through silica gel. The determination of % ee was performed by comparison of the $[\alpha]_D$ of the isolated alcohol with the $[\alpha]_D$ of a solution made of the known standard: **8**, 38% ee for (*S*)-phenylethanol (standard from Aldrich);[20] **9**, 59% ee for (*R*)-phenylethanol (standard from Aldrich);[20] **11**, 0% ee for (*R*)-2-butanol (standard from Aldrich);[20] **12**, 31% ee for (*R*)-cyclohexenol (literature standard);[27] **13**, 61% ee for (*S*)-cyclohexenol (literature standard);[27] **14**, 74% ee for (1*S*,2*S*,4*R*)-*exo*-2-norbornanol (literature standard);[28] **15**, 60% ee for (1*R*,2*R*,4*S*)-*exo*-2-norbornanol (literature standard).[28]

In those experiments where the isolation of hydroxybutanoic acid was desired, the hydrolysis mixture was extracted with ether prior to acidification and extraction of the carboxylic acid. The determination of % ee was performed by comparison of $[\alpha]_D$ of the isolated hydroxybutenoic acid with the $[\alpha]_D$ of a solution made of the known standard:[20] **9**, 77% ee; **15** (from Noyori hydrogenation), 94% ee.

General Procedure for the Preparation of the Mosher Esters. To the alcohol (obtained from the β-keto ester or the β-hydroxy ester by base hydrolysis) in CH_2Cl_2 were added DCC, DMAP, and the (*R*)- or the (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid. The reaction mixture was stirred for 12 h at room temperature and worked up with H_2O, 1 N HCl, aqueous $NaHCO_3$. The organic phase was separated, dried, and concentrated. The determination of the enantiomeric excess was determined by ^1H NMR: **8**, 60% ee; **9**, 75% ee; **11**, 14% ee; **12**, 21% ee; **13**, 76% ee; **14**, 25–30% ee; **15**, 44–55% ee.

Procedure for the Determination of the % ee or % de with Eu(hfc)$_3$ as a Chiral Shift Reagent. Each NMR sample was prepared by mixing 100 μL of Eu(hfc)$_3$ (100 mg/mL $CDCl_3$), 10 mg of the β-keto ester or β-hydroxy ester, and 0.4 mL of $CDCl_3$. The % ee or % de was determined by the difference in the integration of those signals in the ^1H NMR spectrum that had been resolved in the NMR experiment done on the racemic sample and optimized for the relative concentration of the shift reagent (**8**, 73% ee; **9**, 74% ee).

Procedure for Hydrogenation (Noyori's Conditions). $RuCL_2$–COD (38 mg, 0.13 mM) and (*S*)-BINAP (100 mg, 0.16 mM) were transferred to a Schlenk vessel in an inert atmosphere (glovebox). A solution of triethylamine in toluene (0.135 mL, 1.87 mM, 5.5 mL, freshly distilled and degassed in three freeze–thaw cycles) was added by means of a cannula, and the mixture was refluxed under N_2 for 8 h. The solvent and triethylamine were removed by high vacuum, leaving behind a solid, red-brown residue. A solution of the requisite keto ester (for **2**, 200 mg, 1.03 mM; for **5**, 200 mg, 1.02 mM) in triply degassed 2-propanol freshly distilled from CaH_2 was added via a cannula to the Schlenk vessel and refluxed until it appeared that all catalyst had dissolved and the solution had acquired a bright orange color. The solution was added directly to a Parr pressure bomb, which was flushed with argon and then pressurized to 100 atm H_2. The reaction was stirred at 25–30 °C for 58 h, then the pressure was released and the solvents removed in vacuo. Kugelrohr distillation afforded a yellowish oil, which appeared homogeneous by TLC and NMR, and corresponded to the diastereomers of **9** (196 mg, 97%) and **15** (198 mg, 98%). This material was analyzed for % ee by spectral methods. Attempts at the separation of diastereomers proved unsuccessful (see Discussion Section).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds **6**–**15** and ^1H NMR spectra of *S*-Mosher ester of **9** (23 pages). Ordering information is given on any current masthead page.

