homogenized at 4 °C in 5–7 volumes of medium containing sucrose (250 mM), EDTA (1 mM), Tris (25 mM), pH 7.4, and BSA (10 mg/mL). The homogenate was centrifuged at 600g for 10 min, and the resulting supernatant was centrifuged at 15000g for 10 min to sediment mitochondria. Mitochondria were suspended in a buffer of sucrose (200 mM), $MgCl_2$ (5 mM), KCl (20 mM), EDTA (0.2 mM), Tris (25 mM), BSA (1 mg/mL), and Na_2HPO_4 (10 mM), pH 7.4. Aliquots (1 mL) containing mitochondrial protein (between 100 and 1000 μg/mL) were combined (100:1) with sodium isocitrate (10 mM) and inhibitor (10:1) when relevant and preincubated at 37 °C for 15 min prior to addition of 1. The substrate was added from a stock solution (100 μM in methoxyethanol) to give a final concentration of 1–2 μM with a final solvent concentration of 1–2%. To establish the background (substrate) fluorescence the emission spectrum of the mitochondrial suspension was taken immediately following addition of 1 to the suspension. Between measurements the mitochondria were kept at 37 °C in a shaking water bath. Control samples which did not contain 1 were treated with 1–2% methoxyethanol. Inhibitors were added from stock solutions (10 and 100 mM) in water (pH 4, HCl). Some cloudiness was evident with ketoconazole when diluted into the mitochondrial incubate.

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Interconversion and Phosphoester Hydrolysis of 2′,5′- and 3′,5′-Dinucleoside Monophosphates: Kinetics and Mechanisms

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First-order rate constants for the interconversion and hydrolytic cleavage of several 2′,5′- and 3′,5′-dinucleoside monophosphates (UpU, UpA, ApU, ApA) have been determined over an acidity range from $H_0 = -0.2$ to $H_- = 12.4$ at 363.2 K. Both reactions proceed at comparable rates at pH < 2 and are of first order with respect to hydronium ion at pH < pK_a of the phosphate moiety (0.7) and second-order under less acidic conditions (pH 1–2). With dinucleoside monophosphates derived from adenosine, acid-catalyzed depurination of the starting material competes with the phosphate migration and phosphoester hydrolysis at pH < 3. The migration rates extrapolated to zero buffer concentration become pH-independent at pH > 4. Under these conditions the migration is considerably faster than the phosphoester hydrolysis, which exhibits acid catalysis at pH < 5 and base catalysis under more basic conditions. By contrast, hydrolysis of the 5′-phosphoester bond is the only reaction detected in alkaline solutions (pH > 8). The reaction is first order with respect to hydroxide ion at $[OH^-] < 0.01$ mol dm^{-3} and approaches zero-order dependence at higher alkalinities, where the unesterified 2′- or 3′-hydroxyl group becomes ionized. The mechanisms of different partial reactions, and the effects of base moiety structure (purine vs pyrimidine) on their rates are discussed. The data are compared to the known reaction kinetics of monoalkyl esters of adenosine 2′- and 3′-monophosphates.

Introduction

Internucleosidic 3′,5′-phosphodiester bonds play a central role in chemistry and biochemistry of nucleic acids. In particular, their hydrolytic reactions have been the subject of considerable interest as a model system for the action of ribonucleases. Breslow and co-workers have shown that uridylyl(3′,5′)uridine[1,2] and adenylyl(3′,5′)-adenosine[3] undergo under neutral or mildly acidic condition a buffer-catalyzed isomerization to the corresponding 2′,5′-dinucleoside monophosphates and a buffer-catalyzed hydrolysis to form a nucleoside cyclic 2′,3′-monophosphate with cleavage of the 5′-linked nucleoside. Both reactions have been shown to proceed through the same phosphorane intermediate obtained by nucleophilic attack of the neighboring 2′/3′-hydroxyl group on the tetracoordinated phosphorus atom.[2,3] Formation of this intermediate exhibits a specific-acid/general-base catalysis, its breakdown to isomeric dinucleoside monophosphates a general-acid catalysis, and cleavage of the 5′-linked nucleoside a specific-base/general-acid catalysis.

