition depending on the acidity of the reaction solution. Rate constants for the partial reactions have been calculated and the mechanisms of the individual steps are discussed.

Several lines of evidence[1–12] suggest that the acidic hydrolysis of nucleosides generally involves a rapid initial protonation of the base moiety of the substrate, giving a mono- or dication, and a subsequent rate-limiting formation of a cyclic glycosyl oxocarbenium ion. Accordingly, intact purine or pyrimidine bases are released as initial reaction products, though further degradation in acidic solutions may occur.[1,13] For example, the hydrolysis of several 6-substituted 9-(β-D-ribofuranosyl)purines have been shown to yield corresponding purines over a wide acidity range.[1] This is also the situation with the unsubstituted 9-(β-D-ribofuranosyl)purine in concentrated acid solutions, whereas at low oxonium ion concentrations 4-amino-5-formamidopyrimidine appears to be the first isolable product.[1] The aim of the present study is to obtain more quantitative information about the competition of these two routes, and to elucidate the mechanisms of the partial reactions involved.

RESULTS AND DISCUSSION

Fig. 1 shows the pH-rate profile for the disappearance of 9-(β-D-ribofuranosyl)purine under acidic conditions. As described earlier,[1] the observed first-order rate constant is proportional to the oxonium ion concentration in highly acidic solutions, but exhibits a marked positive deviation at low acid concentrations. Examination of the product mixtures by LC, and ^{1}H NMR and UV spectroscopy indicated purine and 4,5-diaminopyrimidine to be the only stable products, besides D-ribose, in the whole acidity range studied. The results are given in Fig. 1 in terms of the mole fraction of purine. The proportion of the intact purine initially decreases with the decreasing oxonium ion concentration, goes through a minimum at $[H^+]=10^{-3}$ mol dm^{-3}, and begins to increase thereafter. In order to rationalize this kind of product distribution the hydrolysis of 4-amino-5-formamidopyrimidine, shown earlier to be formed at low acid concentrations,[1] is considered in the following. The pH-rate profile for the hydrolysis of 4-amino-5-formamidopyrimidine is presented in Fig. 2. In highly acidic solutions the observed

* Part X, see Ref. 1.

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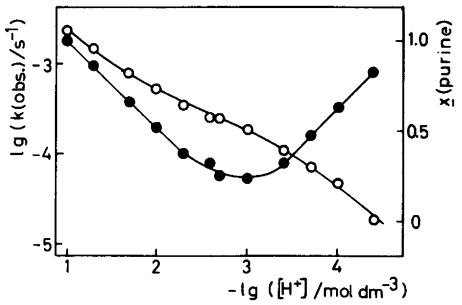


Fig. 1. pH-rate profile for the hydrolysis of 9-(β-D-ribofuranosyl)purine at 363.2 K (○), and the mole fractions of free purine formed (●). Zero mole fraction of purine corresponds to the formation of 4,5-diaminopyrimidine. The ionic strength adjusted to 0.10 mol dm^{-3} with sodium perchlorate.

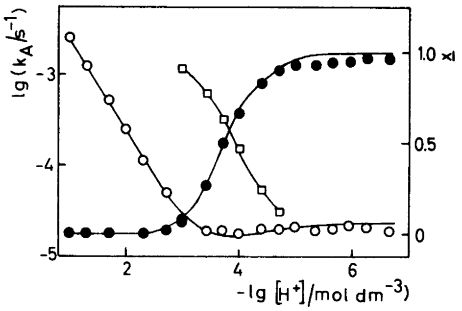
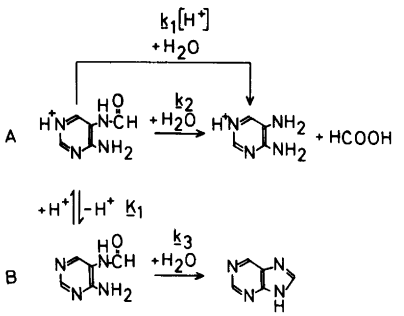


Fig. 2. pH-rate profile for the hydrolysis of 4-amino-5-formamidopyrimidine at 363.2 K (○), the extent of protonation of the starting material (□), and the mole fractions of free purine formed (●). Zero mole fraction of purine corresponds to the formation of 4,5-diaminopyrimidine. The ionic strength adjusted to 0.10 mol dm^{-3} with sodium perchlorate.

