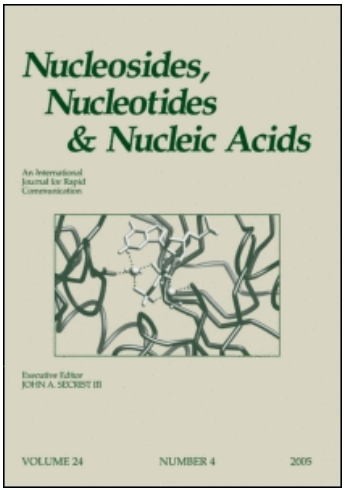

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Acid-Catalyzed Hydrolysis of 2-Amino-9-(α-D-Ribofuranosyl)-purine and its Acyclo and 2-Methyl Analogues: Competition Between Depurination and Opening of the Imidazole Ring

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ACID-CATALYZED HYDROLYSIS OF 2-AMINO-9-(β-D-RIBOFURANOSYL)-PURINE AND ITS ACYCLO AND 2-METHYL ANALOGUES: COMPETITION BETWEEN DEPURINATION AND OPENING OF THE IMIDAZOLE RING.

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Abstract. Hydrolytic decomposition of 2-amino- and 2-methyl-9-(β-D-ribofuranosyl)purines, 2-methyladenosine, 2-amino-7- and -9-(2-hydroxyethoxymethyl)purines and 9-ethoxymethyl-purine has been followed by HPLC under acidic conditions. 2-Methyladenosine undergoes depurination over the entire acidity range studied (pH 4.7 to H_0 -0.7). The other compounds are depurinated only at high hydronium ion concentrations, while under mildly acidic conditions the imidazole ring is opened to give 2-substituted 4-amino-5-formamidopyrimidine. The latter compound finally undergoes competitive deformylation to 4,5-diaminopyrimidine and cyclization to 2-substituted purine. The mechanism of the imidazole ring opening is discussed on the basis of the pH-rate profiles and structural effects.

It is widely accepted that purine nucleosides[1] and some of their isosteric analogues, such as benzimidazole and indazole nucleosides,[2,3] are hydrolyzed under acidic conditions by a mechanism involving a rapid initial protonation of the base moiety and a subsequent rate-limiting heterolysis to the free base and a resonance stabilized glycosyl carbonium ion. The same mechanism has also been proposed for hydrolysis of a hypermodified purine nucleoside, wyosine.[4] However, some exceptions to this normal behavior of purine nucleosides are known. 7-Deaza-2'-deoxyadenosine has been shown to undergo anomerization to pyranoid and α-furanoid derivatives concurrent with acid hydrolysis, which strongly suggests

that hydrolysis proceeds via a cationic acyclic Schiff base, and not via a glycosyl oxocarbenium ion.[5] One may speculate that the π-electron delocalization at N9 is less efficient with this compound than with unmodified purine nucleosides, and hence it reacts like a glycosylamine,[6] and not like a nucleoside. It is more difficult to explain why unsubstituted 9-(β-D-ribofuranosyl)purine under mildly acidic conditions (pH > 2) undergoes opening of the imidazole ring instead of depurination, which prevails in more acidic solutions.[7,8] With the corresponding 2'-deoxyribonucleoside this anomalous behavior could not be detected at pH < 4,[1] most probably due to the fact that deoxyribosides are depurinated almost 3 orders of magnitude faster than ribonucleosides. It is worth noting that none of the 6-substituted purine nucleosides studied, including amino, dimethylamino, methoxy and chloro derivatives, exhibits this kind of behavior.[7] 2-Amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine has more recently been shown to undergo imidazole ring-opening at a higher pH range (5.8 < pH < 7.2), although 2'-deoxyadenosine is depurinated under the same conditions.[9] The latter finding suggests that the exceptional mechanism established for the acid-catalyzed hydrolysis of the unsubstituted purine riboside is not limited to this compound, but may be general for all purine nucleosides with no substituent at C6. To ascertain this, and to elucidate the factors that result in the change in mechanism, hydrolysis of 2-amino- and 2-methyl-9-(β-D-ribofuranosyl)purines, 2-methyladenosine, 2-amino-7- and -9-(2-hydroxyethoxymethyl)purines and 9-ethoxymethylpurine was studied over a wide acidity range. Detailed understanding of the hydrolytic behavior of 2-aminopurine derivatives is also of interest in view of the known mutagenic action of this nucleobase.[10]

RESULTS AND DISCUSSION

pH-Rate profiles and product distribution. FIG. 1 and 2 show the pH-rate profiles and product distributions determined by

