3-Methoxy-1-butanol from Reduction of 25. A solution of 8.7 g (48 mmol) of 1-acetoxy-3-(chloromethoxy)butane (**25**) in 150 mL of anhydrous diethyl ether was added dropwise to a stirred slurry of 4.1 g (110 mmol) of lithium aluminum hydride in 200 mL of anhydrous diethyl ether. After addition was completed the mixture was stirred for 2 h at room temperature and then hydrolyzed by sequential dropwise addition of 4.1 mL of water, 4.1 mL of 15% aqueous sodium hydroxide, and 12.4 mL of water. The mixture was filtered and the precipitate washed with five 20-mL portions of ether. The combined filtrate and washings were dried ($MgSO_4$), filtered, and then concentrated to give an oil which was distilled to give 4.0 g (80%) of 3-methoxy-1-butanol, bp 34–37 °C (17 mm) [lit.[29] bp 158–159 °C]. The IR and ^1H NMR spectra of the product were identical with those of an authentic sample (Aldrich Chemical Co.) of the title compound.

Acetolysis of *trans*-2-Deuterio-4-methyl-1,3-dioxane (26). A solution of 114 mg (1.1 mmol) of *trans*-**26** (containing 10% of the cis isomer) and 120 mg (1.2 mmol) of acetic anhydride in 1.5 mL of $CDCl_3$ containing 1% Me_4Si was placed in a 5-mm NMR tube. The reaction was initiated by the addition of 2.0 μL of concentrated sulfuric acid and the progress was monitored by ^1H NMR (Table II). The 270-MHz spectrum of the product mixture is shown in Figure 1 (supplementary material).

Acknowledgment. This investigation was supported by a grant from the Research Foundation of the University of Connecticut. We thank Allan Croteau for preparing some of the cyclic acetals used in this study.

Supplementary Material Available: ^1H NMR of **6** and a mixture of **27** and **28** (1 page). Ordering information is given on any current masthead page.

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Reaction of 9-(β-D-Ribofuranosyl)purine with Alkalies: Kinetics and Mechanism[1]

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The progress of the alkaline hydrolysis of 9-(β-D-ribofuranosyl)purine has been studied by LC analyses, NMR spectroscopy, and isotopic labeling techniques. Comparison of the results of different experimental approaches reveals that the hydrolysis consists of three consecutive reactions, viz., transformation of the starting material to 5-formamido-4-ribosylaminopyrimidine, its deformylation to 5-amino-4-ribosylaminopyrimidine, and the hydrolysis of the latter intermediate to free sugar and 4,5-diaminopyrimidine. Both of the intermediates involved have been shown to be equilibrium mixtures of anomeric furanoid and pyranoid derivatives. Pseudo-first-order rate constants have been determined for the consecutive reactions at different temperatures and hydroxide ion concentrations. The role that the glycosyl hydroxyl groups play in different stages of the hydrolysis reaction has been elucidated by comparing the kinetic data with those observed for 9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)purine. The mechanisms for the consecutive reactions have been discussed.

Introduction

Several methods employed for the determination of nucleotide distribution in nucleic acids involve treatment of nucleic acids in alkali.[2-5] For this reason quantitative information about the degradation of the monomeric constituents, nucleosides, in basic solutions is desirable. Particularly purine nucleosides have been shown to be relatively susceptible to the action of hydroxide ion.[6-11] However, the data on the kinetics and mechanisms of their alkaline solvolyses are very limited.[11] One of the most labile nucleosides is unsubstituted 9-(β-D-ribofuranosyl)-purine.[6] Brown et al. have presented paper chromatographic and UV spectroscopic evidence for the appearance of 5-formamido-4-ribosylamino- and 5-amino-4-ribosylaminopyrimidine during the alkaline cleavage of 9-(β-D-ribofuranosyl)purine[6] and its 5′-monophosphate.[12] Presumably, nucleophilic attack of hydroxide ion at C8 of the starting material results in opening of the imidazole ring. Deformylation of the resulting 5-formamido derivative and rupture of the ribosyl–nitrogen bond would give 4,5-diaminopyrimidine as the final reaction product. The aim of the present study is to verify the suggested, partly tentative pathway by NMR, LC, and isotopic labeling studies. Kinetics of the consecutive partial reactions are determined as a function of temperature and hydroxide ion concentration. The role of the ribosyl hydroxyl groups is examined by comparing the kinetics of the hydrolysis of 9-(β-D-ribofuranosyl)purine and its 2′,3′-*O*-isopropylidene derivative. The factors affecting the accumulation of the intermediates are discussed on the bases