We have recently studied the buffer-independent isomerization and phosphoester hydrolysis of the monomethyl and monoisopropyl esters of adenosine 2′- and 3′-monophosphates over a wide acidity range ($H_0 = -0.2$ to pH 11).[4] The alkylphosphate migration and hydrolysis to cyclic 2′,3′-monophosphate and alcohol proceed at comparable rates under acidic conditions. At neutral pH a pH-independent phosphate migration prevails, in striking contrast to the buffer-catalyzed reactions investigated by Breslow.[1,2] By contrast, in alkaline solutions only phosphoester hydrolysis was observed to take place, consistent with earlier studies on hydrolysis of dinucleoside monophosphates.[5] In all likelihood both migration and hydrolysis proceed through a common phosphorane intermediate. However, in aqueous alkali, where no phosphate migration could be

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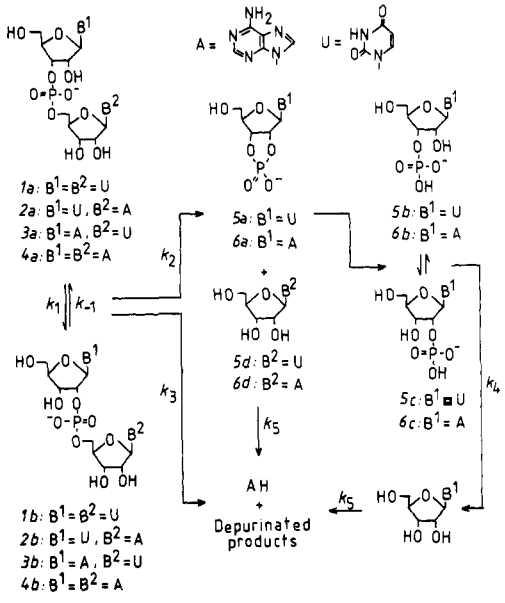
Scheme I



detected, the phosphoester hydrolysis may take place via a pentacoordinated transition state rather than a pentacoordinated intermediate. The recent ab initio calculations of Lim and Karplus[6] for hydrolysis of ethylene phosphate show that the dianionic phosphorane intermediate is too unstable to exist, in contrast to monoanionic phosphorane. The studies on isomerization and hydrolysis of adenosine 2'- and 3'-alkylphosphates under various conditions are now extended to dinucleoside monophosphates, which more closely mimic the structure of ribonucleic acids. Mechanisms of different partial reactions are discussed, and the effects of base moiety structure on their rates elucidated. The data are compared to those reported for monoalkyl[4] and monoaryl[7] esters of nucleoside 2'- and 3'-monophosphates.

Results and Discussion

Product Distributions. HPLC analyses of the aliquots withdrawn at appropriate intervals from acidic aqueous solutions ($[H^+] > 0.01$ mol dm^{-3}) of uridylyl(3',5')uridine (3',5'-UpU, **1a**), uridylyl(3',5')adenosine (3',5'-UpA, **2a**), adenylyl(3',5')uridine (3',5'-ApU, **3a**), and adenylyl(3',5')adenosine (3',5'-ApA, **4a**) revealed that all these compounds react in essentially the same manner: the starting material is partially rearranged to the corresponding 2',5'-isomer (**1b**, **2b**, **3b**, **4b**), and the resulting isomeric mixture is hydrolyzed to free 5'-linked nucleoside (**5d** or **6d**) and a mixture of nucleoside 2'- and 3'-monophosphates (**5b,c** or **6b,c**) derived from the 3'-linked nucleoside (Scheme I). The latter compounds are most probably formed via a cyclic 2',3'-monophosphate, either 2',3'-*c*UMP (**5a**) or 2',3'-*c*AMP (**6a**), the hydrolysis of which is fast enough to prevent its accumulation.[8-10] This assumption is supported by the fact that the 2'- and 3'-monophosphates are produced in exactly the same concentration ratio as in the much faster hydrolysis of 2',3'-*c*UMP and 2',3'-*c*AMP.[9-11] Moreover, the product composition was observed to be independent of the isomeric nature of the starting material, although the mutual isomerization of 2'- and 3'-monophosphates is too slow to result in complete equilibration under the reaction conditions.[10,11] Accordingly, it appears clear that dinucleoside monophosphates (**1-4**) undergo two concurrent reactions in aqueous acid: (i) interconversion between 2',5'- and 3',5'-isomers and (ii) hydrolysis to a nucleoside cyclic 2',3'-monophosphate with cleavage of the 5'-linked nucleoside. Dinucleoside monophosphates derived from adenosine (**2-4**) are additionally susceptible to depurination, as indicated by appearance of adenine and depurinated dinucleoside monophosphates among the products. At $[H^+] < 0.01$ mol dm^{-3}, nucleoside 2'- and 3'-monophosphates are rather rapidly dephosphorylated to the corresponding nucleoside.[10,11] For this reason, nucleosides were accumulated instead of nucleoside 2'/3'-monophosphates under neutral and slightly acidic conditions.

