in 5 mL of nitromethane was added. The orange suspension turned yellow within a few minutes and after 1 h the solution was filtered through Celite. Removal of the nitromethane solvent at room temperature and reduced pressure afforded 0.87 g (~100%) of yellow crystals. The compound decomposed slowly at room temperature on standing. ^{1}H NMR (δ, ppm in $CDCl_3$): 8.16 (s, 1 H), 8.06 (dd, J = 2.1 and 8.3 Hz, 1 H), 7.82 (bs, 1 H), 7.65 (d, J = 8.3 Hz, 1 H), 7.23 (m, 4 H), 2.39 (s, 3 H).

When recovered from ether solution, a nicely crystalline monoetherate is obtained. ^{1}H NMR (CD_3OD): 8.30 (s, 1 H), 7.96 (dd, J = 2.1 and 8.3 Hz, 1 H), 7.65 (bs, 1 H), 7.63 (d, J = 8.3 Hz, 1 H), 2.53 (q, J = 7.4 Hz, 4 H), 2.27 (s, 3 H), 1.20 (t, J = 7.4 Hz, 6 H).

Cyclopalladated *N*-Methylbenzaldimine Tetrafluoroborate Bis(dimethylformamide solvate). A mixture of 0.80 g (1.5 mmol) of the cyclopalladated *N*-methylimine chloro dimer[7] and 0.75 g (3.9 mmol) of silver tetrafluoroborate was stirred in 10 mL of DMF at room temperature for 1 h. The solution was filtered through Celite and the solvent was evaporated under reduced pressure at 25 °C. The yellow crystals obtained were recrystallized from methylene chloride-ether to give 1.21 g (88%) of product, mp 147–8 °C dec. Figure 1 (Supplementary Material) shows the X-ray structure of this product.[12]

^{1}H NMR ($CDCl_3$, ppm): 8.01 (bs, 2 H), 7.83 (bs, 1 H), 7.25–7.22 (m, 1 H), 7.07–7.02 (m, 2 H), 6.79–6.75 (m, 1 H), 3.28 (s, 3 H), 3.14 (s, 6 H), 2.99 (s, 6 H). ^{13}CNMR ($CDCl_3$, ppm): 176.07, 167.10, 151.57, 146.12, 130.58, 129.75, 127.53, 125.18, 46.92, 38.31, 32.73. Anal. Calcd for $C_{14}H_{22}N_3O_2PdBF_4$: C, 36.74; H, 4.81; N, 9.18. Found: C, 36.74; H, 5.13; N, 8.79.

Dimerization of Styrene. A solution of 0.43 g (1.0 mmol) of the cyclopalladated tetrafluoroborate from *N*-(*p*-tolyl)-*p*-nitrobenzaldimine in 12 mL of styrene was heated at 100 °C in an oil bath for 19 h. Analyses by GLC showed less than 5% styrene remaining at this time. The mixture was dissolved in hexane and chromatographed on silica gel. There was obtained 7.0 g (70%) of nearly colorless, liquid 2,3-diphenyl-1-butene which was quite pure by NMR.[4]

Isomerization of 3-Phenyl-1-propene. This reaction was carried out as in the styrene dimerization above substituting 3-phenyl-1-propene for the styrene. Analyses of the reaction mixture by GLC showed the alkene was 93% isomerized after 19 h at 100 °C to a 89:4 trans to cis mixture of 1-phenyl-1-propenes.

Addition of 3-Hexyne to Cyclopalladated *N*-Phenylbenzaldimine Chloro Dimer. A solution of 0.65 g (1.0 mmol) of the chloro dimer[7] in 12 mL of DMF was magnetically stirred under nitrogen at 150 °C while 0.52 g (6.3 mmol) of 3-hexyne in 5 mL of DMF was slowly added over a 2.5-h period. After a further 30 min at 150 °C, the reaction mixture was cooled and the DMF was removed at 25 °C under reduced pressure. The residue was chromatographed on silica gel. Elution with methylene chloride–methanol gave 0.17 g (29%) of crude product for which the NMR spectrum was similar to that of the tetrafluoroborate. The chloride was very hygroscopic so it was converted to the known tetrafluoroborate by treating it with 0.12 g of silver tetrafluoroborate (0.62 mmol) in 7 mL of nitromethane. After being stirred for 1 h, the mixture was filtered through Celite with nitromethane rinsing. The filtrate was concentrated under reduced pressure and the residue was recrystallized from methanol–ether to give 0.12 g (63%) of off-white crystals for which the ^{1}H NMR and ^{13}C NMR spectra were identical with the spectra obtained from the compound prepared from the cyclopalladated tetrafluoroborate.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. Additional support was provided by the Center for Catalytic Science and Technology of the University of Delaware. We, also, gratefully acknowledge the loan of the palladium chloride used in this study by Johnson Mathey Inc.

Supplementary Material Available: Table of the yields and properties of intermediates prepared (Table 2) and the properties of isoquinolinium tetrafluoroborates (Table 3), Figures 1 and 2 showing the X-ray crystal structures of the cyclopalladated *N*-(*p*-tolyl)-*p*-nitrobenzaldimine bis(dimethylformamide) tetrafluoroborate and 7-benzoxy-3-(*p*-benzoxyphenyl)-6-methoxy-4-(methoxycarbonyl)-2-methylisoquinolinium tetrafluoroborate, respectively, and Tables 4, 5, 6, 7, and 8 giving crystallographic data for the isoquinolinium salt (13 pages). Ordering information is given on any current masthead page.