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Interconversion and Hydrolysis of Monomethyl and Monoisopropyl Esters of Adenosine 2′- and 3′-Monophosphates: Kinetics and Mechanisms

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First-order rate constants for mutual isomerization and hydrolytic cleavage of the monomethyl and monoisopropyl esters of adenosine 2′- and 3′-monophosphates (2′- and 3′-AMP) have been determined by HPLC over a wide pH range. Both reactions proceed at comparable rates under acidic conditions, exhibiting a second-order dependence of rate on hydronium ion concentration at $1 < pH < 2$, and a first-order dependence in more acidic solutions. Moreover, hydrolytic depurination takes place at $pH < 3$. In the pH range 4 to 9 a pH-independent phosphate migration prevails. By contrast, in alkaline solutions the methyl esters are hydrolyzed to a mixture of 2′- and 3′-AMP, the reaction rate being proportional to the hydroxide ion concentration at $[OH^-] < 0.1$ mol dm^{-3}. No sign of mutual isomerization was detected under these conditions. With the isopropyl esters alkaline degradation of the adenine moiety is considerably faster than the phosphodiester hydrolysis. Mechanisms of phosphate migration and phosphodiester hydrolysis under various conditions have been discussed.

Introduction

Phosphodiester bonding plays a vital role in biological chemistry by linking nucleoside units to nucleic acids. Numerous kinetic and mechanistic works on hydrolysis of simple mono-, di-, or triesters of phosphoric acid refer, in their introduction, to the importance of detailed understanding of the chemical behavior of this bond.[1] However, surprisingly few attempts have been made to apply the

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results of these fundamental studies to hydrolytic reactions of compounds that would more closely mimic the fragments of nucleic acids. Anslyn and Breslow[2] have studied the buffer-catalyzed hydrolysis and isomerization of uridylyl(3',5')uridine as a model of ribonuclease action. Hydrolysis to uridine and uridine 2',3'-cyclic monophosphate has been shown to prevail in imidazole buffers and isomerization to uridylyl(2',5')uridine in more acidic carboxylate buffers.[2,3] Both reactions proceed through the same five-coordinate phosphorane monoanion intermediate, the formation of which has been suggested to exhibit a specific acid/general base catalysis. The decomposition of this species to uridine and uridine 2',3'-cyclic monophosphate show, in turn, a specific base/general acid catalysis, and the breakdown to 2',5'- and 3',5'-diesters a general acid catalysis.[2]

Besides the buffer-catalyzed hydrolysis, kinetics for the alkaline cleavage of dinucleoside monophosphates have also been studied. The initial hydrolysis product is a 2',3'-cyclic monophosphate, and no isomerization of 3',5'-phosphodiester bonds to 2',5'-bonds takes place concurrent with hydrolysis,[4-6] suggesting that the 2'-oxyanion displaces the 5'-linked nucleoside by an in-line associative mechanism. Dinucleoside monophosphates derived from purine nucleosides are more stable than their pyrimidine counterparts, the chemical nature of the 5'-linked nucleoside playing the major role.[6-8] Some semiquantitative observations are also mechanistically relevant. Internucleosidic 3',5'-phosphodiester bonds have been shown to undergo partial isomerization to the 2',5'-bond during the chemical synthesis of oligoribonucleotides, when the 2'-*O*-protecting group is removed under very acidic conditions.[9,10] It is also known that dinucleoside 3',5'-monophosphates derived from pyrimidine nucleoside 3'-monophosphates are hydrolyzed in aqueous acid from 2 to 3 times as fast as their purine counterparts.[11,12] 2-Chlorophenyl esters of dinucleoside 3',5'-monophosphates have been shown to yield a mixture of 2',5'- and 3',5'-diesters and 2'- and 3'-monoesters under acidic conditions.[13,14]

We have previously given a kinetic and mechanistic description for competitive interconversion and hydrolytic dephosphorylation of nucleoside 2'- and 3'-monophosphates over the acidity range from pH 7 to H_0 −0.7.[15,16] As an extension of these studies we now report on interconversion and hydrolysis of corresponding phosphodiesters, monomethyl and monoisopropyl esters of adenosine 2'- and 3'-monophosphates. An analogous study has earlier been carried out with the phenyl ester of *cis*-4-hydroxytetrahydrofuran 3-phosphate.[17] However, the phenyl group is a so much better leaving group than a 5'-linked nucleoside that this compound cannot be regarded as a good model for dinucleoside monophosphates. For example, no migration of the phenylphosphate group could be detected.