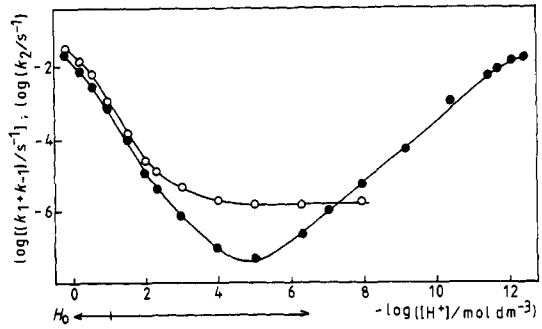


Figure 1. The pH–rate profile for the interconversion of 2',5'- and 3',5'-UpU ($k_1 + k_{-1}$, ○) and hydrolysis of 3',5'-UpU (k_2, ●) at 363.2 K. The ratio k_1/k_{-1} remained 1.0 ± 0.1 over the whole pH range studied.

The time-dependent product distribution was in aqueous alkali similar to all the dinucleoside monophosphates studied. No isomerization was observed. Disappearance of the starting material was accompanied with release of the 5'-linked nucleoside and the 2'- and 3'-monophosphates of the 3'-linked (or 2'-linked) nucleoside. The monophosphates were obtained in the same concentration ratio as in the alkaline hydrolysis of nucleoside cyclic 2',3'-monophosphates. At low hydroxide ion concentrations an intermediary appearance of the cyclic phosphate could even be detected.

Kinetics of the Interconversion and Hydrolysis of 2',5'- and 3',5'-UpU. Figure 1 shows the pH–rate profiles for hydrolysis and interconversion of 2',5'- (**1b**) and 3',5'-UpU (**1a**). The rate constants referring to buffer solutions (pH 3–8) are those extrapolated to buffer concentration zero. The rate profile of mutual isomerization is almost identical with that reported previously[4] for adenosine 2'- and 3'-methylphosphates. The reaction order with respect to hydronium ion is 1 on the acidic side of the phosphate pK_{a1} value (0.7), 2 at pH 1–2, and 0 at pH > 3. The only relevant difference is that between pH 2 and 3, a first-order dependence of rate on hydronium ion concentration is detected. The observed first-order rate constant, k_1, may thus be expressed by eq 1, where the

$$k_1 = \left[\frac{k_a}{K_{a1}}[H^+]^2 + \frac{k_b}{K_{a1}}[H^+] + k_c\right] \Big/ \left[1 + \frac{[H^+]}{K_{a1}}\right] \quad (1)$$

partial rate and equilibrium constants are those depicted in Scheme II. The first term in the numerator of the right-hand side of eq 1 refers to hydronium ion catalyzed isomerization of neutral phosphodiester (SH), the second one to hydronium ion-catalyzed reaction of monoanionic phosphodiester (S^-), and the last one to uncatalyzed reaction of the same species. An analogous equation may

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(8) Abrash, H. I.; Cheung, C.-C. S.; Davis, J. C. *Biochemistry* **1967**, *6*, 1298.
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Scheme II

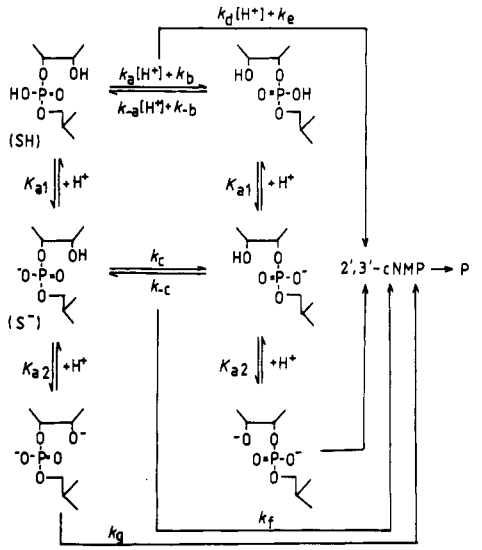


Table I. Partial Rate Constants for the Interconversion and Hydrolysis of 2',5'- and 3',5'-UpU and Adenosine 2'- and 3'-Methylphosphates at 363.2 K[a]

	UpU	AMP methyl esters[b]
pK_{a1}	0.7[c]	1.0[c]
$k_a/10^{-3}$ dm^3 mol^{-1} s^{-1}	13.3	3.2
$k_{-a}/10^{-3}$ dm^3 mol^{-1} s^{-1}	11.7	3.9
$k_b/10^{-3}$ s^{-1}	0.19	*d*
$k_{-b}/10^{-3}$ s^{-1}	0.16	*d*
$k_c/10^{-6}$ s^{-1}	0.66	1.3
$k_{-c}/10^{-6}$ s^{-1}	0.67	1.6
$k_d/10^{-3}$ dm^3 mol^{-1} s^{-1}	15.4	11.6
$k_e/10^{-3}$ s^{-1}	0.066	*d*
$k_f/10^{-6}$ s^{-1}	0.029	<0.1
k_g/s^{-1}[e]	0.017	
pK_{a2}[e]	11.5	

[a] The ionic strength adjusted to 0.10 mol dm^{-3} with sodium chloride. For the partial rate and equilibrium constants see Scheme II. [b] From ref 4. [c] For both isomers. [d] Could not be obtained with a reasonable accuracy. [e] For the 3',5'-isomer.