first-order rate constant is linearly related to the oxonium ion concentration, but becomes completely pH-independent at low acidities. Fig. 2 also includes the data on the protonation of the starting material under the conditions of the kinetic measurements, and the compositions of the product mixtures in terms of the mole fraction of purine formed. Besides purine the only detected product was 4,5-diaminopyrimidine. Comparison of the curves in Fig. 2 reveals that protonated 4-amino-5-formamidopyrimidine yields 4,5-diaminopyrimidine, while the neutral substrate is converted to purine. The preceding observations can well be accounted for by the reaction pathways depicted in Scheme 1. The monocation of the substrate is decomposed to 4,5-diaminopyrimidine either unimolecularly or after rapid initial formation of the dication (Route A). The sites of protonation have been



Scheme 1.

assigned tentatively. Alternatively, the neutral substrate undergoes unimolecular cyclization to purine. The rate-law obeyed can thus be expressed by eqn. (1), where [S(tot.)]

$$-\frac{d[S(tot.)]}{dt} = k_1[H^+][SH^+]+k_2[SH^+]+k_3[S]= \frac{k_1[H^+]^2+k_2[H^+]+k_3K_1}{K_1+[H^+]}[S(tot.)] \quad (1)$$

stands for the sum of [S] and [SH^+]. The constants K_1, k_1, k_2 and k_3 are defined in Scheme 1. Substitution of the spectrophotometrically determined acidity constant, $K_1=1.15\times10^{-4}$ mol dm^{-3}, in eqn. (2) enables the calculation of k_1, k_2 and k_3 by least-squares fitting. In eqn. (2) k_A is the first-order rate constant observed for the disappearance of 4-amino-5-formamidopyrimidine.

$$k_A = \frac{k_1[H^+]^2+k_2[H^+]+k_3K_1}{K_1+[H^+]} \quad (2)$$

The constants obtained, *viz.* $k_1=2.50\times10^{-2}$ dm^3 mol^{-1} s^{-1}, $k_2=8\times10^{-6}$ s^{-1} and $k_3=2.2\times10^{-5}$ s^{-1}, give the curve of lg k_A/s^{-1} *vs.* $-$lg [H^+] drawn in Fig. 2. Substitution of the same values in eqn. (3) gives the mole fraction of purine, x_1, as a function of oxonium ion concentration.

$$x_1 = \frac{k_3 K_1}{k_1[H^+]^2+k_2[H^+]+k_3K_1} \quad (3)$$

Table 1. First-order rate constants at different temperatures, and the entropies of activation for the hydrolysis of 4-amino-5-formamidopyrimidine (I) and 9-(β-D-ribofuranosyl)purine (II) in aqueous perchloric acid.

Compound	$[H^+]$/mol dm^{-3}	T/K	$k/10^{-4}$ s^{-1}	$\Delta S^{\neq}$/J K^{-1} mol^{-1} [a]
I	0.10	363.2	25.1 ±0.2	−102±10 [b]
	0.10	358.2	17.1 0.2	
	0.10	353.2	14.2 0.3	
	0.10	348.2	9.84 0.20	
	0.10	343.2	6.75 0.09	
II	0.10	363.2	24.0 ±0.2	11±10 [b,c]
	0.10	358.2	13.8 0.1	
	0.10	353.2	7.82 0.06	
	0.10	348.2	5.04 0.06	
	0.10	343.2	2.97 0.05	
	0.0010 [d]	363.2	2.73 ±0.03	−109± 7 [e]
	0.0010	358.2	1.93 0.03	
	0.0010	353.2	1.42 0.02	
	0.0010	348.2	0.949 0.017	
	0.0010	343.2	0.609 0.012	

[a] At 298.15 K. [b] Refers to the second-order rate constants. [c] In lit.[8] 6 J K^{-1} mol^{-1}. [d] The ionic strength adjusted to 0.10 mol dm^{-3} with sodium perchlorate. [e] Refers to the first-order rate constants.

As seen from Fig. 2, the experimental points fall fairly well on the calculated curve over the whole acidity range studied.

The preceding data indicate that in 0.1 mol dm^{-3} perchloric acid the reaction *via* the dication of 4-amino-5-formamidopyrimidine prevails. Under these conditions the entropy of activation for the hydrolysis reaction is strongly negative, −(102±10) J K^{-1} mol^{-1} (Table 1). Accordingly, it appears reasonable to assume that the rapid initial formation of the dication is followed by rate-limiting nucleophilic attack of a molecule of water at the carbonyl carbon, either prior to or concerted with the departure of the formic acid. On the basis of the available data it is impossible to deduce definitely whether the participation of water as a nucleophile occurs in the decomposition of the monocationic substrate.