Hydrolysis of 2-Aminopurine Deoxyribonucleoside in Neutral Solution

Peter C. Ratsep and Reynaldo C. Pless*,†

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received March 17, 1988

At various temperatures between 50 and 110 °C, 2-aminopurine deoxyribonucleoside is severalfold more prone to degradation in aqueous sodium cacodylate buffer, pH 6.9, than is its structural isomer, deoxyadenosine, as determined by HPLC analysis of hydrolysates. It is calculated that, at 37 °C, the rate of hydrolysis of 2-aminopurine deoxyribonucleoside is 5 times as large as the rate of hydrolysis of deoxyadenosine. The decomposition of 2-aminopurine deoxyribonucleoside is almost unaffected by changes in ionic strength or buffer concentration, but is clearly accelerated by increasing acidity in the range from pH 5.8 to pH 7.15. While deoxyadenosine decomposes to produce the corresponding free base, adenine, as the sole UV-absorbing product, 2-aminopurine deoxyribonucleoside yields primarily 2,4-diamino-5-formamidopyrimidine rather than 2-aminopurine. The latter compound is also formed during hydrolysis of 2-aminopurine deoxyribonucleoside, but it arises largely in a secondary reaction from 2,4-diamino-5-formamidopyrimidine. The increased propensity to depurination evinced by 2-aminopurine deoxyribonucleoside in comparison to deoxyadenosine as well as the alteration of the heterocyclic base occurring during the hydrolysis is of interest in view of the mutagenic activity of 2-aminopurine.

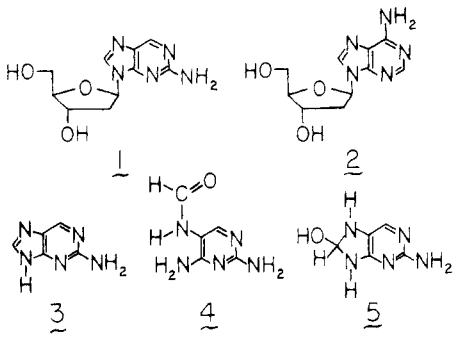
The *N*-glycosyl bond in 2-aminopurine 2′-deoxyribonucleoside (**1**, see Scheme I) is significantly more prone to cleavage in neutral or basic aqueous medium at elevated temperatures than are the glycosyl linkages in 2′-deoxyadenosine (**2**) and 2′-deoxyguanosine.[1] While this observation was initially used as the basis for an analytical procedure for the quantitation of 2-aminopurine moieties

†Present address: Bethesda Research Laboratories, Gaithersburg, Maryland 20877.

(1) Pless, R. C.; Bessman, M. J. *Biochemistry* **1983**, *22*, 4905–4915.

0022-3263/88/1953-3241$01.50/0

Scheme I



incorporated at adenine sites in known DNA sequences,[1] the pronounced hydrolytic lability of the glycosyl bond in **1** is of intrinsic interest, both from a mechanistic point of view and because of the mutagenic character of 2-aminopurine (**3**). We have, therefore, examined the degradation of **1** in aqueous solutions under a variety of conditions, and compared it to the hydrolysis of **2**. The two reactions were found to differ markedly not only in their rates but also in their product distributions.

Experimental Section

Materials. The following materials were purchased and used without further purification: 2′-deoxyadenosine (**2**) from P-L Biochemicals, cacodylic acid, 2,4-diamino-5-nitropyrimidine, and 2′-deoxycytidine from Sigma Chemical Co., 2-aminopurine (**3**) from Pfaltz & Bauer, HPLC-grade acetonitrile and methanol from Burdick & Jackson, 2-deoxyribose and Me_2SO-d_6 (99.96% D) from Aldrich Chemical Co.

1 was prepared from **3** and 2′-deoxycytidine with use of *trans*-*N*-glycosylase from *Lactobacillus helveticus*,[2] as previously described.[3] The enzyme was the gift of Professor Maurice J. Bessman, The Johns Hopkins University. The product was homogeneous on HPLC analysis (PAC column, isocratic elution with acetonitrile/methanol (90:10, v/v), monitoring A_{280}). Its UV-absorption spectrum in neutral aqueous solution, had λ_{max} = 304 nm, λ_{min} = 262 nm, and A_{max}/A_{min} = 6.5, in agreement with the spectrum of Frederiksen.[4] For the 200-MHz ^1H NMR spectrum of 60 mM **1** in Me_2SO-d_6 at 25 °C, chemical shifts (δ values, relative to internal Me_4Si standard), integration, and assignment of bands are as follows: 8.59 ppm, singlet, 1.0, H-6; 8.28 ppm, singlet, 1.0, H-8; 6.52 ppm, singlet, 1.9, NH_2 group; 6.29 ppm, multiplet, 1.0, H-1′; 5.29 ppm, doublet, 0.8, 3′-OH; 4.97 ppm, multiplet, 0.7, 5′-OH; 4.38 ppm, multiplet, 1.0, 3′-H; 3.85 ppm, multiplet, 1.1, H-4′; multiplet centered on 3.56 ppm, 2.0, H-5′ and H-5″; singlet at 3.33 ppm, water; 2.64 ppm, multiplet, 1.0, H-2′; multiplet at 2.50 ppm, Me_2SO; 2.26 ppm, multiplet, 1.0, H-2″. The assignment of H-6 and H-8 was based on the exchangeability of the latter with D_2O and on significant nuclear Overhauser effects of the latter, but not of the former, on H-1′, H-2′, and H-3′.