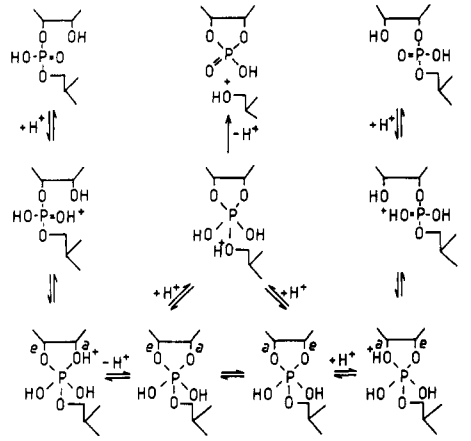
be written for the rate constant, k_{-1}, of the reverse reaction. The partial rate and equilibrium constants obtained by least-squares fitting[12] are listed in Table I.

The rate profile of the hydrolysis of isomeric UpUs is similar to that of their mutual isomerization at pH < 3, whereas under less acidic conditions these two reactions respond in a very different manner to changes in pH. As stated above, isomerization is pH independent at pH > 3. By contrast, hydrolysis is first order with respect to hydronium ion at pH 3–5 and first order with respect to hydroxide ion at pH 6–11. At higher alkalinities the hydrolysis rate levels off to a constant value. Accordingly, the observed first-order rate constant obeys eq 2. The first

$$k_2 = \left[\frac{k_d}{K_{a1}}[H^+]^2 + \frac{k_e}{K_{a1}}[H^+] + k_f + \frac{k_g K_{a2}}{[H^+]}\right] \Big/ \left[\frac{[H^+]}{K_{a1}} + 1 + \frac{K_{a2}}{[H^+]}\right] \quad (2)$$

three terms in the numerator of the right-hand side of eq 2 are equivalent to those in eq 1, while the last term refers to hydroxide ion catalyzed hydrolysis of monoanionic phosphodiester (S^-). Table I records the partial rate and equilibrium constants obtained by least-squares fitting.

Scheme III



Mechanisms of Phosphate Migration and Phosphoester Hydrolysis. According to Anslyn and Breslow,[2] the buffer-catalyzed hydrolysis and interconversion of 2',5'- and 3',5'-UpU proceed via a common pentacoordinated intermediate formed by a nucleophilic attack of the neighboring hydroxyl group on the tetracoordinated phosphorus atom. For the following reasons, this also seems to be the case with the corresponding hydronium ion catalyzed reactions. Firstly, the initial hydrolysis product of isomeric UpUs is 2',3'-cUMP and the reaction proceeds under acidic conditions two orders of magnitude faster than hydrolysis of dimethyl phosphate.[13] Both observations are consistent with nucleophilic attack of the neighboring hydroxyl group on the phosphorus atom of UpU and are difficult to explain without this kind of an intramolecular participation. Secondly, the pH–rate profiles of phosphate migration and phosphoester hydrolysis are very similar in the acidic range (Figure 1), suggesting that the products of hydrolysis and isomerization are formed from a common intermediate, most likely a pentacoordinated phosphorane.

The values of partial rate constants in Table I reveal that at high acid concentrations (pH < 2) the terms $(k_a/K_{a1})[H^+]^2$ and $(k_d/K_{a1})[H^+]^2$ are the predominant ones in eqs 1 and 2, respectively. Accordingly, both hydrolysis and mutual isomerization of 2',5'- and 3',5'-UpU proceed under these conditions by hydronium ion catalyzed reaction of neutral phosphodiester (SH). Most likely a rapid initial protonation of SH is followed by nucleophilic attack of the neighboring hydroxyl group on the phosphorus atom, resulting in formation of a pentacoordinated intermediate (Scheme III). According to the pseudorotation concept of Westheimer,[14] the attacking hydroxyl group adopts initially an apical and the esterified neighboring hydroxyl group an equatorial position. Since the electronegativity difference between 2'-O, 3'-O and 5'-O ligands is small, any one of them may subsequently adopt an apical position via pseudorotation of the phosphorane intermediate and hence leave after protonation. In fact, all these ligands depart approximately as readily, as shown by comparable values of the rate constants k_a, k_{-a}, and k_d. For comparison, with adenosine 2'- and 3'-methylphosphates the cleavage of methoxy group as methanol is three times as fast as cleavage of 2'- and 3'-hydroxyl groups.