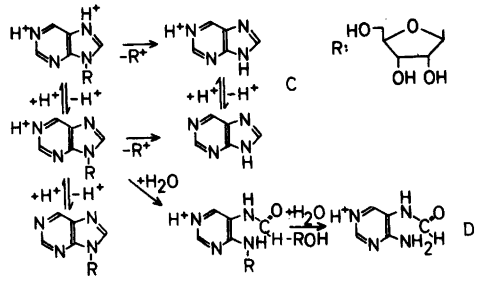
The most attractive mechanism for the decomposition of the neutral substrate is the intramolecular nucleophilic attack of the 4-amino group on the carbonyl carbon with concomitant loss of a molecule of water (Route B in Scheme 1). The carboxylic acid and triethanolamine buffers employed did not markedly affect the rate of this reaction. However, in phosphate buffers the disappearance of 4-amino-5-formamidopyrimidine was accelerated and 4,5-diaminopyrimidine was formed besides purine (Table 2). Possibly hydrogen phosphate dianion acts as an intermolecular nucleophilic catalyst in the cleavage of the *N*-formyl group. For comparison, it should be noted that 2,4-diaminopyrimidine has been suggested to form ion pairs with dihydrogen phosphate anion in aqueous solution.[14]

Table 2. First-order rate constants for the hydrolysis of 4-amino-5-formamidopyrimidine in $H_2PO_4^-/HPO_4^{2-}$-buffers [a] at 363.2 K, and the mole fractions of purine formed.

$[HPO_4^{2-}]$/mol dm^{-3}	$k_{obs}/10^{-5}$ s^{-1}	x(purine)
0 [b]	1.98±0.02	0.96
0.0025	2.28±0.03	0.60
0.0050	3.31±0.07	0.51
0.010	4.00±0.09	0.43
0.025	5.22±0.11	0.37

[a] The buffer ratio 1:1. The ionic strength adjusted to 0.1 mol dm^{-3} with sodium perchlorate. [b] The oxonium ion concentration adjusted with a triethanolamine buffer.

The influences that variations of the oxonium ion concentration exert on the kinetics and product compositions of the hydrolysis of 9-(β-D-ribofuranosyl)purine (Fig. 1) can be accounted



Scheme 2.

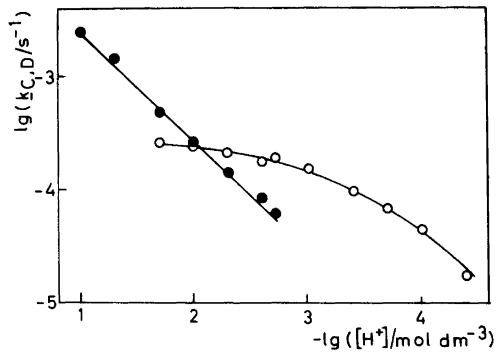


Fig. 3. pH-rate profiles for the decomposition of 9-(β-D-ribofuranosyl)purine by Routes C (●) and D (○) depicted in Scheme 2. The data refer to 363.2 K and the ionic strength of 0.10 mol dm^{-3}.

for by the two competitive pathways depicted in Scheme 2. Either rapid initial formation of mono- and dications of the substrate is followed by unimolecular rate-limiting formation of a cyclic glycosyl oxocarbenium ion (Route C), analogous to the hydrolysis of 6-substituted purine nucleosides, or opening of the imidazole ring of the base moiety leads to formation of 4-amino-5-formamidopyrimidine, the cleavage of which occurs as described above (Route D). At $[H^+] \geq 0.10$ mol dm^{-3} purine is the only detectable product, indicating that Route C is utilized. On going to less acidic solutions the proportion of 4,5-diaminopyrimidine, the hydrolysis product of Route D at $[H^+] > 2\times10^{-3}$ mol dm^{-3}, is gradually increased. Accordingly, Route D becomes the major pathway. At $[H^+] < 10^{-3}$ mol dm^{-3} the mole fraction of 4,5-diaminopyrimidine begins to diminish again, since Route D at low oxonium ion concentrations yield purine besides 4,5-diaminopyrimidine.