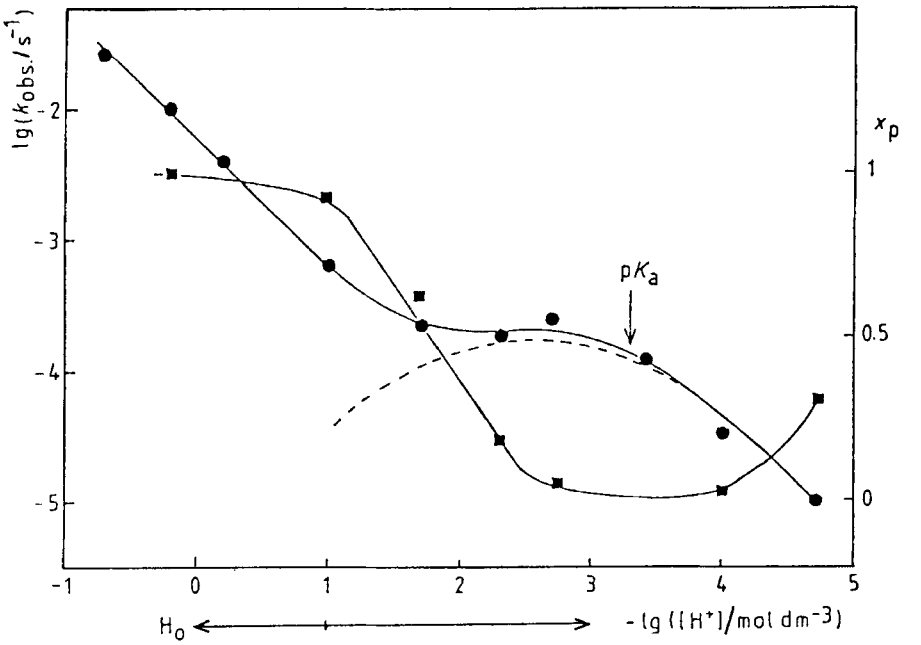


FIG. 1: pH-Rate profile (●) and product distribution (■) for the hydrolytic decomposition of 2-amino-9-(β-D-ribofuranosyl)purine (1b) at 363.2 K (I = 0.1 M with NaCl). The mole fraction, x, stands for 2-aminopurine. x = 0 corresponds to formation of 2,4,5-triaminopyrimidine (3b).

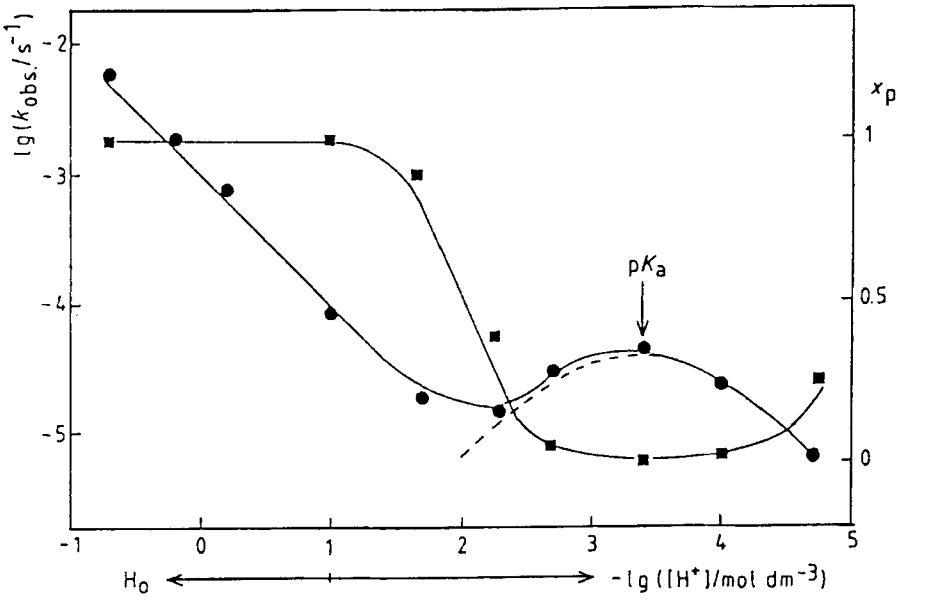
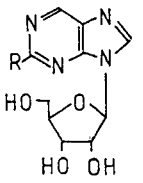
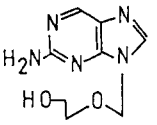


FIG. 2: pH-Rate profile (●) and product distribution (■) for the hydrolytic decomposition of 2-amino-9-(2-hydroxyethoxymethyl)purine (2). For the experimental conditions and definition of x see the legend of FIG. 1.