At low acid concentrations (pH > 2), the terms $(k_b/K_{a1})[H^+]$ and $(k_e/K_{a1})[H^+]$ predominate, indicating that

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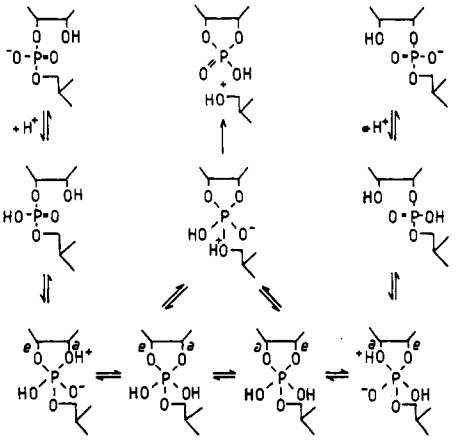
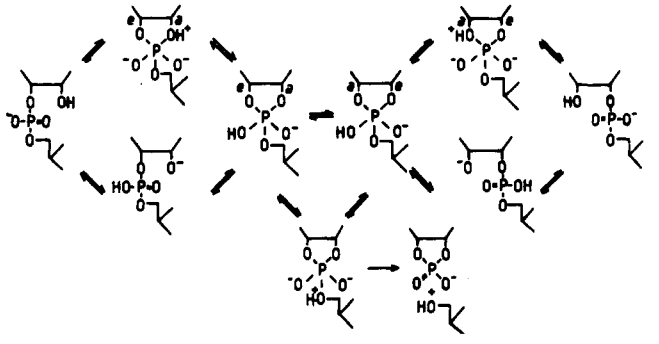
(14) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.

Scheme IV



Scheme V



the reaction involves a rapid initial protonation of the monoanionic phosphodiester (S^-) and subsequent attack of the neighboring hydroxyl group (Scheme IV). The rate constants k_b and k_{-b} are considerably higher than k_e, which means that the resulting phosphorane intermediate is decomposed faster to isomeric phosphodiesters than to 2',3'-*c*UMP and uridine. With adenosine 2'- and 3'-methylphosphates the existence of this reaction pathway could not be experimentally verified,[4] but with uridine 3'-(2-chlorophenyl)phosphate it constitutes the main route under moderately acidic conditions.[7]

Over a rather wide pH range from 4 to 7 the phosphate migration proceeds predominantly by uncatalyzed rearrangement of anionic phosphodiesters (S^-), analogous to isomerization of adenosine methylphosphates.[4] The values of rate constants, k_c and k_{-c}, indicate that the uncatalyzed migration of uridine 5'-phosphate group is slightly slower than that of methylphosphate group. Two kinetically indistinguishable mechanisms may be written for the reaction: (i) attack of unionized hydroxyl group on monoanionic phosphodiester grouping (upper route in Scheme V) or (ii) attack of ionized hydroxyl group on neutral phosphodiester grouping (lower route in Scheme V). For the reasons discussed previously,[4] the former alternative seems more attractive.

It is difficult to decide on the basis of available data whether the hydrolysis of isomeric UpUs also utilizes an uncatalyzed pathway, described above for the pH-independent migration. If this reaction really occurs, it must be at least 1 order of magnitude slower than the migration (compare k_c and k_{-c} with k_f). The situation was observed to be similar with adenosine 2'- and 3'-methylphosphates.[4] In other words, breakdown of the monoanionic phosphorane favors isomeric phosphodiesters over cyclic 2',3'-monophosphate (Scheme V), whereas breakdown of the neutral species yields comparable amounts of hydrolysis and isomerization products (Schemes III and IV). One

Scheme VI

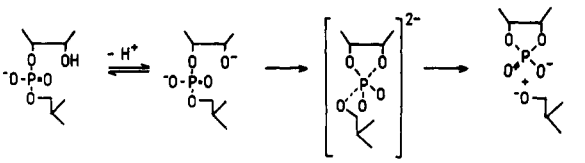


Table II. First-Order Rate Constants for the Mutual Isomerization and Hydrolysis of 2',5'- and 3',5'-Dinucleoside Monophosphates at Different Hydronium Ion Concentrations at 363.2 K[a]

compd	$[H^+]$ (mol dm^{-3})	k_1 (10^{-3} s^{-1})	k_{-1} (10^{-3} s^{-1})	k_2 (10^{-3} s^{-1})	k_3 (10^{-3} s^{-1})
UpU	1.0	15.1	13.9	19.0	
	0.03	0.068	0.072	0.094	
	1×10^{-5}	0.000 80	0.000 71	0.000 039	
UpA	1.0	5.43	5.43	20.0	3.1
	0.03	0.042	0.042	0.058	0.11
	1×10^{-5}	0.000 70	0.000 78	0.000 14	
ApU	1.0	5.38	4.97	11.3	2.85
	0.03	0.050	0.046	0.058	0.063
	1×10^{-5}	0.000 50	0.000 66	0.000 027	
ApA	1.0	5.04	5.31	15.0	2.35
	0.03	0.036	0.037	0.029	0.12
	1×10^{-5}	0.000 42	0.000 60	*b*	

[a] For the rate constants see Scheme I. [b] Could not be determined.

may tentatively assume that the anionic form of cyclic 2',3'-monophosphate is more strained than the protonated forms, the hence its formation is impeded.