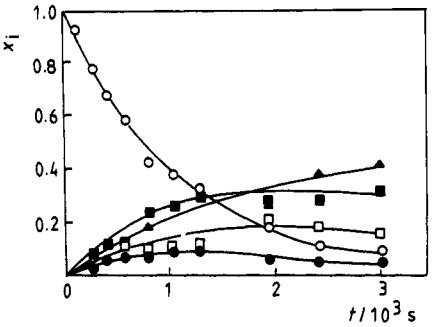
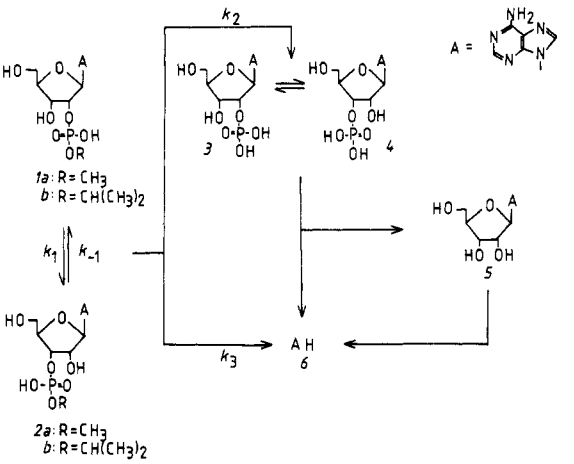


Figure 1. Time-dependent product distribution for reactions of the monomethyl ester of 2'-AMP (**1a**) in 0.10 mol dm^{-3} aqueous hydrogen chloride at 363.2 K. Notation: monomethyl ester of 2'-AMP (open circles), monomethyl ester of 3'-AMP (**2a**, filled circles), 2'-AMP (**3**, open squares), 3'-AMP (**4**, filled squares), and adenine (**6**, filled triangles).

Scheme I



Results and Discussion

Product Distributions. Figure 1 shows the time-dependent product distribution obtained by HPLC for decomposition of the monomethyl ester of 2'-AMP (**1a**) in 0.10 mol dm^{-3} aqueous hydrogen chloride at 363.2 K. As seen, disappearance of the starting material is accompanied with formation of 2'-AMP (**3**), 3'-AMP (**4**), monomethyl ester of 3'-AMP (**2a**), and adenine (**6**). Similarly, the monomethyl ester of 3'-AMP yielded **1a**, **3**, **4**, and **6** under the same conditions. The concentration ratio of 2'- and 3'-AMP remained constant ([2'-AMP]/[3'-AMP] = 0.6) throughout the kinetic run and was independent of the starting material employed. In other words, the reaction yields 2'- and 3'-AMP in the same ratio as the much faster hydrolysis of adenosine cyclic 2',3'-monophosphate (2',3'-cAMP).[16] Accordingly, it appears clear that the monomethyl esters of 2'- and 3'-AMP undergo three competitive reactions in aqueous acid: (i) mutual isomerization, (ii) hydrolysis to a mixture of 2'- and 3'-AMP via cyclic 2',3'-monophosphate, and (iii) depurination (Scheme I). Hydrolysis of **1a** and **2a** to adenosine (**5**) appears to be insignificant. The half-time for the hydrolysis of adenosine is about 2000 s under the experimental conditions employed.[18] In other words, adenosine should have

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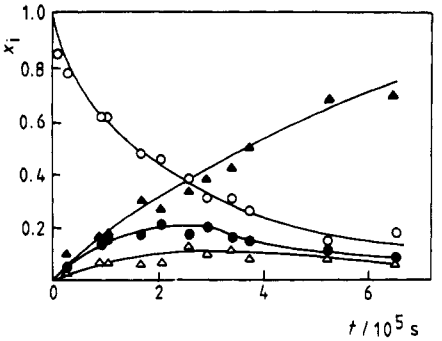

Figure 2. Time-dependent product distribution for reactions of the monomethyl ester of 2′-AMP (**1a**) in a formic acid buffer ([HCOOH]/[HCOONa] = 0.05/0.01 mol dm^{-3}, I = 0.1 mol dm^{-3} with NaCl) at 363.2 K. Notation: monomethyl ester of 2′-AMP (open circles), monomethyl ester of 3′-AMP (**2a**, filled circles), adenosine (**5**, open triangles), and adenine (**6**, filled triangles).