HPLC for hydrolytic decomposition of 2-amino-9-(β-D-ribofuranosyl)purine (1b) and the corresponding acyclo nucleoside, 2-amino-9-(2-hydroxyethoxymethyl)purine (2). Both compounds undergo depurination at high hydronium ion



1a: R=H
1b: R=NH_2
1c: R=CH_3



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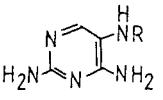


3a: R=CHO
3b: R=H

concentrations (pH < 1), but under less acidic conditions the major product is 2,4-diamino-5-formamidopyrimidine (3a). 3a then undergoes acid-catalyzed hydrolysis to 2,4,5-triaminopyrimidine (3b), with which spontaneous intramolecular cyclization to 2-aminopurine starts to compete at pH pK_a (FIG. 3). The latter reaction is in all likelihood responsible for the increment of the mole fraction of 2-aminopurine in the product mixture of 1b and 2 at low hydronium ion concentrations.The mechanisms of deformylation and recyclization of 4-amino-5-formamidopyrimidine have been discussed earlier.[8] HPLC analyses showed that 3a is formed from the acyclo nucleoside, 2, without appearance of any detectably stable intermediate, whereas the conversion of 2-aminopurine riboside, 1b, to 3a proceeds by intermediary formation of an equilibrium mixture of four unstable compounds, which were UV spectroscopically similar to 3a. When the decomposition of 1b was followed by ^1H NMR in D_2O, the disappearance of the anomeric proton signal at 5.98 (ppm from external TMS at pD 3) was accompanied by appearance of four new anomeric doublets at 5.72, 5.54, 5.26 and 5.16, and two broad singlets at 7.76 and 8.28. The shifts of the latter signals closely resembled those of the H6 and N^5-CHO resonances of 3a, and hence these intermediates were tentatively assigned as anomeric N^4-ribofuranosyl and N^4-ribopyranosyl derivatives of 2,4-di-amino-5-formamidopyrimi-

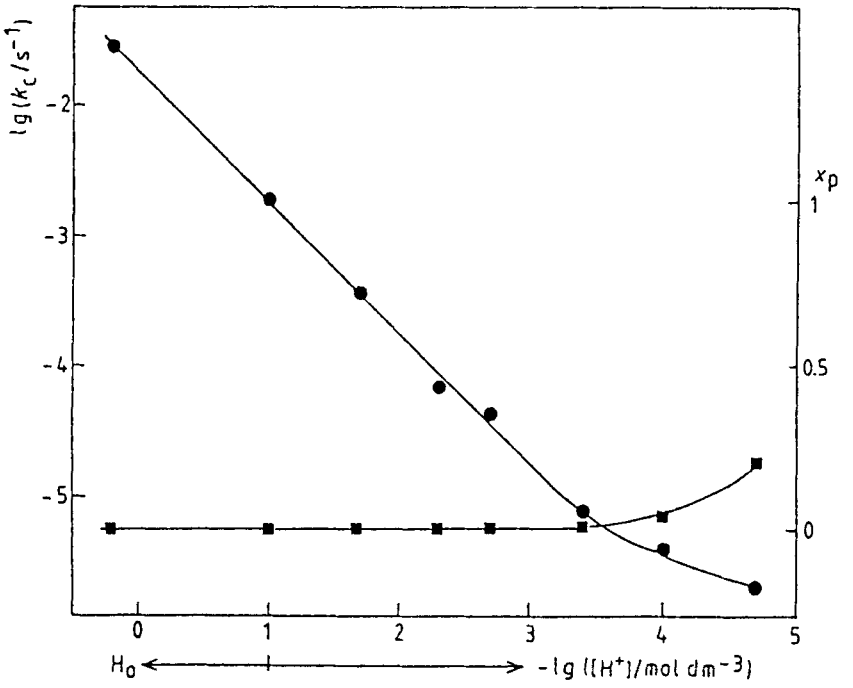
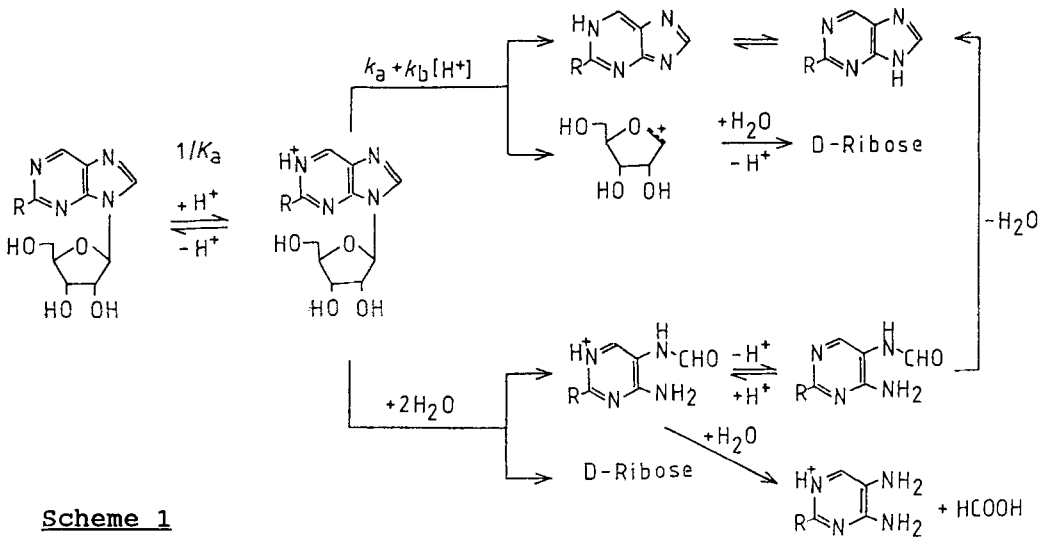