The first-order rate constants for the decomposition of 9-(β-D-ribofuranosyl)purine by Routes C and D can be expressed at any given oxonium ion concentration by eqns. (4) and (5), respectively.

$$k_C = (1-x_D)k(\text{obs}) \quad (4)$$

$$k_D = x_D k(\text{obs.}) \quad (5)$$

Here k(obs) is the observed first-order rate constant and x_D is the mole fraction of the products produced by Route D. Since 4,5-diaminopyrimidine is formed by Route D only, x_D can be calculated *via* eqn. (6), where x_2 is the mole fraction of 4,5-diaminopyrimidine

$$x_D = \frac{k_1[H^+]^2 + k_2[H^+] + k_3K_1}{k_1[H^+]^2 + k_2[H^+]}\, x_2 \quad (6)$$

formed in the hydrolysis of 9-(β-D-ribofuranosyl)purine. Substitution of eqn. (6) in eqns. (4) and (5) enables calculation of the partial rate constants k_C and k_D.

Fig. 3 shows the values obtained for k_C and k_D by the method described above. k_C continuously increases with the increasing oxonium ion concentration, analogous to the hydrolysis of 6-substituted purine nucleosides.[1] In contrast, k_D levels off to a constant value in solutions where the starting material is present mainly as its monocation. At $[H^+] = 1.0\times10^{-3}$ ml dm^{-3}, *i.e.* under conditions where route D prevails, the entropy of activation for the hydrolysis of 9-(β-D-ribofuranosyl)purine is strongly negative in striking contrast to the slightly positive value that Route C exhibits (Table 1). Accordingly, the rate-limiting step of the reaction leading to formation of 4-amino-5-formamidopyrimidine appears to be bimolecular. The most attractive mechanism involves a nucleophilic attack of a molecule of water at C8 of the protonated substrate. Opening of the imidazole ring results in cleavage of the glycosyl-nitrogen bond with concomitant release of 4-amino-5-formamidopyrimidine. At $[H^+] < 10^{-3}$ mol dm^{-3} the latter compound is the first product detectable by LC. In more acidic solutions an additional intermediate preceding 4-amino-5-formamidopyrimidine is stable enough to be observed, but its concentra-

tion remains low during all stages of the hydrolysis reaction. The structure of this intermediate is unknown, but the tentative suggestion given in Scheme 2 seems the most attractive alternative.

In summary, the monocation of 9-(β-D-ribofuranosyl)purine appears to be more susceptible to the nucleophilic attack of water than protonated 6-substituted purine nucleosides. For comparison, a similar difference has been observed in the alkaline cleavage of 9-(1-ethoxyethyl)purines, the nucleophilic attack of hydroxide ion on the unsubstituted purine ring being more rapid than expected on the basis of the reactivity-basicity correlation determined for the 6-substituted derivatives.[15]

EXPERIMENTAL

Purine, 4,5-diaminopyrimidine and 9-(β-D-ribofuranosyl)purine were products of Sigma Chemical Company, and they were employed as received. 4-Amino-5-formamidopyrimidine (m.p. 182–183 °C, lit.[16] 183–185 °C) was prepared by refluxing 4,5-diaminopyrimidine in formic acid and crystallizing the neutralized product from ethanol.[16] The hydrolyses of 9-(β-D-ribofuranosyl)purine and 4-amino-5-formamidopyrimidine were carried out as described previously.[1] The compositions of the cooled aliquots were analyzed by reversed phase LC (Varian Aerograph 5020 Liquid Chromatograph) using a commercial Micropak MCH-5 column and a variable wavelength UV detector (Varian UV-100). Elution was performed with an acetic acid buffer (0.02 mol dm^{-3}, buffer ratio 1:1) containing 12 % (v/v) acetonitrile. The flow rate was 0.8 cm^3 min^{-1} throughout. The signals were assigned on the basis of retention times and UV-spectra. The heights of the peaks were calibrated by comparing with authentic samples of known concentrations.

The concentrations of the reaction components were also determined by multilinear regression from the UV spectra of the alkalized aliquots (Cary D17), employing the absorbances measured from 230 to 320 nm at 5 nm intervals. The results were consistent with those obtained by LC.

The compositions of the product mixtures were ascertained by following the progress of the hydrolysis reactions in D_2O by ^1H NMR spectroscopy (Jeol JNM-PMX 60) as described earlier.[1]

The oxonium ion concentrations of the buffer solutions at 363.2 K, namely formate, acetate, phosphate and triethanolamine buffers, were calculated on the basis of the data in literature.[17]

The acidity constant, K_1, for the conjugate acid of 4-amino-5-formamidopyrimidine was determined spectrophotometrically at 363.2 K (Unicam SP 800 spectrophotometer) at the ionic strength of 0.10 mol dm^{-3} (NaCl) by the method described earlier.[18]

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