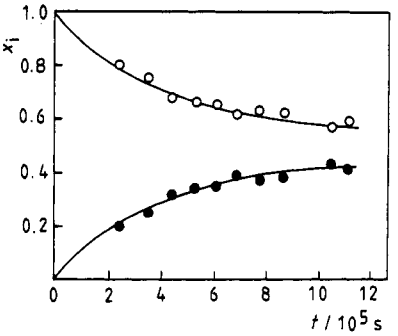

Figure 3. Time-dependent product distribution for reactions of the monomethyl ester of 2′-AMP (**1a**) in a triethanolamine buffer ([TEAHCl]/[TEA] = 0.02/0.01 mol dm^{-3}, I = 0.1 mol dm^{-3} with NaCl) at 363.2 K. Notation: monomethyl ester of 2′-AMP (open circles) and monomethyl ester of 3′-AMP (**2a**, filled circles).

been accumulated if formed under the experimental conditions. Clearly this is not the case. The product distributions observed in 1.0 and 0.010 mol dm^{-3} hydrogen chloride were qualitatively similar, suggesting that Scheme I is a sufficient description for reactions taking place at pH < 2. At pH 3 (formic acid/sodium formate buffer, 0.05/0.01 mol dm^{-3}) 2′- and 3′-AMP were not accumulated. By contrast, adenosine (**5**) appeared as an intermediate, although its mole fraction remained below 0.15 during the kinetic run (Figure 2). In principle adenosine may be produced by P–O$^{3'}$ fission of either the phosphodiesters (**1a**, **2a**) or phosphomonoesters (**3**, **4**). Phosphomonoesters are generally hydrolyzed at pH 3 much faster than phosphodiesters. For example, monomethyl phosphate is cleaved under these conditions 100 times as fast as dimethyl phosphate.[19,20] Accordingly, it appears probable that adenosine is formed via dephosphorylation of 2′- and 3′-AMP. Since their dephosphorylation is known to be fast (k = 1.3 × 10^{-5} s^{-1})[15] compared to formation of adenosine in the present case ($k \approx 10^{-6}$ s^{-1}), it may well be that the mole fractions of isomeric AMPs do not reach the limit of detection (0.03) during a kinetic run.

Under neutral conditions, i.e. at pH 4–9, the only significant reaction is mutual isomerization of **1a** and **2a** (Figure 3), the 2′-isomer being slightly more stable ([**1a**]/[**2a**] = 1.2). Evidently the hydrolysis to adenosine monophosphates is at least 1 order of magnitude slower. By contrast, in alkaline solutions **1a** and **2a** were observed to undergo a base-catalyzed hydrolysis to a mixture of 2′- and 3′-AMP ([2′-AMP]/[3′-AMP] = 0.8). The product distribution was similar to that observed for alkaline hydrolysis of 2′,3′-cAMP and independent of the identity of the starting material. No phosphate migration could be detected (Figure 4), consistent with hydrolysis of dinucleoside monophosphates.[4-6]

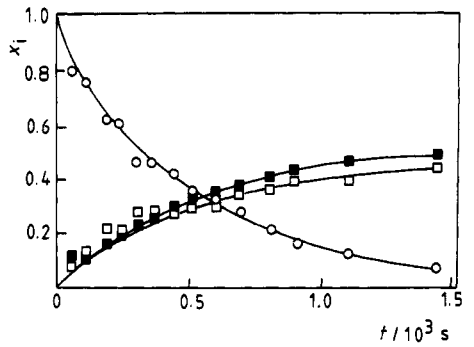

Figure 4. Time-dependent product distribution for reactions of the monomethyl ester of 2′-AMP (**1a**) in 0.10 mol dm^{-3} aqueous sodium hydroxide at 363.2 K. Notation: monomethyl ester of 2′-AMP (open circles), 2′-AMP (**3**, open squares), and 3′-AMP (**4**, filled squares).