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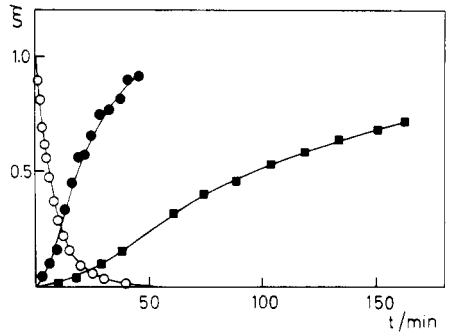


Figure 1. Release of the $^{14}C8$ atom from 9-(β-D-ribofuranosyl)[8-^{14}C]purine (●) and [1-^{14}C]D-ribose from 9-([1′-^{14}C]β-D-ribofuranosyl)purine (■) in 0.15 mol dm^{-3} aqueous sodium hydroxide at 343.2 K. Disappearance of the starting materials (○).

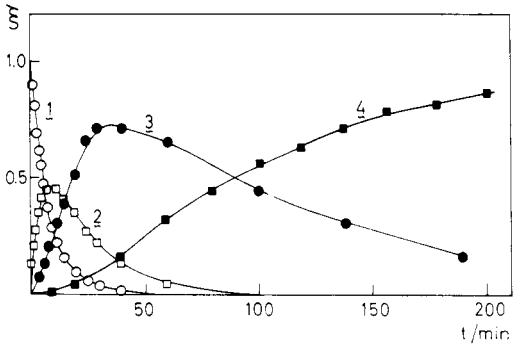


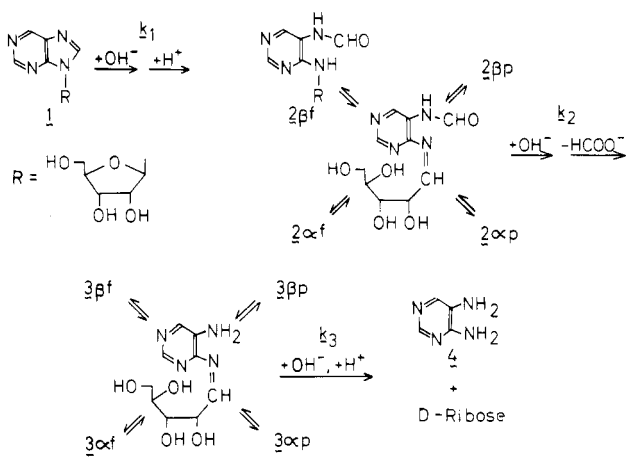
Figure 2. Appearance of isomeric 5-formamido-4-ribosylaminopyrimidines (□), 5-amino-4-ribosylaminopyrimidines (●), and 4,5-diaminopyrimidine (■) during the hydrolysis of 9-(β-D-ribofuranosyl)purine (○) in 0.15 mol dm^{-3} aqueous sodium hydroxide at 343.2 K.

of the results observed earlier[13,14] for 9-(1-alkoxyalkyl)-purines.

Results and Discussion

Figure 1 shows the results of the kinetic studies with isotopically modified 9-(β-D-ribofuranosyl)purines in 0.15 mol dm^{-3} aqueous sodium hydroxide. The disappearance of the starting material (1) obeys strictly first-order kinetics, the half-life being about 340 s at 343.2 K. In contrast, the release of the $^{14}C8$ atom from 9-(β-D-ribofuranosyl)[8-^{14}C]purine occurs much slower, and the extent of the reaction depends sigmoidally on time. Accordingly, the decomposition of the starting material must be followed by accumulation of an intermediate, which still contains the $^{14}C8$ atom. The existence of such an intermediate is clearly seen by the LC analyses of the reaction mixture. Comparison of the distribution curves depicted in Figures 1 and 2 reveals that the release of the $^{14}C8$ atom is preceded by formation of a relatively stable intermediate, indicated by **2** in Figure 2. In fact, the LC analyses show that this initial product is a mixture of four different species, the concentration ratios of which remain constant during the kinetic run. Their UV spectra are identical and closely resemble that reported for 4-amino-5-formamidopyrimidine.[15] However, the ribosyl moiety must still be present in compounds **2**, since they are formed much faster than the ribosyl group is released from 9-([1′-^{14}C]β-D-ribofuranosyl)purine, as can be seen from Figure 1. Most

Scheme I



probably nucleophilic attack of a hydroxide ion yields 5-formamido-4-(β-D-ribofuranosyl)aminopyrimidine (**2**βf), which is rapidly anomerized to α-furanosyl and α- and β-pyranosyl derivatives, indicated by **2**αf, **2**αp and **2**βp in Scheme I.