FIG. 3: pH-Rate profile (●) and product distribution (■) for the hydrolysis and cyclization of 2,4-diamino-5-formamidopyrimidine (3). For the experimental conditions and definition of x see the legend of FIG. 1.

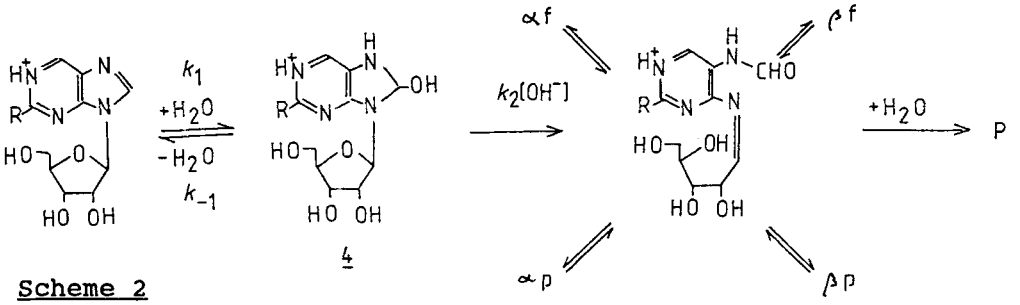
dine. Accordingly, the mechanism presented previously[8] for the unsubstituted 9-(β-D-ribofuranosyl)purine, 1a, (Scheme 1) also applies to the hydrolysis of 2-aminopurine riboside and its acyclic analogue, although adenosine, the corresponding 6-aminopurine riboside, undergoes depurination over the entire acidity range studied.[7] Protonation of both 1a and adenosine at N1 has been established by ^{15}N NMR spectroscopy,[1,11-13] and in all likelihood N1 protonation also prevails with 1b and 2, as evidenced by IR spectroscopy in aqueous solution[14] and by ^{1}H NMR spectroscopic studies on base-pairing of 2-aminopurine residue in oligodeoxynucleotides.[15]

We have suggested previously[8] that opening of the imidazole ring is initiated by rate-limiting nucleophilic



Scheme 1

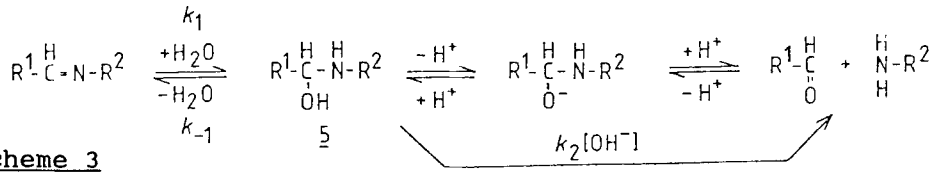
attack of a molecule of water at the C8 atom of the N1 protonated purine ring (Scheme 2). Accordingly, the rate of this reaction would become pH-independent at pH < pK_a, which seemed to be the case with unsubstituted 9-(β-D-ribofuranosyl)purine, 1a. By contrast, the hydrolytic decomposition of 2 shows an inverse dependence of rate on acidity at 2.2 < pH < 3.5. This behavior, which cannot be accounted