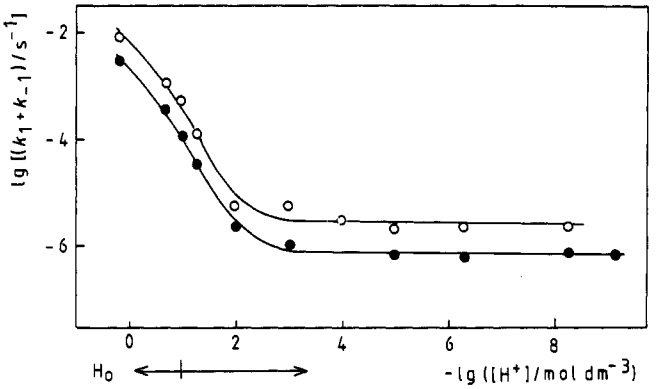

Figure 5. pH–rate profiles for interconversion of the monoalkyl esters of 2′- and 3′-AMP at 363.2 K (I = 0.1 mol dm^{-3} with NaCl). Notation: methyl esters (**1a**/**2a**, open circles) and isopropyl esters (**1b**/**2b**, filled circles).

The product distributions obtained with the monoisopropyl esters of 2′- and 3′-AMP (**1b**, **2b**) resembled those of the corresponding methyl esters under neutral and acidic conditions. In alkaline solutions the hydrolysis to 2′- and 3′-AMP could not be followed owing to considerably faster degradation of the base moiety.

In the following discussion possible buffer catalysis has been neglected. All the rate constants were measured at buffer concentrations lower than 0.05 mol dm^{-3}. The results of Anslyn and Breslow[2,3] clearly indicate that under such conditions the buffer-dependent rates are hardly detectable compared to buffer-independent rates.

pH–Rate Profile for Phosphate Migration. We have shown previously[15,16] that the pH–rate profile for migration of a dihydrogen phosphate group between the 2′- and 3′-hydroxyl functions of ribofuranose ring consists of four distinct regions. The reaction is first order with respect to hydronium ion at pH < 1, i.e. on the acidic side of the pK_{a1} value of the alkyl phosphate group, approaches a second-order dependence between pH 1 and 2, becomes pH-independent at pH 3–6, and shows again a first-order dependence on acidity at pH > 7. As seen from Figure 5, the pH dependence for migration of monoalkyl phosphate groups is rather similar. In fact, the only difference is that the reaction does not undergo rate retardation at pH > 7. This is expected, since alkylation of one of the phosphate oxygens prevents the dianion formation, which takes place

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Scheme II

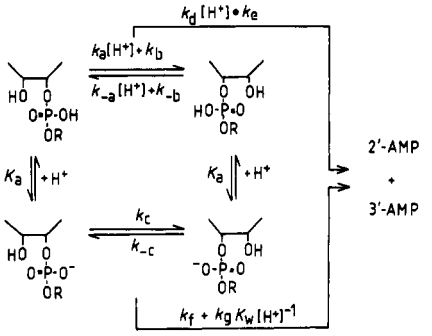

Table I. Partial Rate Constants for the Interconversion, Hydrolysis, and Depurination of Monoalkyl Esters of 2'- and 3'-AMP at 363.2 K[a]

	methyl esters (1a, 2a)	isopropyl esters (1b, 2b)
pK_a	1.0	1.0
$k_a/10^{-3}$ dm^3 mol^{-1} s^{-1}	3.2	0.9
$k_{-a}/10^{-3}$ dm^3 mol^{-1} s^{-1}	3.9	1.1
$k_c/10^{-6}$ s^{-1}	1.3	0.34
$k_{-c}/10^{-6}$ s^{-1}	1.6	0.41
$k_d/10^{-3}$ dm^3 mol^{-1} s^{-1}	11.6	4.3
$k_f/10^{-7}$ s^{-1}	<1	<1
$k_g/10^{-3}$ dm^3 mol^{-1} s^{-1}	18.0	*b*
$k_h/10^{-3}$ dm^3 mol^{-1} s^{-1}	2.0	1.8