^{1}H NMR spectroscopic measurements lend additional support to the preceding suggestion. Diminution of the purine proton signals at δ 8.67, 8.79, and 8.90 (in D_2O) was observed to be accompanied with appearance of three singlets at δ 8.27, 8.40, and 8.44. For comparison, the protons of 4-amino-5-formamidopyrimidine resonate at δ 8.30, 8.45, and 8.48. Simultaneously the anomeric proton doublet at δ 6.17 was decreased with concomitant formation of four new signals in the anomeric proton region, viz., a broad singlet at δ 5.47 and a doublet at δ 5.54 (J = 9 Hz), 5.80 (J = 6 Hz), and 5.95 (J = 3 Hz). The last two signals were weak and probably refer to the thermodynamically less stable furanoid derivatives. It is generally known that carbohydrates having five-membered rings exhibit their anomeric proton signals at a lower field than their counterparts having six-membered rings.[16] The major components, assumingly α- and β-pyranosyl derivatives, were separated preparatively by LC and characterized by ^{13}C NMR spectroscopy. As seen from Table I, the chemical shifts of the ribosyl carbons closely resemble those observed[17] for anomeric N-phenyl-D-ribopyranosylamines, inconsistence with the proposed structure of intermediate **2**.

The rapid anomerization of **2**βf under alkaline conditions is expected, since it is a glycosylamine derived from a primary amine. It has been shown that this kind of compounds are anomerized rapidly in basic solutions via acyclic Schiff bases.[18-20] For example, the second-order rate constant for the base-catalyzed anomerization of anomeric N-(4-methylphenyl)-D-glucopyranosylamines has been reported to be 3.6 × 10^{-2} dm^3 mol^{-1} s^{-1} at 298.2 K.[19] At the temperatures of the present investigation the anomerization would thus be completed in a few seconds. In contrast, no anomerization of the starting material (1) is observed under the conditions employed. This is expected, since the acyclic Schiff bases derived from nucleosides are much less stable than those derived from glycosylamines. The crucial factor that facilitates the opening of the glycon ring with glycosylamines is the mesomeric electron release of the amino nitrogen. In nucleosides the corresponding

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Table I. ^{13}C NMR Chemical Shifts[a] for the Intermediates of the Alkaline Hydrolysis of 9-(β-D-Ribofuranosyl)purine

compd	C1′	C2′	C3′	C4′	C5′	base moiety
2αp[b]	78.2	69.8	68.7	67.3	63.5	164.4, 156.6, 156.4, 152.6, 115.9[c]
2βp	77.9	70.7	69.4	66.7	63.8	164.5, 156.6, 156.5, 152.7, 115.8
3αp	78.8	69.5	69.5	67.7	64.9	153.6, 148.8, 138.1, 127.4[d]
3βp	78.1	70.8	69.4	66.7	63.8	153.9, 148.4, 137.5, 127.6
e	81.3	69.9	69.9	67.8	62.1	
f	81.6	70.4	70.1	67.4	63.1	

[a] Taken as ppm from Me_4Si in D_2O. [b] See Scheme I. [c] For 4-amino-5-formamidopyrimidine in Me_2SO-d_6 165.6, 162.5, 160.1, 154.4, 120.9. [d] For 4,5-diaminopyrimidine in D_2O 157.6, 151.9, 141.4, 128.9. [e] For *N*-phenyl-α-D-ribopyranosylamine in Me_2SO-d_6.[17] [f] For *N*-phenyl-β-D-ribopyranosylamine in Me_2SO-d_6.[17]

nitrogen atom is part of an aromatic ring system, and hence the electron release is impeded.

Comparison of the data in Figures 1 and 2 reveals that the release of the formyl group from intermediate **2** is followed by accumulation of another intermediate, indicated by **3**, which still contains the glycosyl moiety. LC analyses show that this intermediate is also a mixture of four different species, which are in a rapid equilibrium with each other. Their UV spectra are identical and resemble that of 4,5-diaminopyrimidine.[21] The major components, which exhibit the anomeric proton signals at δ 5.51 (d, J = 9 Hz) and 5.54 (br s), were separated preparatively by LC and characterized by ^{13}C NMR. The shifts of the ribosyl carbons (Table I) are almost identical with those observed for intermediate **2**, but the N^5-CHO signal at 164.5 is absent. The two minor components, exhibiting the anomeric proton signals at δ 5.81 (d, J = 6 Hz) and 6.02 (d, J = 3 Hz), are probably the corresponding furanoid isomers **3**αf and **3**βf. The conversion of mixture **2** to mixture **3** thus simply involves the departure of the *N*-formyl group as formate ion (Scheme I).

Further examination of Figures 1 and 2 shows that the last stage in the alkaline hydrolysis of 9-(β-D-ribofuranosyl)purine is the release of the ribosyl group with concomitant formation of 4,5-diaminopyrimidine (**4**). UV, LC, and ^1H NMR analyses show that the conversion of the starting material to **4** is quantitative.