Scheme 2

for by a rate-limiting attack of water on the protonated substrate, is characteristic for the hydrolysis of Schiff bases (Scheme 3) and refers to a change in the rate-limiting step.[16] It has been well established that formation of the carbinolamine intermediate (5) is the slow step at low

hydronium ion concentrations ($k_2[OH^-] > k_{-1}$), but in highly acidic solutions its decomposition becomes rate-limiting ($k_{-1} > k_2[OH^-]$). Since the latter partial reaction proceeds



Scheme 3

by abstraction of a proton from the hydroxyl group, it is base-catalyzed, and hence an inverse dependence of rate on acidity is observed. Most probably the same mechanism may be applied to the imidazole ring opening. At low acidities, attack of water at the C8 atom of the protonated purine ring is rate-limiting, as suggested previously,[8] while under sufficiently acidic conditions decomposition of the resulting 7,8-covalent hydrate (4) may become the slow step. This change in the rate-limiting step is clearly detected with 2, and most probably the situation is analogous with 1a and 1b, though depurination becomes the predominant reaction under conditions where the inverse dependence of rate on acidity could otherwise be detected.

FIG. 4 shows the kinetic data for the hydrolytic decomposition of 2-methyl-9-(β-D-ribofuranosyl)purine (1c). The pH-rate profile and the product distribution both closely resemble those of the corresponding amino derivative, suggesting that the reactions of 1b and 1c are mechanistically similar.

Kinetics of partial reactions. By applying the steady-state approximation to intermediate 4 in Scheme 2, the first-order rate constant, k_{ro}, for the opening of the imidazole ring may be expressed by eqn. (1). Here K_a is the acidity constant of the substrate monocation, K_w is the ionic product of water under the experimental conditions ($7.2\ 10^{-13}\ M^2$

$$k_{ro} = \frac{k_1 K_w}{(k_{-1}/k_2 + K_w/[H^+])(K_a + [H^+])} \qquad (1)$$

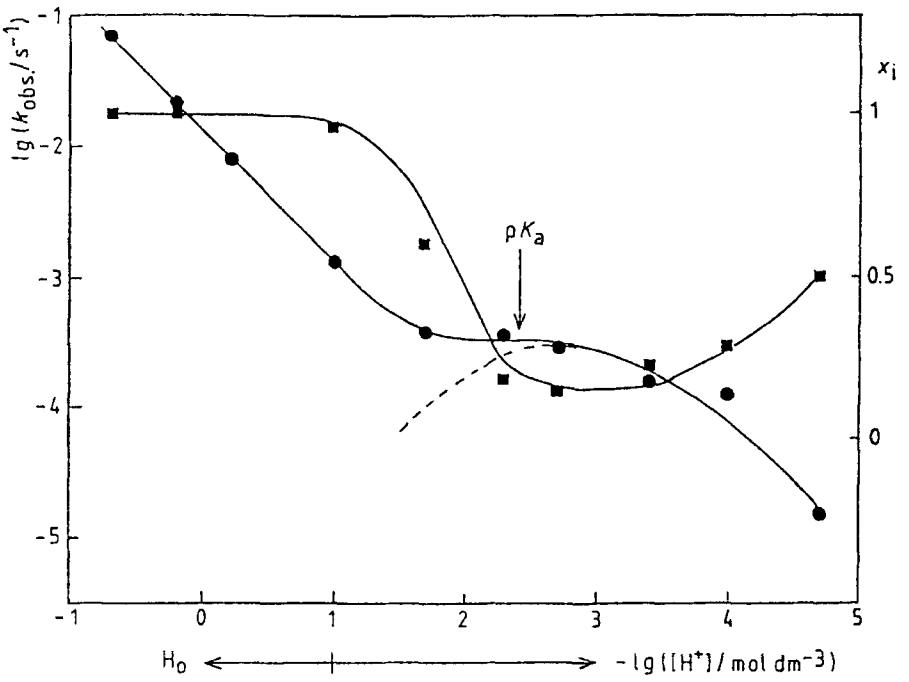


FIG. 4: pH-Rate profile (●) and product distribution (■) for the hydrolytic decomposition of 2-methyl-9-(β-D-ribofuranosyl)purine (1c). For the experimental conditions and definition of x see the legend of FIG. 1.

at 363.2 K, I = 0.1 M)[17,18], and the partial rate constants are those indicated in Scheme 1.