Under alkaline conditions, 2',5'- and 3',5'-UpU are hydrolyzed by the mechanism described originally by Brown et al.[15] Accordingly, the 2'-(or 3'-)hydroxy group is ionized in a rapid initial stage, and the oxyanion formed then intramolecularly displaces the 5'-linked nucleoside (Scheme VI). No phosphate migration takes place, suggesting that the reaction proceeds *via* a pentacoordinated transition state rather than *via* a pentacoordinated intermediate. According to ab initio calculations on ethylene phosphate, the dianionic phosphorane intermediate is too unstable to exist.[6]

Effect of Base Moiety Structure on Reaction Kinetics. Table II records the first-order rate constants for the hydrolysis and interconversion of 2',5'- and 3',5'-isomers of UpU, UpA, ApU, and ApA at three different hydronium ion concentrations at 363.2 K. The data in Table I indicate that the hydrolysis and isomerization of dinucleoside monophosphates proceed at $[H^+] = 1.0$ mol dm^{-3} ($H_0 = -0.2$) by hydronium ion catalyzed reactions of neutral phosphodiester (Scheme III) at $[H^+] = 0.03$ mol dm^{-3} partly by this route and partly by hydronium ion catalyzed reactions of monoanionic phosphodiester (Scheme IV) and at $[H^+] = 1.0 \times 10^{-5}$ mol dm^{-3} by uncatalyzed reaction of the monoanionic phosphodiester. As seen, the effect of base moiety structure on the rates of these reactions is only a moderate one. Consistent with the semiquantitative observations of Witzel,[16] the dinucleoside monophosphates derived from 3'-UMP (or 2'-UMP) are hydrolyzed at high acid concentrations slightly faster than those derived from 3'-AMP. Under neutral and slightly acidic conditions experimental evidence for a similar conclusion is even more scanty. Accordingly, the data do not lend support to the suggestion[17] that O2 of

(15) Brown, D. M.; Magrath, D. I.; Neilson, A. H.; Todd, A. R. *Nature* **1956**, *177*, 1124.
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Table III. First-Order Rate Constants, k_2, for Hydrolysis of Dinucleoside Monophosphates in Aqueous Alkali at 333.2 K

	$k_2/10^{-3}$ (s^{-1})							
H_-[a]	2',5'-UpU	3',5'-UpU	2',5'-UpA	3',5'-UpA	2',5'-ApU	3',5'-ApU	2',5'-ApA	3',5'-ApA
11.71	0.96		1.11	1.01	0.51	0.61	0.72	0.45
12.01	1.72	1.61	1.98	1.71	0.93	0.96	1.32	0.72
12.32	2.91	2.53	3.28	3.17	1.48	1.32	2.15	1.04
12.73	5.40	4.11	6.81	4.27	2.48	1.69	3.70	1.43
13.04	8.01	5.24	10.14	5.79	3.45	1.78	5.13	1.68

[a] The H_- values determined at 298.2 K[18] were corrected with respect to the change of the ionic product of water on going to 333.2 K.[19]

3'-linked pyrimidine nucleoside would enhance the phosphoester hydrolysis and isomerization by hydrogen bonding with the 2'-hydroxyl group. The effects of base moiety structure appear to be small and rather nonsystematic.

Table II also includes rate constants for depurination of dinucleoside monophosphates derived from adenosine. With all these compounds depurination competes efficiently with the phosphate migration and phosphodiester hydrolysis in the acidic range. Since depurination of adenine nucleotides is a first-order reaction with respect to hydronium ion,[20] while phosphoester hydrolysis and phosphate migration exhibit a second-order dependence of rate on $[H^+]$ between pH 1 and 2, the proportion of depurination is more marked at low acid concentrations, i.e., around pH 2.