LC analyses of the reaction mixtures of 9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)purine indicated that the reaction pathway for its alkaline cleavage is similar to that described above for 9-(β-D-ribofuranosyl)purine.

Figure 3 shows the first-order rate constants, k_1, obtained at different hydroxide ion concentrations for the disappearance of 9-(β-D-ribofuranosyl)purine. At [OH^-] < 0.1 mol dm^{-3} the plot of k_1 vs. [OH^-] is curvilinear, but turns linear at higher alkalinities. The marked curvature cannot be accounted for by common salt effects. For example, the rate constant obtained in 0.05 mol dm^{-3} sodium hydroxide was increased by less than 5%, when the ionic strength was adjusted to 0.2 mol dm^{-3} with sodium chloride. In the whole basicity range studied **2** was the first product detected. Accordingly, the reaction pathway is not changed with the hydroxide ion concentration. Since blocking of the 2′- and 3′-hydroxyl groups with an isopropylidene group makes k_1 proportional to [OH^-], as seen from Figure 3, it appears clear that ionization of either of these groups is responsible for the curvilinear dependence of k_1 on [OH^-]. The acidity constant for the 2′-hydroxyl group of purine nucleosides is known to be of the order of 10^{-12} mol dm^{-3} at 298.2 K.[22-25] Taking the ionization

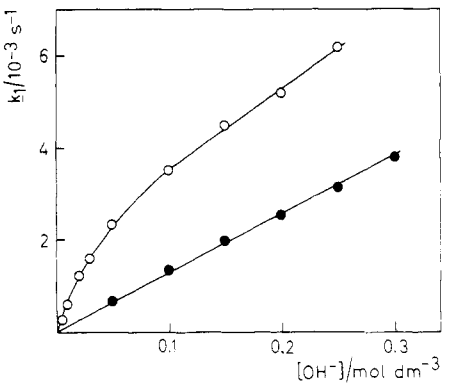

Figure 3. First-order rate constants for the disappearance of 9-(β-D-ribofuranosyl)purine (○) and its 2′,3′-*O*-isopropylidene derivative (●) in aqueous sodium hydroxide at 353.2 K and 333.2 K, respectively.

of this group into account, the rate law for the hydrolysis reaction can be described by eq 1, where k_1(SH) and k_1(S^-)

$$-\frac{d[S(total)]}{dt} = k_1(SH)[SH][OH^-] + k_1(S^-)[S^-][OH^-] \quad (1)$$

denote the second-order rate constants for decomposition of the neutral and anionic form of the substrate, respectively. When [SH] and [S^-] are expressed in terms of K and [S(total)], defined by eq 2 and 3, eq 4 is obtained for

$$K = \frac{[S^-]}{[SH][OH^-]} \quad (2)$$

$$[S(total)] = [SH] + [S^-] \quad (3)$$

$$k_1 = \frac{k_1(SH)[OH^-] + k_1(S^-)K[OH^-]^2}{K[OH^-] + 1} \quad (4)$$

the observed first-order rate constant, k_1. The latter equation can easily be transformed to eq 5, which indicates that the left hand side of eq 5 should be linearly related to [OH^-]. Least-squares fitting by various values of K

$$k_1\frac{K[OH^-] + 1}{K[OH^-]} = k_1(S^-)[OH^-] + \frac{k_1(SH)}{K} \quad (5)$$

shows that K = 35 dm^3 mol^{-1} gives the best linear correlation. The slope and intercept of the correlation line yield the values of (1.9 ± 0.1) × 10^{-2} and (8.0 ± 0.5) × 10^{-2} dm^3 mol^{-1} s^{-1} for k_1(S^-) and k_1(SH), respectively.

We have shown previously that the alkaline cleavage of 9-(1-alkoxyalkyl)purines proceeds by initial rate-limiting attack of hydroxide ion at C8 of the purine ring.[13,14] Most probably the situation is the same in the hydrolysis of purine riboside. The kinetic data treated above indicate that the neutral substrate is attacked four times more readily than its 2′-oxy anion. The lower reactivity of the

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Table II. First-Order Rate Constants at Different Temperatures and Hydroxide Ion Concentrations for the Consecutive Steps of the Alkaline Hydrolysis of 9-(β-D-Ribofuranosyl)purine[f]

T, K	[OH⁻], mol dm⁻³	$10^{-3}k_1$, s⁻¹	$10^{-3}k_2$, s⁻¹		$10^{-3}k_3$, s⁻¹	
353.2	0.20	5.24 ± 0.08[a]	2.57[b]		0.514[c]	
343.2	0.050	0.934 ± 0.015	0.429	0.393[d]	0.0490	0.0472[e]
	0.10	1.50 ± 0.018	0.742	0.722	0.107	0.119
	0.15	2.05 ± 0.02	1.24	1.18	0.161	0.150
	0.20	2.47 ± 0.02	1.57	1.53	0.197	0.220
333.2	0.20	1.18 ± 0.02	0.802		0.105	
323.2	0.20	0.397 ± 0.017	0.384		0.0571	