The observed first-order rate constant, k_{dp}, of depurination may, in turn, be expressed by eqn. (2),[7] where the partial rate constants k_a and k_b refer to the spontaneous and oxonium ion catalyzed heterolysis of the substrate monocation, respectively. Depurination is significant compared to imidazole ring-opening only at $[H^+] >> K_a$. Under these conditions eqn. (2) is reduced to eqn. (3). The first-order rate constant, $k_{obs.}$, observed for the disappearance of the starting material, is the sum of k_{ro} and k_{dp} (eqn. 4). The values obtained by least-squares

$$k_{dp} = \frac{(k_a + k_b[H^+])[H^+]}{K_a + [H^+]} \qquad (2)$$

$$k_{dp} = k_a + k_b[H^+] \sim k_b[H^+] \quad (3)$$

$$k_{obs.} = k_{ro} + k_{dp} \quad (4)$$

fitting[19] for k_1, k_{-1}/k_2 and k_3 are listed in TABLE 1, and the fitted lines are presented in FIG. 1, 2 and 4.

Structural effects. Comparison of the values of k_1 in TABLE 1 reveals that insertion of a methyl or amino group at C2 of unsubstituted purine riboside retards the nucleophilic attack of water on the protonated substrate by a factor of 3 and 30, respectively. This is expected, since these substituents are electron-donating, and hence increase the electron density at C8. However, the basicity of the purine ring, and thus the pre-equilibrium concentration of the protonated substrate, is simultaneously increased. Owing to partial cancellation of these two opposing effects, the influence on the observed rate constant k_{ro} for imidazole ring opening remains low. The rate constants representing the maxima of the bell-shaped pH-rate profiles of 1a, 1b and 1c are 2.0 10^{-4} s^{-1}, 1.6 10^{-4} s^{-1} and 3.2 10^{-4} s^{-1}, respectively (Ref. 8 and FIG. 1 and 4). For the same reason the C2 substituent has only a moderate effect on the rate of depurination (TABLE 1); electropositive methyl and amino groups increase the basicity of the leaving group, but

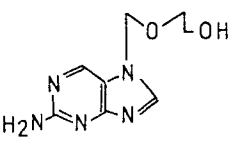
TABLE 1: Partial rate constants for the hydrolytic decomposition of 2-substituted 9-(β-D-ribofuranosyl)purines and their acyclic analogues at 363.2 K.[a]

Compd.	pK_a	k_1 / 10^{-3} s^{-1}	k_{-1}/k_2 / M	k_b / 10^{-3} M^{-1} s^{-1}
1a[b]	1.7	9.5	1.3 10^{-9}	27
1b	3.3	0.29	1.3 10^{-10}	5.8
1c	2.4	3.3	1.0 10^{-9}	13
2	3.4	0.14	1.5 10^{-9}	1.0
8	1.7	5.8	8.7 10^{-9}	14

[a]Ionic strength adjusted to 0.1 M with NaCl. For the partial rate constants see Scheme 2 and eqn. 2. [b]Calculated from the data in Ref. 8.

simultaneously the electron density at N9 is increased, and the heterolysis step thus retarded. Accordingly, the competition between depurination and imidazole ring-opening is rather insensitive to the polar nature of the C2 substituent.

2-Amino-7-(2-hydroxyethoxymethyl)purine (6) is depurinated 50 times as fast as its 9-isomer (2), the second order rate constant, k_b, being 4.25 10^{-2} M^{-1} s^{-1} at 363.2 K (I = 0.1 M). The imidazole ring opening becomes detectable only



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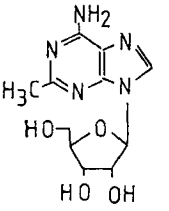

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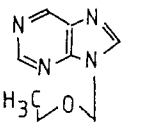

8

at pH > 3.5, and even then it remains as a minor side reaction. At pH 4 the first-order rate constant for the disappearance of 6 is 1.4 10^{-5} s^{-1}, and about one third of the reaction proceeds by opening of the imidazole ring. Comparison of the latter finding with the data in FIG. 2 indicates that the imidazole ring opening of 6 is slower than that of 2, but still of the same order of magnitude. In other words, imidazole ring opening competes with depurination also with 7-substituted analogues of purine nucleosides, as long as C6 remains unsubstituted. Some further evidence for the suggestion that substitution at C6 plays a decisive role in determining the mechanism of the hydrolytic decomposition comes from studies on 2-methyladenosine (7). While 2-methyl-9-(β-D-ribofuranosyl)purine undergo imidazole ring opening at low hydronium ion concentrations, its 6-amino derivative is depurinated over the entire acidity range studied, i. e. from pH 3.5 to H_o - 0.7. The first-order rate constant is strictly proportional to hydronium ion concentration, depurination being 2.5 times as fast as that of adenosine.[7] It is also clear that the 5'-hydroxyl group of 6-unsubstituted purine ribonucleosides,

or the 2-hydroxy group of the ethoxymethyl moiety of their acyclo analogues, do not participate as nucleophiles in opening of the imidazole ring. 9-(1-Ethoxymethyl)purine (8), with no hydroxyl functions in the carbonyl moiety, behaves like its ribofuranosyl counterpart, depurination prevailing at pH < pK_a and imidazole ring opening at pH > pK_a. The rates of both reactions are considerably smaller than those of the corresponding riboside (1a).[8] The values obtained for the partial rate constants are included in TABLE 1.