The first-order rate constants observed for the hydrolysis of dinucleoside monophosphates in aqueous alkali at 333.2 K are collected in Table III. Table IV, in turn, records the pK_{a2} values calculated for the unesterified 2'- or 3'-hydroxyl group, and the first-order rate constants, k_g, for cleavage of the ionized species (Scheme II). As seen, the 2'-OH of 3',5'-isomers is from 0.3 to 0.7 log units more acidic than the 3'-OH of 2',5'-isomers. In contrast, the 2'-O-ionized 3',5'-dinucleoside monophosphates are cleaved less readily than their 3'-O-ionized 2',5'-counterparts. Accordingly, at low hydroxide ion concentrations the hydrolytic stabilities of 2',5'- and 3',5'-isomers are usually almost equal, as also shown previously for adenosine 2'- and 3'-methylphosphates.[4]

The effect of base moiety structure on reactivity is more marked under alkaline than under acidic conditions. Dinucleoside monophosphates derived from 2'- or 3'-AMP are slightly more acidic than those derived from 2'- or 3'-UMP, the nature of the 5'-linked nucleoside playing a less important role. The enhanced acidity is, however, overcompensated by considerably slower heterolysis of the former compounds. Accordingly, UpU and UpA are hydrolyzed more rapidly than ApU and ApA at pH < pK_{a2}. Adenosine appears to leave slightly more readily than uridine. Comparison with the previous data[4] on hydrolysis of adenosine 2'- and 3'-methylphosphates shows that replacing the methyl group with a 5'-linked nucleoside enhances the phosphoester cleavage by a factor of ranging from 1.4 to 2.0.

In summary, 2',5'- and 3',5'-dinucleoside monophosphates undergo under acidic conditions competitive mutual isomerization and hydrolysis to free 5'-linked nucleoside and a mixture of 2'- and 3'-monophosphates of the 3'-linked nucleoside. Reactions proceed via a common phosphorane intermediate, which is formed at high acid concentrations by an intramolecular attack of the neighboring hydroxyl group on monocationic phosphodiester and at low acid concentrations by an attack on neutral phosphodiester. With dinucleoside monophosphates derived from adenosine depurination competes with these reactions. Under neutral conditions pH-independent isomerization of monoanionic phosphodiester prevails, whereas in alkaline solutions phosphoester hydrolysis is the only reaction detected. The latter reaction proceeds by nucleophilic attack of the ionized 2'-/3'-hydroxyl group on the monoanionic phosphodiester grouping with concomitant cleavage of the 5'-linked nucleoside. The effect of base moiety structure on rate of hydrolysis and isomerization is relatively small.

Experimental Section

Materials. The dinucleoside monophosphates used in kinetic measurements and the nucleic acid bases, nucleosides, and nucleotides employed as reference materials were commercial products of Sigma. They were used as received after checking their purity by HPLC.

Kinetic Measurements. Reactions were followed by the HPLC technique described previously[21] in detail. Chromatographic separations were carried out on a Hypersil ODS5 column (4 × 250 mm, 5 μm). An acetic acid/sodium acetate buffer (0.045/0.015 mol dm^{-3}, pH 4.2), containing 0.1 mol dm^{-3} ammonium chloride and 2–3% (v/v) acetonitrile, was employed as eluent. Complete separation of all reaction components was achieved under these isocratic conditions, the retention times being varied from 2 to 50 min. Signal areas were converted to concentrations with the aid of calibration solutions of known concentrations.

The hydronium ion concentrations of reaction solutions, adjusted with hydrogen chloride and sodium hydroxide, and formate, acetate, thiethanolamine, and glycine buffers, were calculated from pK_a values of the buffer acids under experimental conditions.[22-25]

Calculation of the Rate Constants. First-order rate constants, k_1 and k_{-1}, for the interconversion of 2',5'- and 3',5'-dinucleoside monophosphates were calculated by eqs 3 and 4, where

$$(k_1 + k_{-1})t = \ln [(1 - x_e)/(x - x_e)] \quad (3)$$

$$k_1/k_{-1} = (1 - x_e)/x_e \quad (4)$$

x stands for the mole fraction of the 3',5'-isomer in the isomeric mixture at moment t, and x_e is the same quantity at equilibrium. Usually, a two-parameter fitting by the method of least-squares was applied to obtain both $(k_1 + k_{-1})$ and x_e. The procedure is based on the observation that both 2',5'- and 3',5'-isomers undergo hydrolysis of the phosphodiester bond at approximately equal rates.

First-order rate constants, k_2, for hydrolysis of isomeric UpUs were calculated by applying the integrated first-order rate equation to diminution of the total concentration of these species as a function of time. Under alkaline conditions no isomerization took place, and the rate constants were obtained separately for each of the isomers.