[a] Calculated from the LC data by eq 6. [b] Calculated from the LC data by eq 7. [c] Calculated from the LC data by eq 9. [d] Calculated from the radiochemical data by eq 8. [e] Calculated from the radiochemical data by eq 9. [f] The rate constants k_1, k_2, and k_3 refer to the partial reactions indicated in Scheme I.

latter species probably results from the electrostatic repulsion between the anionic substrate and the attacking nucleophile.

Application of the rate law for two consecutive first-order reactions enables the calculation of the rate constants, k_2, for the disappearance of the equilibrium mixture of isomeric compounds **2**. The results based on the LC data are collected in Table II. The rate constants calculated from the release of the ^{14}C8 atom from 9-(β-D-ribofuranosyl)[8-^{14}C]purine are listed in the same table. The two sets of values agree within the limits of experimental errors. Rate constants, k_2, are, in contrast to k_1, proportional to the hydroxide ion concentration, indicating that ionization of the ribosyl hydroxyl groups doesn't play any important role in the decomposition of **2**. Presumably destruction of the purine ring and isomerization to ribopyranosyl derivatives reduce the acidity of the 2′-hydroxyl group. The tentative mechanism for the deformylation stage consists of a nucleophilic attack of hydroxide ion at the carbonyl carbon of the formyl group and subsequent departure of a formate ion. The reaction may thus be compared to the alkaline deformylation of 4-amino-5-formamidopyrimidine, the rate constants of which are given in Table III. Obviously insertion of a ribosyl group at the 4-amino group of the latter compound has only a minor effect of the rate of the deformylation reaction. However, with 4-amino-5-formamidopyrimidine a pH-independent intramolecular cyclization to purine competes with the displacement of the formyl group. At low base concentrations the proportion of this reaction becomes marked. It remains obscure whether such a reaction takes place with the corresponding 4-ribosyl derivatives **2**. It appears, however, reasonable to assume that the 4-ribosylamino group is a less efficient intramolecular nucleophile than the 4-amino group. The fact that 9-(β-D-ribofuranosyl)purine is not isomerized during hydrolysis supports the latter argument.

The rate constants calculated for the decomposition of compounds **3** via the rate law of three concecutive first-order reactions are also included in Table II. The values based on the formation of 4,5-diaminopyrimidine agree satisfactorily with those obtained for the release of [1-^{14}C]ribose from 9-([1′-^{14}C]-β-D-ribofuranosyl)purine. Again the first-order rate constants are proportional to the hydroxide ion concentration. The situation is thus analogous to the alkaline hydrolysis of *N*-arylglycosylamines, which shows linear pH–rate profiles at [OH⁻] > 0.01 mol dm⁻³.[26] Most probably the reaction proceeds via the acyclic Schiff base, and the mechanism described for the Schiff bases

Table III. First-Order Rate Constants for the Conversion of 4-Amino-5-formamidopyrimidine to 4,5-Diaminopyrimidine and Purine in Aqueous Sodium Hydroxide at 343.2 K

[OH⁻], mol dm⁻³	$10^{-3}k$, s⁻¹		
	a	*b*	*c*
0.050	0.504 ± 0.003	0.306	0.198
0.10	0.773 ± 0.007	0.583	0.190
0.15	1.13 ± 0.03	0.913	0.217
0.20	1.27 ± 0.03	1.10	0.173

[a] For the disappearance of 4-amino-5-formamidopyrimidine. [b] For the formation of 4,5-diaminopyrimidine. [c] For the formation of purine.

Table IV. First-Order Rate Constants at Different Hydroxide Ion Concentrations for the Consecutive Steps of the Alkaline Hydrolysis of 9-(2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)purine at 333.2 K[d]

[OH⁻], mol dm⁻³	$10^{-3}k_1$, s⁻¹	$10^{-3}k_2$, s⁻¹	$10^{-3}k_3$, s⁻¹
0.050	0.648 ± 0.009[a]	0.129[b]	0.0164[c]
0.10	1.33 ± 0.01	0.225	0.0290
0.15	2.00 ± 0.03	0.385	0.0368
0.20	2.60 ± 0.02	0.500	0.0528

[a] Calculated from the LC data by eq 6. [b] Calculated from the LC data by eq 7. [c] Calculated from the LC data by eq 9. [d] The rate constants k_1, k_2, and k_3 refer to the partial reactions indicated in Scheme I.

of aromatic amines[27] is utilized. Accordingly, rate-limiting attack of hydroxide ion at C1′ of the acyclic ribosyl moiety leads to formation of a carbinolamine, which is rapidly heterolyzed to free sugar and 4,5-diaminopyrimidine.