2,4-Diamino-5-formamidopyrimidine (**4**) was prepared in 88% overall yield from 2,4-diamino-5-nitropyrimidine. The nitro compound was reduced by hydrogenation in anhydrous methanol over Raney nickel,[5] and the resulting 2,4,5-triaminopyrimidine (mp 177–178.5 °C; lit.[6] mp 176–179 °C) was formylated by treatment with 95% formic acid at reflux, as described by Isay,[6] to give **4** (mp 226–227 °C; lit.[6] mp 224 °C). Aqueous solutions of **4** had UV-absorbance maxima at 272, 287, and 296 nm, and minima at 261, 261, and 274 nm, at pH 1, 7, and 13, respectively (lit.[7] λ_{max} = 270 nm and λ_{max} = 287 nm for the monocation and the neutral species, respectively). For the ^1H NMR spectrum of 50 mM **4** in Me_2SO-d_6 at 25 °C, chemical shifts (relative to internal Me_4Si), relative intensity, multiplicity, coupling constants, and assignments are as follows: 9.11 ppm, 3.0, broad, HCON*H* (trans); 8.76 ppm, 1.1, doublet, 11.0 Hz, HCON*H* (cis); 8.13 ppm, 2.8, doublet, 1.6 Hz, *H*CO (trans); 7.95 ppm, 1.0, doublet, 11.0 Hz, *H*CO (cis); 7.69 ppm, 2.2, singlet, H-6 (trans); 7.57 ppm, 0.9, singlet, H-6 (cis); 6.30 ppm, 2.5, singlet, 4-N*H*$_2$ (cis); 6.18 ppm, 6.1, singlet, 4-N*H*$_2$ (trans); 5.92 ppm, 2.8, singlet, 2-N*H*$_2$ (cis); 5.85 ppm, 6.4, singlet, 2-N*H*$_2$ (trans). Here, cis and trans refer to the geometric relationship between amide hydrogen and carbonyl oxygen in the formamido group.[8] Assignment of peaks was based on D_2O exchange experiments (bands at 8.13, 7.95, 7.69, and 7.57 ppm remained) and consideration of coupling constants, peak areas, and chemical shifts. The bands at 8.76 and 7.95 ppm, which showed coupling constants of 11.0 Hz, were assigned to the cis form of the formamido group, while the band at 8.13 ppm, with a coupling constant of 1.6 Hz, was assigned to the trans form, in accord with the coupling constants reported for the cis and trans conformations in various N-substituted formamides.[8,9]

Methods. Hydrolysis mixtures were prepared in deionized, degassed water with a weighed amount of anhydrous nucleoside and the appropriate volume of 174 mM aqueous sodium cacodylate buffer, pH 6.9. Nucleoside concentration of the final solution was verified by UV-absorption measurement after appropriate dilution with water (ϵ_{259} = 15 000 M^{-1} cm^{-1} for **2**, ϵ_{304} = 7100 M^{-1} cm^{-1} for **1**) and the pH was determined with a glass pH electrode standardized with pH 7.00 buffer. Aliquots of 300-μL volume were sealed in 0.7-mL borosilicate glass ampules (Wheaton Scientific) and kept at the selected temperature in silicone oil filled wells in constant temperature heat blocks (Lab-Line Instruments). The temperature variation during hydrolysis was maximally ±1 °C. After different reaction times, one or two ampules were withdrawn from the heat block and stored at −20 °C until the analysis. All ampules from one kinetic experiment were analyzed consecutively, with a Glenco System I HPLC apparatus. The ampules were opened, and 50-μL aliquots of the hydrolysates were diluted with 500 μL of the appropriate HPLC elution solvent. Samples were loaded onto the column (a 25 cm × 0.46 cm Whatman column, Partisil PAC, 10 μm, fitted with a Whatman PAC guard precolumn) via a 15-μL injection loop, and resolved by isocratic elution with acetonitrile/methanol (90:10, v/v) in the case of hydrolysates of **1** and with acetonitrile/methanol (77:23, v/v) in the case of hydrolysates of **2**, with a flow rate of 1.0 mL/min. The effluent was monitored in a flow-through cell of 24-μL volume and 12-mm path length, at 280 nm in the case of hydroysates of **1** and at 254 nm for hydrolysates of **2**. Absorbance was continuously traced on a Linear recorder. The linearity of the response of the detection system was verified.

The disappearance of the starting nucleoside was quantitated by measuring either the height or the area of the corresponding peak; the two methods gave closely similar results. The natural logarithms of the nucleoside peak sizes and the corresponding reaction times were entered as coordinates in a linear regression analysis program for the determination of the slope of the least-squares straight line. Between 10 and 20 coordinate points were used in each instance. All slopes were calculated with correlation coefficients r greater than 0.99; in the case of poorer correlation, the kinetic run was repeated. For the degradation of **1** at 37 °C and at 50 °C, and for the degradation of **2** at 50 °C, the reaction was followed for at least 1 half-time. In all other hydrolysis experiments, time points were taken covering at least 2 half-times.

Arrhenius enthalpies of activation were calculated as $\Delta H^{\ddagger} = -R[\Delta(\ln k)/\Delta(1/T)] - RT$, where R is 1.987 cal mol^{-1} deg^{-1}, T is the absolute temperature, and $\Delta(\ln k)/\Delta(1/T)$ is the slope of the least-squares straight line in the plot of the natural logarithm of the apparent pseudo-first-order rate constant vs the inverse of the absolute temperature.