First-order rate constants for hydrolysis, k_2, and depurination, k_3, of isomeric UpAs were calculated by eqs 5 and 6,

$$\frac{[\text{Ado}]}{[\text{UpA}]_0} = \frac{k_2}{k_5 - k_d}(e^{-k_d t} - e^{-k_5 t}) \quad (5)$$

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$$k_d = k_2 + k_3 \quad (6)$$

where [Ado] denotes the concentration of adenosine at moment t and $[UpA]_0$ is the initial concentration of the starting material (either 2',5'-UpA or 3',5'-UpA). The rate constants, k_2, k_3, and k_5, are defined in Scheme I. The values of k_5 were taken from literature.[26] Under alkaline conditions depurination is extremely slow compared to phosphoester hydrolysis. In other words, k_3 and k_5 are negligible compared to k_2, and eq 5 thus reduces to the first-order rate law.

First-order rate constants for hydrolysis, k_2, and depurination, k_3, of isomeric ApU at pH < 2 were calculated by eqs 6 and 7,

$$\frac{[AMP]}{[ApU]_0} = \frac{k_2}{k_4 - k_d}(e^{-k_d t} - e^{-k_4 t}) \quad (7)$$

where [AMP] stands for the total concentration of 2'- and 3'-AMP at moment t and $[ApU]_0$ is the initial concentration of the starting material. The rate constants, k_2, k_3, and k_4, are defined in Scheme I. The values for k_4 were taken from literature.[10] It should be noted that 2',3'-cAMP is not accumulated at pH < 2. At higher pH, dephosphorylation of 2'- and 3'-AMP is, in turn, fast enough to prevent their accumulation.[10] Under these conditions eq 8 was applied. Again the rate constants refer to Scheme I. Under

$$\frac{[Ado]}{[ApU]_0} = \frac{k_2}{k_5 - k_d}(e^{-k_d t} - e^{-k_5 t}) \quad (8)$$

alkaline conditions k_3 and k_5 are negligible compared to k_2, and a simple first-order rate law is obtained.

First-order rate constants for hydrolysis, k_2, and depurination, k_3, of isomeric ApAs at pH < 2 were calculated by eqs 6 and 9.

$$\frac{[Ado]}{[ApA]_0} = \frac{k_2}{k_5 - k_d}(e^{-k_d t} - e^{-k_5 t}) \quad (9)$$

Here [Ado] is the concentration of adenosine at moment t, and $[ApA]_0$ is the initial concentration of the starting material. Under these conditions the dephosphorylation of 2'- and 3'-AMP is so slow compared to phosphoester hydrolysis that the concentration of adenosine is not increased by this route in the course of a kinetic run. In alkaline solutions eq 9 is reduced to a first-order rate equation.

Table IV. Kinetically Determined pK_a values for 2'-OH of 3',5'-Dinucleoside Monophosphates and 3'-OH of Their 2',5'-Isomers and First-Order Rate Constants for Hydrolysis of the Ionized Species at 333.2 K.[a]

compd	pK_{a2}	$k_g/10^{-3}$ (s^{-1})
2',5'-UpU	12.84	12.8
3',5'-UpU	12.55	6.92
2',5'-UpA	12.97	18.8
3',5'-UpA	12.52	7.41
2',5'-ApU	12.73	5.13
3',5'-ApU	12.04	2.00
2',5'-ApA	12.70	7.38
3',5'-ApA	12.24	5.68

[a] See Scheme II.

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Selenoxide Elimination for the Synthesis of Unsaturated-Sugar Uracil Nucleosides

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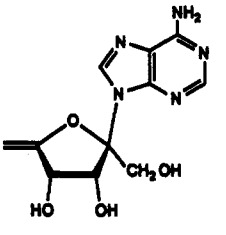
Introduction of a phenylseleno group to the sugar portion of uracil nucleosides and selenoxide elimination reactions of the resulting selenium-containing derivatives are described. A phenylselenide anion prepared by reducing $(PhSe)_2$ with $LiAlH_4$ was found to be highly reactive. By using this selenide as a nucleophile, ring openings of various types of cyclonucleosides and nucleosides having an anhydro structure in the sugar portion were accomplished. The products, which contain a phenylseleno group in the sugar portion, were oxidized with *m*-CPBA in CH_2Cl_2, and their susceptibility to the selenoxide elimination and regiochemistry of the reaction was investigated.

Selenium-containing organic molecules have been known to be versatile synthons in organic synthesis. Among various synthetic utilities of the organoseleniums,[1] selenoxide elimination would constitute one of the most frequently used synthetic operations leading to unsaturated organic molecules such as allylic alcohols.[2] It can be carried out under very mild reaction conditions, and its syn elimination pathway provides an additional merit for regio- and stereodefined synthetic planning of a target molecule.

Although the occurrence of nucleoside antibiotics bearing unsaturated sugars, such as angustmycin A (1),[3-5] has stimulated the synthesis of unsaturated-sugar nucleosides of various types,[6-8] the methods available until recently have been based on base-promoted elimination of the corresponding halogeno or sulfonyl derivatives.[9] An



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