The kinetic data for the alkaline hydrolysis of 9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)purine are listed in Table IV. The second-order rate constant for the decomposition of the starting material falls between the values reported above for the disappearance of 9-(β-D-ribofuranosyl)purine and its 2′-oxy anion. The effects of the 2′,3′-*O*-isopropylidene group on the rate constants, k_2 and k_3, for the subsequent partial reactions are rate retarding but small. Evidently the nucleophilic attack of the hydroxide ion is retarded sterically.

We have shown previously[13,14] that 5-formamido derivatives are not accumulated during the alkaline hydrolysis of 9-(1-alkoxyalkyl)purines. However, this is what happens with purine riboside. The observed difference may partly result from the fact that purine riboside is attacked by hydroxide ion about three times more readily than, for example, 9-(1-ethoxyethyl)purine. If the decomposition rates of the resulting intermediates do not differ as much, the formamido compounds are more markedly accumulated in the hydrolysis of 9-(β-D-ribofuranosyl)purine. Moreover, the recyclization of the formamido intermediate to the starting material may play a more important role with 9-(1-alkoxyalkyl)purines than with the corresponding ribofuranosyl derivative. As mentioned above, the ribosyl group probably reduces the ability of the 4-amino group to act as an intramolecular nucleophile. Possibly the influence of an alkoxyalkyl group is not as marked. In summary, the present investigation corroborates the earlier suggestions[6] concerning the mechanism for the alkaline cleavage of 9-(β-D-ribofuranosyl)purine. A complete kinetic description for the multistage reaction is given.

Experimental Section

Materials. 9-(β-D-Ribofuranosyl)purine employed in kinetic studies, and 4,5-diaminopyrimidine employed as reference material

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Table V. Retention Times for the Compounds Detected during the Alkaline Hydrolysis of 9-(β-D-Ribofuranosyl)purine, Its 2′,3′-O-Isopropylidene Derivative, and 4-Amino-5-formamidopyrimidine[a]

compd	t_R, min			
	90/10[b]	80/20	60/40	20/80
9-(β-D-ribofuranosyl)purine	3.6	2.4		
isomeric 5-formamido-4-ribosylaminopyrimidines	2.4	2.1		
	2.7	2.1		
	2.9[c]	2.1		
	3.1[c]	2.1		
isomeric 5-amino-4-ribosylaminopyrimidines	3.9	2.7[c]		
	3.9	2.9		
	4.7	3.1		
	6.2	3.6[c]		
9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine			3.2	
isomeric 5-formamido-4-(2′,3′-O-isopropylideneribosyl)aminopyrimidines			2.1[c]	
			2.3[c]	
			2.5	
			2.7	
isomeric 5-amino-4-(2′,3′-O-isopropylideneribosyl)aminopyrimidines			3.1[c]	
			3.3[c]	
			3.9	
			6.2	
4-amino-5-formamidopyrimidine				2.2
4,5-diaminopyrimidine				4.5
purine				2.5

[a] On a Hibar column (250 mm, 4-mm diameter) packed with LiChrosorb RP-18 (10 μm). Flow rate 1 cm^3 min^{-1}. [b] Composition of the eluant expressed as the ratio of the volumes of the acetic acid buffer (pH 4.4) and acetonitrile. [c] Minor components in the isomeric mixture.

were commercial products of Sigma Chemical Company. The purity of the compounds was checked by LC, and they were used as received.

9-([1′-^{14}C]β-D-Ribofuranosyl)purine was prepared as its triacetate by fusing the isotopically modified tetra-O-acetyl-β-D-ribofuranose with purine (Sigma) at 180 °C in the presence of a catalytic amount of p-toluenesulfonic acid.[28] The product was deacetylated with sodium methoxide in methanol, as described for acetylated aryl glycofuranosides.[29] Crystallization from methanol yielded a compound, which was identical with authentic 9-(β-D-ribofuranosyl)purine by LC and UV and ^{1}H NMR spectroscopy. Tetra-O-acetyl-β-D-ribofuranose was synthesized from [1-^{14}C]D-ribose (NEN) according to the method of Guthrie and Smith.[30]

9-(β-D-Ribofuranosyl)[8-^{14}C]purine was prepared as described above using [8-^{14}C]purine and tetra-O-acetyl-β-D-ribofuranose (Fluka) as starting materials. [8-^{14}C]Purine was obtained by refluxing 4,5-diaminopyrimidine in [^{14}C]formic acid (NEN)[31] and cyclizing the resulting 5-formamido derivative in boiling formamide.[32] The preparation of 4-amino-5-formamidopyrimidine used in kinetic measurements has been described earlier.[33]

9-(2′,3′-O-Isopropylidene-β-D-ribofuranosyl)purine was prepared by acid-catalyzed condensation of commercial 9-(β-D-ribofuranosyl)purine with acetone.[34] The product was homogenous, as deduced by LC and ^{1}H NMR spectroscopy.