UV-absorption spectra were measured in 1-cm cuvettes in a Hitachi 100-80A recording spectrophotometer, against a reference cuvette filled with the appropriate solvent. Proton NMR spectra

(2) Uerkvitz, W. *Eur. J. Biochem.* **1971**, *23*, 387–395.
(3) Pless, R. C.; Levitt, L. M.; Bessman, M. J. *Biochemistry* **1981**, *20*, 6235–6244.
(4) Frederiksen, S. *Biochem. Pharmacol.* **1965**, *14*, 651–660.
(5) Brown, D. J. *J. Appl. Chem.* **1957**, *7*, 109–113.
(6) Isay, O. *Ber. Dtsch. Chem. Ges.* **1906**, *39*, 250–265.
(7) Roth, B.; Strelitz, J. Z. *J. Org. Chem.* **1969**, *34*, 821–836.
(8) Cadet, J.; Nardin, R.; Voituriez, L.; Remin, M.; Hruska, F. E. *Can. J. Chem.* **1981**, *59*, 3313–3318.
(9) Siddall, T. H.; Stewart, W. E.; Marston, A. L. *J. Phys. Chem.* **1968**, *72*, 2135–2141.

Table I. Pseudo-First-Order Rate Constants for Hydrolysis of 5 mM Nucleosides in 15 mM Sodium Cacodylate, pH 6.9

	k, s^{-1}					
nucleoside	37 °C	50 °C	70 °C	90 °C	110 °C	111 °C
1	2.54×10^{-8}	1.50×10^{-7}	9.8×10^{-7}	6.8×10^{-6}	3.44×10^{-5}	3.59×10^{-5}
2		2.66×10^{-8}	1.95×10^{-7}	1.87×10^{-6}	8.4×10^{-6}	

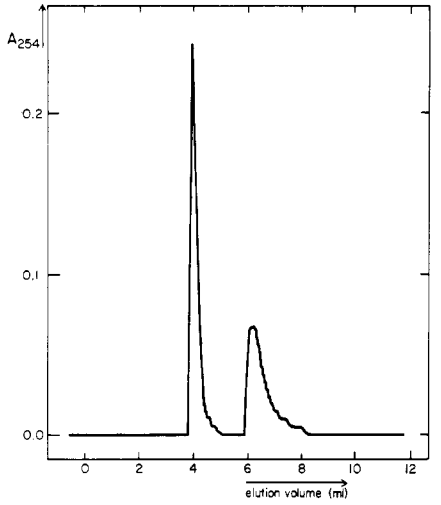


Figure 1. Elution profile obtained for standard hydrolysis of **2** at 70 °C for 37 days. **2** elutes at 4.1 mL; adenine elutes at 6.2 mL.

were recorded on a Bruker 200-MHz FT NMR spectrometer or on a Bruker 500-MHz FT instrument. ^{13}C NMR spectra were determined at 75 MHz on a CPX300 NMR spectrometer equipped with a wide-bore probe. Mass spectra were obtained with a Kratos MS-30 mass spectrometer.

Results

The standard initial concentrations in the hydrolysis of **1** and **2** were 5 mM nucleoside and 15 mM sodium cacodylate buffer, pH 6.9. This buffer was chosen because of its transparence in the spectral region of interest (254 and 280 nm) and because its pH is practically independent of temperature, as determined by us for the range from 20 °C to 93 °C. After different reaction times, hydrolysates were analyzed by high-pressure liquid chromatography, monitored by UV-absorbance measurement.

For all reaction temperatures examined, hydrolysates of **2** contained only two species absorbing at 254 nm (Figure 1); these were identified as the unreacted nucleoside and the corresponding free base, adenine, on the basis of their elution volume and their UV-absorption spectra. In contrast, hydrolysates of **1** contained, apart from the starting nucleoside and its free base, **3**, considerable quantities of a third species absorbing at 280 nm, which eluted between the nucleoside and the free base (Figure 2, panel A). This material was identified as 2,4-diamino-5-formamidopyrimidine (**4**), as described below.

Similar molar amounts of **3** and **4** were present after intermediate times (6 h) in hydrolyses performed at 110 °C (Figure 2, panel A). Longer reaction times (22 h) brought about almost complete consumption of the starting nucleoside and a slow increase in **3**, apparently at the expense of **4**. No free **3** was detected in hydrolysis at 37 °C (Figure 2, panel B), at least for the reaction time analyzed, which was 1 half-time. In hydrolyses at intermediate temperatures (50, 70, 90 °C), **4** was the first product to form, followed in the later stages of the reaction by the appearance of **3**.

Hydrolysis of **1** and of **2** was carried out in the standard mixture at various fixed temperatures, and the disappearance of the starting nucleoside was monitored as a

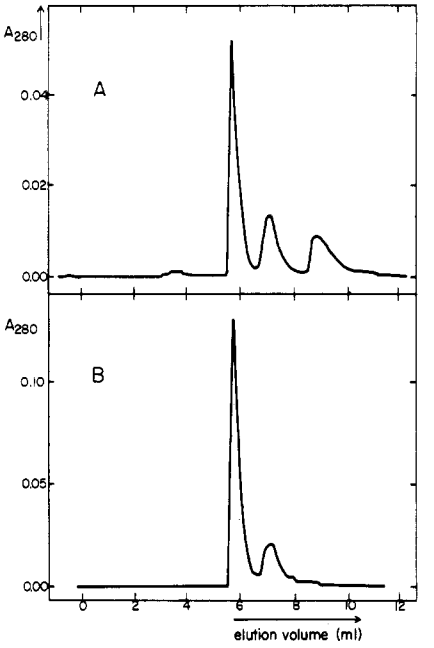


Figure 2. Elution profile obtained for standard hydrolysis of **1** at 110 °C for 6 h (panel A) and at 37 °C for 175 days (panel B). **1** elutes at 5.7 mL, **4** elutes at 7.2 mL, and **3** elutes at 9.0 mL.