Conclusions. In summary, purine ribonucleosides bearing no substituent at C6 are hydrolyzed at low hydronium ion concentrations by opening of the imidazole ring, irrespective of whether C2 is substituted or not, whereas 6-substituted and 2,6-disubstituted purine ribosides are depurinated under these conditions. It is, however, difficult to explain why transfer of a substituent from C6 to C2 results in such a mechanistic change. The basicities of 2- and 6-aminopurine ribosides, for example, are almost identical (pK_a 3.7 at 298.2 K), and replacement of C8H by deuterium was observed to take place at comparable rates with both nucleosides, the first-order rate constants obtained in an acetate buffer (pD 5.4, T=363 K) being 5 10^{-4} s^{-1} and 3 10^{-4} s^{-1} for 1b and adenosine, respectively. As mentioned above, the site of protonation is most probably the same with 1a, 1b and adenosine. These comparisons of electronic properties of the base moieties naturally applies to the ground state only. The relative basicities of the nitrogen atoms of 2- and 6-substituted purine residues may be different in the transition state. It is also noteworthy that at high hydronium ion concentrations both 1b and adenosine are depurinated at comparable rates. In other words, diprotonated 2-aminopurine is as good a leaving-group as diprotonated adenine, and most likely the same applies to the monoprotonated species. The question that remains to be answered is why 1b, as well as 1a and 1c, undergo at low hydronium ion concentrations imidazole ring opening, which is much faster than the depurination of adenosine. One might

speculate that the ring-opening is preceded by a preequilibrium process at unsubstituted C6, which markedly enhances the susceptibility of C8 to nucleophilic attack. A possible reaction could be hydration of the 1,6-double bond. Wolfenden et al.[20] have shown that 1a equilibrates with a 1,6-covalent hydrate, although the concentration of the latter species remains low. It is also known[21] that purine and 2-aminopurine undergo addition of sulfur nucleophiles, such as hydrogen sulphite ion, across the 1,6-double bond in aqueous solution. The mole fraction of the 1,6-covalent hydrate has been estimated to be less than 10^{-7} by assuming that the equilibrium constants for the addition of hydroxide ion to N1 protonated purine riboside and 1-methylpurinium riboside are equal.[20] The value of this magnitude clearly argues against 1,6-hydrate as a reaction intermediate. It is possible, however, that 1-methylpurinium riboside is not a sufficiently good model for N1 protonated purine riboside. N1 methylation may lower the electron density at C6 less efficiently than N1 protonation, and it may also form a steric hindrance to the approach of hydroxide ion. The mole fraction of the 1,6-hydrate may thus be underestimated, and its appearance as a reaction intermediate cannot be strictly excluded.

EXPERIMENTAL

Materials. 2-Amino-9-(β-D-ribofuranosyl)purine[14] (1b), 2-amino-7-(2-hydroxyethoxymethyl)purine[22] (6) and 2-amino-9-(2-hydroxyethoxymethyl)purine[22] (2) were synthesized as described earlier. The preparation of the other compounds was as follows:

2-Methyl-9-(β-D-ribofuranosyl)purine (1c). 4-Amino-2-methylpyrimidin-6-one, obtained by refluxing a mixture of ethyl cyanoacetate and acetamidine hydrochloride in methanolic sodium methoxide,[23] was nitrosoated according to Traube[24] and reduced to 4,5-diamino-2-methylpyrimidin-6-one with aqueous sodium bisulfite.[25] Prolonged refluxing of the product in concentrated formic acid gave 2-methylhypoxan-