NMR Spectroscopic Measurements. The progress of the alkaline cleavage of 9-(β-D-ribofuranosyl)purine was followed by ^{1}H NMR spectroscopy as follows. Samples of 1 mmol of the starting material were hydrolyzed at different times in 10 cm^3 of aqueous sodium hydroxide (0.1 mol dm^{-3}) at 343.2 K. Cooled solutions were neutralized with aqueous hydrogen chloride and evaporated to dryness under reduced pressure. Deuterium oxide was added and the evaporation repeated to deuterate the rapidly exchangeable protons. The residues were dissolved in 0.6 cm^3 of deuterium oxide containing *tert*-butyl alcohol as internal standard. The spectra were recorded on a Jeol JNM-PMX 60 spectrometer.

The ^{13}C NMR spectra for the intermediates of the alkaline hydrolysis were obtained as follows. The reaction mixture neutralized at an appropriate time was lyophilized and the residue was dissolved in water (200 mg cm^{-3}). The resulting solution was fractionated in 20-mm^3 portions by preparative reversed-phase LC (Varian Aerograph 5000, UV-100 detector) on a Spherisorb RP-18 column (250 mm, 8-mm diameter, particle size 5 μm). The elution (1 cm^3 min^{-1}) was carried out with acetic acid buffer (0.05 mol dm^{-3}, pH 5.7) containing 7% acetonitrile. The fractions obtained were lyophilized and the spectra recorded in D_2O on a Jeol GX-400 spectrometer.

Kinetic Studies with Isotopically Modified Compounds. The hydrolyses of 9-(β-D-ribofuranosyl)[8-^{14}C]purine at different hydroxide ion concentrations were carried out in stoppered bottles immersed in a water bath, the temperature of which was kept constant within 0.05 K. The initial substrate concentration was 2×10^{-3} mol dm^{-3}. The release of the ^{14}C8 atom was followed by treating the aliquots (5 cm^3) withdrawn at suitable intervals with a strong cation exchange resin (Dowex 50W X2, mesh 100–200, H^+ form) until complete neutralization took place. The unreacted starting material and all nitrogen containing degradation products were retained in the resin, as deduced by UV spectroscopy. The radioactivities of the solutions, containing the released ^{14}C8 atom, were determined by liquid scintillation counting (LKB 81000 counter) in lumagel. The cpm values obtained were corrected to a constant quenching by the method of external standardization.

The release of the ^{14}C8 atom was also followed by passing the aliquots through a strong anion exchange resin (Dowex 1X2, mesh 100–200, Cl^- form). Besides the hydroxide ions, the released ^{14}C8 atoms were retained in the resin, probably as formate ions. The radioactivities of the eluates, containing the starting material and the neutral and cationic products, were determined by liquid scintillation counting as described above.

The hydrolyses of 9-([1′-^{14}C]β-D-ribofuranosyl)purine were performed and the aliquots treated with a strong cation exchange resin as indicated in the foregoing. The starting material and all nitrogen containing products were attached to the resin and the amount of the released sugar was determined from the solutions by liquid scintillation counting.

Kinetic Studies by LC. The hydrolyses of 9-(β-D-ribofuranosyl)purine, its 2′,3′-O-isopropylidene derivative and 4-amino-5-formamidopyrimidine were carried out as described for the isotopically modified purine ribosides. However, the initial substrate concentrations were of the order of 10^{-4} mol dm^{-3}. The compositions of the aliquots (1 cm^3) neutralized with acetic acid were determined by reversed-phase LC using a Hibar column (250

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mm, 4-mm diameter) packed with LiChrosorb RP-18 (10 μm) and a variable wavelength UV detector. Isocratic elution (1 cm^3 min^{-1}) with mixtures of acetonitrile and acetic acid buffer (pH 4.4) was employed throughout, the detection wavelength being generally 260 nm. The retention times observed are given in Table V.