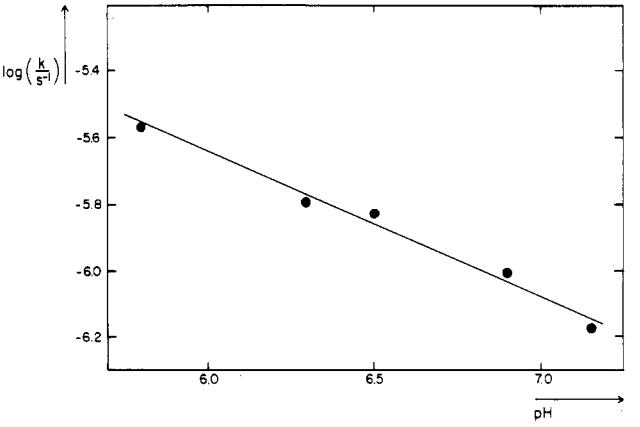


Figure 3. pH dependence of the pseudo-first-order rate constant for the decomposition of **1** in sodium cacodylate buffer at 70 °C.

function of time by HPLC analysis. Semilogarithmic plots of the peak area or of the peak height of the first band (corresponding to unreacted nucleoside) vs time were linear. Pseudo-first-order rate constants, determined as the negative of the slope of these plots, are listed in Table I for the degradation of **1** at temperatures ranging from 37 °C to 111 °C and for the degradation of **2** at temperatures ranging from 50 °C to 110 °C. The Arrhenius plots constructed from these pseudo-first-order rate constants had correlation coefficients r of 0.999, and provided $\Delta H^{\ddagger}$ values of 22.3 and 23.4 kcal/mol for the degradation of **1** and **2**, respectively.

To examine the effect of pH on the rate of decomposition of **1**, the kinetics of hydrolysis of the nucleoside were determined at 70 °C in a series of sodium cacodylate buffers of identical ionic strength (14 mM), spanning the range from pH 5.8 to pH 7.15. As seen in Figure 3, hy-

drolysis of 1 is clearly pH dependent in this range, accelerating as the pH decreases. The product distribution, however, did not change appreciably over the pH range examined.

The rate of degradation of 1 at 70 °C was unaffected by addition of 5 mM deoxyribose to the standard hydrolysis mixture, and rather insensitive to changes in salt concentration (15% rate decrease upon admixture of 120 mM NaCl), to changes in buffer concentration (14% rate decrease upon increase of sodium cacodylate, pH 6.9, from 15 mM to 150 mM), and to changes in nucleoside concentration (16% and 46% rate decrease for nucleoside dilution from 5 mM to 0.75 mM and to 0.054 mM, respectively). A control experiment showed that 5 mM deoxyribose did not produce material absorbing at 280 nm when heated to 70 °C for 18 days in the standard hydrolysis mixture.

To examine if the free base, 3, could be the precursor of the diaminoformamidopyrimidine formed in the reaction, 5 mM 3 was subjected to the standard hydrolytic treatment at 70 °C. 3 decomposed with a pseudo-first-order rate constant of 4×10^{-8} s^{-1}, with formation of 4 as the only major product. Under the same conditions, however, the reverse reaction is much faster; 4 was converted to 3 with a pseudo-first-order rate constant of 8.6×10^{-7} s^{-1}.

Identification of the main product in the hydrolysis of both 1 and 3 as 4 was based on the following data.

1. Hydrolysis of 1 uniformly ^{14}C-labeled in the sugar moiety (prepared enzymatically from uniformly ^{14}C-labeled deoxycytidine and unlabeled 3) yielded the product in unlabeled form; hence it must be a derivative of the aglycon.

2. The products, as obtained by HPLC, agreed with our synthesized sample of 4 in their UV-absorption spectra in aqueous solution at pH 1, 7, and 13.

3. Both chemical-ionization and electron-impact mass spectrometry on the products isolated by HPLC indicated main molecular masses of 153 amu, as expected for 4.

4. The products isolated by HPLC agreed with the synthesized 4 in their NMR spectra, taken in Me_2SO-d_6.

Isay[6] reasoned that formylation of 2,4,5-triaminopyrimidine occurred at the 5-amino group, on account of the distinctly basic character of the product. Because of the importance of 4 in the context of the present work, we have verified the structure by ^{13}C NMR spectroscopy. The proton-decoupled spectrum of 2,4,5-triaminopyrimidine in Me_2SO-d_6 showed bands at 157.2 (C-2), 155.6 (C-4), 140.4 (C-6), and 118.1 ppm (C-5), relative to internal Me_4Si standard. Assignment was based on the characteristic sequence of chemical shifts in pyrimidine carbons[10-12] and, in the case of C-6, on its splitting in the proton-coupled spectrum. The ^{13}C spectrum of 4 in Me_2SO-d_6 showed major peaks, for the trans amide isomer, at 164.0 (H-CO), 160.5 and 159.3 (C-2 and C-4), 150.7 (C-6), and 106.2 ppm (C-5), and smaller peaks for the cis isomer. Assignment in the case of the formyl carbon and C-6 was confirmed by the proton-coupled spectrum. Thus, the C-5 resonance is the only one to shift upfield upon formylation (by 12 ppm), while all other carbon resonances shift downfield. This identifies N-5 as the site of formylation, by analogy to the case of aniline, where N-formylation induces an upfield shift of 10 ppm in the resonance position of C-1 and downfield shifts for C-2 and C-4.[13,14]