thine,[25] which was fused with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose by the method of Vorbruggen using trimethylsilyl trifluoromethanesulfonate as catalyst.[26] The resulting 2-methyl-2',3',5'-tri-O-benzoyl-inosine was converted to the protected 6-chloro-2-methylpurine riboside with phosphorus oxychloride in the presence of N,N-diethylaniline, and further to the corresponding 6-thio derivative with thiourea in boiling ethanol.[27] Debenzoylation in methanolic sodium methoxide, followed by reductive removal of the 6-thio group with Raney nickel,[28] gave crude 1c, which was desalted on Sephadex G10 and purified by TLC on Silica gel plates (Merck, Kieselgel 60 F_{254}, 2 mm) using a mixture of chloroform and methanol (9:1, v/v) as eluant. The product was UV identical with that reported in literature.[28] ^{1}H NMR (DMSO-d_6) δ(CH_3) s 2.51, δ(H5',5'') m 3.70, δ(H4') m 4.05, δ(H2') m 4.25, δ(H3') m 4.60, δ(H1') d 5.90, $J_{1',2'}$ 6 Hz, δ(H8) s 8.35, δ(H6) s 8.70. ^{13}C NMR (DMSO-d_6) δ(CH_3) q 25.5, δ(C5') t 61.4, δ(C2') d 70.4, d δ(C3') 73.6, d δ(C4') 85.9, δ(C1') d 87.2, δ(C5) s 132.1, δ(C8) d 144.7, δ(C6) d 148.1, δ(C4) s 151.4, δ(C2) s 161.3.

2-Methyladenosine (7). Commercial 2-methyladenine (Sigma) was acylated at N^6 with benzoyl chloride,[29] silylated and fused with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose by the method of Vorbruggen.[26] The product was debenzoylated with methanolic sodium methoxide and purified on Silica gel plates (Merck 60 F_{254}, 2 mm) using a 1:1 (v/v) mixture of chloroform and methanol as eluant. The product was UV identical with that reported in literature.[30] ^{1}H NMR (DMSO-d_6) δ(CH_3) s 2.39, δ(H5',5'') m 3.56, δ(H4') m 4.00, δ(H2') m 4.15, δ(H2') m 4.15, δ(H3') m 4.63, δ(H1') d 5.84, $J_{1',2'}$ 7 Hz, δ(H8) s 8.25. ^{13}C NMR (DMSO-d_6) δ(CH_3) q 25.0, δ(C5') t 61.9, δ(C2') d 70.9, δ(C3') d 73.2, δ(C4') d 86.2, δ(C1') d 88.0, δ(C5) s 117.6, δ(C8) d 139.6, δ(C6) s 149.5, δ(C4) s 155.8, δ(C2) s 161.0.

2,4-Diamino-5-formamidopyrimidine (3a). Commercial 2,4-diamino-5-nitropyrimidine (Sigma) was reduced to 2,4,5-triaminopyrimidine by hydrogenation in anhydrous methanol over

Raney nickel,[31] and formylated at N^5 with boiling formic acid.[32] The product crystallized from ethanol exhibited UV and ^1H NMR spectra identical with those reported by Ratsep and Pless.[9]

9-Ethoxymethylpurine (8) was prepared from commercial purine (Sigma) and chloromethyl ethyl ether (Aldrich) by the method described previously for 9-(1-ethoxyethyl)purine.[33] The compound was crystallized as colourless plates from cyclohexane and melted at 79-80 °C. UV(max,pH 7) 264 nm (3.90). ^1H NMR (DMSO-d_6) $\delta(CH_2CH_3)$ t 1.19, $\delta(CH_2CH_3)$ q 3.59, $\delta(OCH_2N)$ s 5.69, δ(H8) s 8.25, δ(H2) s 9.03, δ(H6) s 9.19. ^{13}C NMR (DMSO-d_6) $\delta(CH_2CH_3)$ q 14.8, $\delta(CH_2CH_3)$ t 65.6, $\delta(OCH_2N)$ t 72.5, δ(C5) s 133.8, δ(C8) d 145.8, δ(C6) d 148.9, δ(C4) s 151.7, δ(C2) d 153.1.

Compounds 1c, 7 and 8 may be assigned as N9-isomers on the basis of the ^{13}C shifts of the C4 and C5 atoms. The difference of these two shifts has been shown[34] to be 30 ppm with N9-glycosylated purines and 50 ppm with their N7-isomers.

Kinetic measurements. The progress of acidic hydrolyses was followed by the HPLC technique described previously.[8] The peak heights were transformed to concentrations with the aid of solutions of known concentrations. The hydronium ion concentrations of the buffer solutions employed, viz. formate and acetate buffers, were calculated from the pK_a values of the buffer acids under the experimental conditions.[35,36] The H_o values of concentrated aqueous acids were taken from Ref. 37.

Acidity constants. The pK_a values for the monocations of 1b, 1c, 2, 6 and 8 were determined spectrophotometrically at 298.2 K, as described previously.[38] The results were extrapolated to 363.2 K by assuming that the pK_a value depends on temperature as with adenosine.[7] The pK_a value for monoprotonated 3a was determined at 298.2 and 343.2 K, and extrapolated to 363.2 K via the Van't Hoff equation.

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