Calculation of the Rate Constants. The first-order rate constants, k_1, for the disappearance of the starting materials were calculated from eq 6, where $[S(total)]_0$ is the initial substrate

$$\ln\frac{[S(total)]_0}{[S(total)]_t} = k_1 t \quad (6)$$

concentration and $[S(total)]_t$ the concentration at the moment t. The first-order rate constants, k_2, for the disappearance of the first accumulated intermediate, **2**, in the hydrolysis of 9-(β-D-ribofuranosyl)purine and its 2′,3′-*O*-isopropylidene derivative were obtained by least-squares fitting by eq 7. Here $[B]_t$ denotes the

$$\frac{[B]_t}{[B]_T} = \frac{e^{-k_1 t} - e^{-k_2 t}}{e^{-k_1 T} - e^{-k_2 T}} \quad (7)$$

concentration of **2** at the moment t, and $[B]_T$ is the maximum concentration reached at time t = T. The first-order rate constants, k_2, for the cleavage of the $^{14}C8$ atom from 9-(β-D-ribofuranosyl)[8-^{14}C]purine were obtained by least-squares fitting by eq 8, where $[H^{14}COO^-]$ stands for the concentration of the formate

$$[H^{14}COO^-] = [S(total)]_0\left(1 - \frac{k_2}{k_2 - k_1}e^{-k_1 t} + \frac{k_1}{k_2 - k_1}e^{-k_2 t}\right) \quad (8)$$

ion released at the moment t. First-order rate constants, k_3, for the formation of 4,5-diaminopyrimidine and the release of D-ribose from 9-([1′-^{14}C]β-D-ribofuranosyl)purine were calculated by least-squares fitting by eq 9. [P] stands for the concentration of 4,5-diaminopyrimidine or D-ribose formed at the moment t.

$$[P] = [S(total)]_0\left(1 - \frac{k_2 k_3}{(k_2 - k_1)(k_3 - k_1)}e^{-k_1 t} - \frac{k_1 k_3}{(k_1 - k_2)(k_3 - k_2)}e^{-k_2 t} - \frac{k_1 k_2}{(k_1 - k_3)(k_2 - k_3)}e^{-k_3 t}\right) \quad (9)$$

First-order rate constants for the formation of purine and 4,5-diaminopyrimidine from 4-amino-5-formamidopyrimidine were obtained by multiplying the rate constant for the disappearance of the starting material by the appropriate mole fractions of the products at infinite time.

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Oxidation of *N,N′*-Dialkyl-1,2-bishydroxylamines

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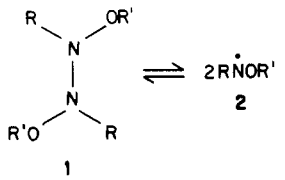
Oxidation of *N,N′*-dialkyl-1,2-bishydroxylamines $RNHOCH_2CH_2ONHR$ gives products that are a function of oxidant. For R = *i*-Pr (**5**) and *m*-chloroperbenzoic acid the products are diisopropyl azo dioxide, $HOCH_2CH_2ONHCH(CH_3)_2$, and ethylene glycol. Product ratios indicate independent oxidation of the two hydroxylamine functions. Na_2WO_4/H_2O_2 in D_2O gives the azo dioxide, ethylene glycol, and acetone oxime. Oxidation with bromine and 1,4-diazabicyclo[2.2.2]octane or *tert*-butyl hypochlorite and triethylamine gives product ratios that are consistent with two pathways to the bis oxime **7**: one that appears to give **7** directly and a second that goes through the monohydroxylamine monooxime **15**. It is proposed that the former reaction proceeds through the 1,4,2,3-dioxadiazine ring, which is oxidized quickly to products. Reaction products from the bishydroxylamine with R = CH_3 are similar to those with R = *i*-Pr.

Dialkoxyhydrazines **1** are a little known class of generally labile molecules.[1] The *N,N′*-dialkyl derivatives have not been seen, but their existence has been inferred from kinetic and product studies of hydroxylamine free radicals **2**.[2] Kaba and Ingold have identified four pathways for



1 **2**

bimolecular self-reaction of **2** involving production of (a) nitrogen and the alcohol (R = H), (b) the azo compound and alcohol (R = Ph), (c) the azo compound, aldehyde, and alcohol (R = CHR_2, Russell fragmentation), and (d) the hydroxylamine and oxime (R′ = CH_2Ph, disproportionation). Thus the *N,N′*-dialkyl-*N,N′*-dialkoxyhydrazines have the interesting property that they exist partly or largely as the hydroxylamine free radical but decompose to give products which generally retain the N–N bond.

One way of forcing the equilibrium toward the hydrazine form is to connect the two hydroxylamine radicals, i.e., to synthesize a cyclic dialkoxyhydrazine. A particularly interesting member of this class would be the six-membered dioxadiazine **3**.[3] If it were to decompose with retention of the N–N bond, an azo compound and the 1,4-dioxygen diradical **4** would be formed. Diradical **4** is the intermediate in dioxetane chemiluminescence.[4,5] On the other

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