Discussion

From the temperature dependence of the rate constants, $\Delta H^{\ddagger}$ values of +23 to +24 kcal/mol have been calculated for the hydrolysis of 2.[15-17] In these instances, the activation parameters pertained to the cleavage of the glycosyl bond in strongly acidic media, probably proceeding from the nucleoside monocations and dications. Our Arrhenius plot for the hydrolysis of 2 at pH 6.9 gives the same value for the enthalpy of activation, +23.4 kcal/mol. This may indicate that even at pH 6.9 the protonated nucleoside is the only reactive species. This notion is supported by the data of Garrett and Mehta,[16] who documented an approximately linear relationship between hydrogen ion activity and apparent first-order rate constant for the hydrolysis of 2 in the range from pH 3.8 to pH 7.5, at 80 °C, as expected if the monocation is the only important reactive species in this pH range.

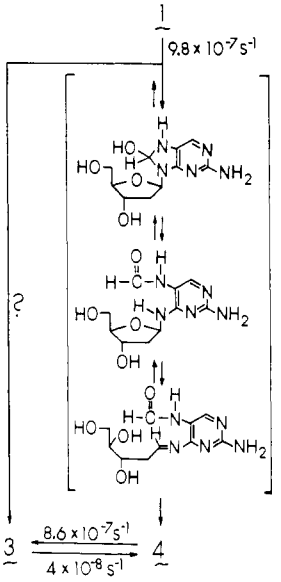
Our calculation of the apparent enthalpy of activation for hydrolysis of 1 at pH 6.9 gave a value of +22.3 kcal/mol, similar to the value of +23.4 kcal/mol determined for hydrolysis of 2. This agreement may be fortuitous, and the calculated $\Delta H^{\ddagger}$ value for hydrolysis of 1 may be a composite activation parameter. This is indicated by the value of −0.44 of the slope, $\Delta(\log k)/\Delta pH$, in Figure 3: if reaction of the monocationic form of 1 were the sole rate-determining process, a value of negative unity would be expected for this slope, because the pH range investigated, pH 5.80–7.15, lies far to the basic side of the pK_a of the monocation of 1, which should be about 4, as a pK_a of 3.4 has been reported for protonated 2-aminopurine ribonucleoside.[18] It is possible that protonated 1 intervenes as only one of several alternative intermediates in the hydrolysis of 1.

In weakly acidic and in neutral media, 2 is believed to decompose by an A-1 mechanism, involving direct conversion of the monocationic form of the nucleoside to an oxycarbonium ion and a tautomer of the free base.[15,16] The course of hydrolysis of 1 is fundamentally different in that a modified form of the corresponding free base, viz. 4, appears as the major or sole UV-absorbing product in the early stages of the reaction. The sequence of appearance of this material and of 3 in hydrolyses of 1 at intermediate temperatures (50, 70, 90 °C) points to 4 as the probable precursor of 3 in these reactions. While we have determined that 3 does produce 4 under the hydrolysis conditions used, the reverse reaction was found to be much faster. Therefore, the presence of 4 in preponderance over 3 after intermediate hydrolysis times for 1 indicates that, at least to a large extent, 4 occurs as a precursor of 3, rather than as its product. Thus, in these hydrolyses, 3 is not formed directly by scission of the glycosyl bond in the starting nucleoside; instead, a reaction at the heterocyclic base precedes cleavage of the glycosyl linkage, a modified base is released, and 3 arises from subsequent processes.

A logical step in the conversion of the 2-aminopurine heterocycle to the 2,4-diamino-5-formamidopyrimidine moiety is hydration of the N(7)–C(8) bond, to produce the 2-amino-7,8-dihydro-8-hydroxypurine (5) moiety. Water addition reactions across C–N double bonds in nitrogen heterocycles have been extensively studied.[19-22] Covalent

(10) Lauterbur, P. C. *J. Chem. Phys.* **1965**, *43*, 360–363.
(11) Mathias, A.; Gil, V. M. S. *Tetrahedron Lett.* **1965**, 3163–3167.
(12) Pugmire, R. J.; Grant, D. M. *J. Am. Chem. Soc.* **1968**, *90*, 697–706.
(13) Sadtler Index 1977, Spectrum No. 1420C.
(14) Sadtler Index 1979, Spectrum No. 6001C.
(15) Zoltewicz, J. A.; Clark, D. F.; Sharpless, T. W.; Grahe, G. *J. Am. Chem. Soc.* **1970**, *92*, 1741–1750.
(16) Garrett, E. R.; Mehta, P. J. *J. Am. Chem. Soc.* **1972**, *94*, 8532–8541.
(17) Hevesi, L.; Wolfson-Davidson, E.; Nagy, J. B.; Nagy, O. B.; Bruylants, A. *J. Am. Chem. Soc.* **1972**, *94*, 4715–4720.
(18) Fox, J. J.; Wempen, I.; Hampton, A.; Doerr, I. L. *J. Am. Chem. Soc.* **1958**, *80*, 1669–1675.

Scheme II



hydration of **3** has not been reported; this may be due to the long reaction times required in this case. Rates for hydration and dehydration vary widely for different heterocyclic systems, and slow conversions have been observed.[23] As determined in the present work, the rates of interconversion of **3** and **4**, proceeding presumably via **5**, are very low.

The probable path by which **1** decomposes ultimately to give **3** is shown in Scheme II. Rate constants are given for 70 °C and 15 mM cacodylate, pH 6.9, as the solvent. **1** degrades to **4** with an apparent pseudo-first-order rate constant of about 9.8×10^{-7} s^{-1}. This process likely occurs via hydration of the N(7)–C(8) double bond, ring opening of the five-membered nitrogen heterocycle to give the 2,4-diamino-5-formamidopyrimidine still glycosylated at the 4-amino group, and cleavage of the glycosyl linkage in this latter species via the Schiff base intermediate.[24] **4** can undergo ring closure to give **3**; this reaction is reversible, with the equilibrium strongly favoring **3**. Direct depurination of **1** to **3**, analogous to the mechanism believed to be operative in the depurination of **2**, cannot be excluded, but can only be a minor path in the conversion of **1** to **3**.

The importance of the structure of the heterocycle for the greater hydrolytic lability of 2-aminopurine nucleosides compared to adenine nucleosides is again stressed by our preliminary observation that, in the ribonucleoside series as well, the 2-aminopurine nucleoside is 7 times as labile as adenosine to hydrolysis at 110 °C and pH 6.9, and gives rise to a product distribution similar to that observed with **1**.

Hydration of the imidazole ring as the initial step in the hydrolysis of purine nucleosides has been suggested previously by Shapiro.[25] It was subsequently argued that this mechanism could not be of general validity in depurinations.[15] While this is probably the case, the present study provides strong evidence that Shapiro's hypothesis may be valid in some instances, dependent on the identity of the aglycon. Reaction at the heterocyclic base prior to cleavage of the glycosyl linkage has been documented for the decomposition of 9-β-D-ribofuranosylpurine[26] and of adenosine[27] in aqueous alkali. Compared to these cases, the pathway for decomposition of **1** in neutral aqueous solution is simpler, because deformylation of 2,4-diamino-5-formamidopyrimidine species is negligible under neutral conditions, and because the recyclization of the formamido group with the 6-amino group, as postulated for adenosine,[27] cannot take place in the case of 2-aminopurine nucleosides.

The reason for the increased hydrolytic lability of 2-aminopurine nucleosides, compared to adenine nucleosides, is not obvious. It appears that 6-unsubstituted purine systems are particularly prone to attack at the imidazole ring. Thus, formation of 4-amino-5-formamidopyrimidine and 4,5-diaminopyrimidine was observed in hydrolysis of 9-β-D-ribofuranosylpurine in weakly acidic medium, while ribofuranosyl derivatives of 6-substituted purines only underwent cleavage of the glycosyl bond under these conditions.[28] Another example is provided by 2-(methylthio)purine, which yields largely 4,5-diamino-2-(methylthio)pyrimidine when heated in concentrated aqueous dimethylamine.[29] A rationale has been given[27] for the increased susceptibility to nucleophilic attack at C-8 evinced by 9-β-D-ribofuranosylpurine compared to adenosine: introduction of an amino substituent at C-6 should increase the electron density at C-8, thus impeding nucleophilic attack. This, however, does not explain the difference in susceptibility of 6-aminopurine and 2-aminopurine nucleosides to attack at C-8, as the 2-amino substituent should also cause increased electron density at C-8.

One of the main purposes of the present study was to determine whether the increased hydrolytic lability of **1** compared to **2**, which was originally observed in aqueous piperidine solution at 90 °C,[1] would also obtain at the physiologically relevant conditions of 37 °C and neutral pH. The measurement of rates of hydrolysis of **1** and **2** over a wide range of temperatures was performed in part to establish a reliable basis for extrapolation of rate constants to 37 °C. In the case of hydrolysis of **1**, it was possible to determine the apparent first-order-rate constant at 37 °C directly, at a value of 2.5×10^{-8} s^{-1}. For hydrolysis of **2**, the lowest temperature at which measurements were made was 50 °C; linear extrapolation of the Arrhenius plot to 37 °C gives a value of 5.3×10^{-9} s^{-1}. Thus, we conclude that in neutral solution at 37 °C, **1** is 5 times as hydrolytically labile as is **2**.

This result is of interest in view of the mutagenic action of **3**, originally demonstrated in bacterial systems,[30] and more recently in animal cells.[31] The main mechanism by which **3** induces mutations is thought to involve its incorporation into DNA. While this normally takes place at adenine positions,[1,32] it may also, at a low but mutagenically significant level, occur at G sites, as demonstrated on model templates by Mhaskar and Goodman;[33] this gives rise to G·C → A·T transitions. Alternatively, **3** correctly

(19) Albert, A.; Armarego, W. L. F. *Adv. Heterocycl. Chem.* **1965**, *4*, 1–42.
(20) Perrin, D. D. *Adv. Heterocycl. Chem.* **1965**, *4*, 43–73.
(21) Albert, A. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 919–928.
(22) Bunting, J. W.; Perrin, D. D. *J. Chem. Soc. B* **1966**, 433–436.
(23) Armarego, W. L. F. *J. Chem. Soc.* **1963**, 4304–4312.
(24) Capon, B.; Connett, B. E. *J. Chem. Soc.* **1965**, 4497–4502.
(25) Shapiro, R. *Prog. Nucleic Acids Res. Mol. Biol.* **1968**, *8*, 73–112.
(26) Lönnberg, H.; Lehikoinen, P. *J. Org. Chem.* **1984**, *49*, 4964–4969.
(27) Lehikoinen, P.; Mattinen, J.; Lönnberg, H. *J. Org. Chem.* **1986**, *51*, 3819–3823.
(28) Lönnberg, H.; Lehikoinen, P. *Nucleic Acids Res.* **1982**, *10*, 4339–4349.
(29) Albert, A.; Brown, D. J. *J. Chem. Soc.* **1954**, 2060–2071.
(30) Freese, E. *J. Mol. Biol.* **1959**, *1*, 87–105.
(31) Caras, I. W.; MacInnes, M. A.; Persing, D. H.; Coffino, P.; Martin, D. W. *Mol. Cell. Biol.* **1982**, *2*, 1096–1103.
(32) Rogan, E. G.; Bessman, M. J. *J. Bacteriol.* **1970**, *103*, 622–633.
(33) Mhaskar, D. N.; Goodman, M. F. *J. Biol. Chem.* **1984**, *259*, 11713–11717.

incorporated at adenine sites may be misread as guanine in a subsequent round of replication, as shown on model templates by Watanabe and Goodman,[34,35] leading to A·T → G·C transition mutations. Accordingly, virtually all of the 2-aminopurine-induced mutations that have been examined in detail have been identified as transitions.[36,37] It has been reported, however, that, apart from its principal mutagenic action as an inducer of transitions, **3** can also cause transversions and frameshift mutations.[38] For these alternative modes of mutagenic action of **3**, the increased lability of the glycosyl linkage demonstrated by us at the level of the 2-aminopurine nucleoside (**1**) may provide a rationale: at the DNA level, incorporation of **3** would cause an increased formation of apurinic sites, and the potential of apurinic sites for mutagenesis, including induction of transversions, first postulated by Bautz Freese,[39] has now been demonstrated with partially depurinated artificial templates or partially depurinated phage DNA using purified DNA polymerase in vitro,[40-47] with partially depurinated ϕX174 DNA in *Escherichia coli*,[48-50] and with partially depurinated SV40 DNA in monkey kidney cells.[51] Apurinic sites have also been postulated as possible intermediates in aflatoxin-induced mutagenesis.[52]

Our observations about the hydrolytic breakdown of **1** are also relevant for the question of 2-aminopurine-induced mutagenesis in a different respect. Our results indicate that the primary event in the hydrolysis of **1** is modification of the base, probably addition of water across the N(7)–C(8) double bond, to give the 2-amino-7,8-dihydro-8-hydroxypurine moiety, with the possibility of a subsequent opening of the five-membered ring to produce the 2,4-diamino-5-formamidopyrimidine moiety. If such processes occur at the DNA level without immediate scission of the glycosidic bond, the modification of the heterocyclic moiety could fundamentally affect the hydrogen bonding relationships that the base can undergo within the constraints imposed by the DNA geometry. It is interesting to observe that the analogous ring-opened products obtained by γ-irradiation of adenine sites in DNA, viz. 4,6-diamino-5-formamidopyrimidine moieties, constitute sufficiently serious lesions to warrant a special repair glycosylase for their excision in *E. coli*.[53]

Acknowledgment. This work was supported by a Public Health Service grant (GM-34450). 500-MHz NMR spectra were obtained by Dr. C. E. Cottrell at the Ohio State University Chemical Instrument Center, using equipment funded in part by NIH Grant No. 1 S10 RR01458-01A1, and mass spectra were recorded by C. R. Weisenberger at the same Center.

Registry No. **1**, 3616-24-8; **2**, 958-09-8; **3**, 452-06-2; **4**, 18620-60-5; 2′-deoxycytidine, 951-77-9; 2,4-diamino-5-nitropyrimidine, 18620-73-0; 2,4,5-triaminopyrimidine, 3546-50-7.

(34) Watanabe, S. M.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2864–2868.
(35) Watanabe, S. M.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6429–6433.
(36) Miller, J. H.; Ganem, D.; Lu, P.; Schmitz, A. *J. Mol. Biol.* **1977**, *109*, 275–301.
(37) Ronen, A. *Mutat. Res.* **1979**, *75*, 1–47.
(38) Persing, D. H.; McGinty, L.; Adams, C. W.; Fowler, R. G. *Mutat. Res.* **1981**, *83*, 25–37.
(39) Bautz Freese, E. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 540–545.
(40) Shearman, C. W.; Loeb, L. A. *J. Mol. Biol.* **1979**, *128*, 197–218.
(41) Kunkel, T. A.; Shearman, C. W.; Loeb, L. A. *Nature (London)* **1981**, *291*, 349–351.
(42) Strauss, B.; Rabkin, S.; Sagher, D.; Moore, P. *Biochimie* **1982**, *64*, 829–838.
(43) Boiteux, S.; Laval, J. *Biochemistry* **1982**, *21*, 6746–6751.
(44) Kunkel, T. A.; Schaaper, R. M.; Loeb, L. A. *Biochemistry* **1983**, *22*, 2378–2384.
(45) Sagher, D.; Strauss, B. *Biochemistry* **1983**, *22*, 4518–4526.
(46) Sagher, D.; Strauss, B. *Nucleic Acids Res.* **1985**, *13*, 4285–4298.
(47) Randall, S. K.; Eritja, R.; Kaplan, B. E.; Petruska, J.; Goodman, M. F. *J. Biol. Chem.* **1987**, *262*, 6864–6870.
(48) Schaaper, R. M.; Loeb, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1773–1777.
(49) Schaaper, R. M.; Kunkel, T. A.; Loeb, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 487–491.
(50) Kunkel, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1494–1498.
(51) Gentil, A.; Margot, A.; Sarasin, A. *Mutat. Res.* **1984**, *129*, 141–147.
(52) Foster, P. L.; Eisenstadt, E.; Miller, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2695–2698.
(53) Breimer, L. H. *Nucleic Acids Res.* **1984**, *12*, 6359–6367.