[a] The ionic strength adjusted to 0.10 mol dm^{-3} with sodium chloride. For the partial rate constants see Scheme II. The values of k_b, k_{-b}, and k_e could not be obtained with a reasonable accuracy. [b] Base moiety is degraded.

with nucleoside monophosphates at this pH. Bearing this difference in mind, the minimal reaction scheme proposed previously[15] for the migration of dihydrogen phosphate group also applies to migration of alkyl hydrogen phosphate group (Scheme II). The plateau between pH 2 and 9 refers to uncatalyzed migration of monoanionic alkyl phosphate group, and the ascending part of the rate profile to hydronium ion catalyzed migration of neutral alkyl phosphate group. As discussed earlier in detail,[15] uncatalyzed migration of neutral alkyl phosphate group (or kinetically equivalent hydronium ion catalyzed migration of monoanionic alkyl phosphate group) does not prevail under any conditions. The reaction is of second-order with respect to hydronium ion at pH > pK_a, indicating that more than one proton is needed to accelerate the migration under conditions where the alkyl phosphate group is predominantly present as a monoanion, and first-order at pH < pK_a. The observed first-order rate constant, k_1, for the 2'–3' migration (see Scheme I) may thus be expressed by eq 1, assuming that the acidity constants of 2'- and 3'-phosphate groups are equal. The partial rate constants

$$k_1 = \frac{(k_a/K_a)[H^+]^2 + (k_b/K_a)[H^+] + k_c}{1 + [H^+]/K_a} \quad (1)$$

are those indicated in Scheme II. An analogous equation may be written for k_{-1}. Table I summarizes the values obtained for the partial rate constants by least-squares fitting.[21] The rate constant, k_b, referring to uncatalyzed migration of the neutral alkyl phosphate group, could not be obtained with a reasonable accuracy. Most likely this partial reaction does not contribute significantly to the observed rate constant under any conditions.

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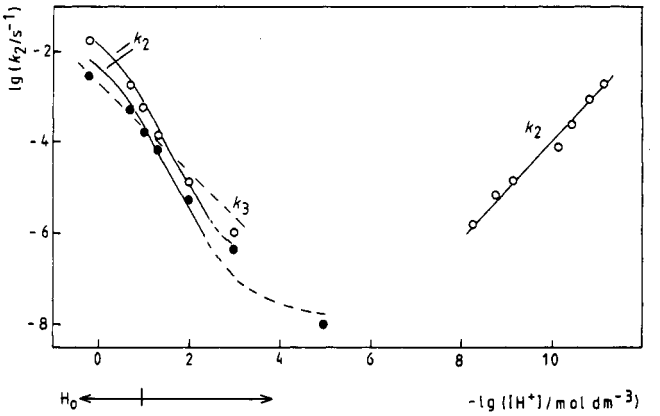


Figure 6. pH–rate profiles for hydrolysis (k_2) and depurination (k_3, dotted line) of the monoalkyl esters of 2'- and 3'-AMP at 363.2 K (I = 0.1 mol dm^{-3} with NaCl). Notation: methyl esters (**1a**/**2a**, open circles) and isopropyl esters (**1b**/**2b**, filled circles).

pH–Rate Profiles for Phosphodiester Hydrolysis and Depurination. The pH–rate profile for the hydrolysis of phosphodiester bond (Figure 6) is similar to that of the phosphate migration at pH < 2, suggesting that the reactive species is a neutral phosphodiester, which undergoes a hydronium ion catalyzed hydrolysis. At pH > 9 a base-catalyzed hydrolysis is observed, the reaction being of first-order with respect to hydroxide ion. It is worth noting that both **1** and **2** react at the same rate. Between pH 2 and 9 the phosphodiester hydrolysis is much slower than the phosphate migration, and no attempts were made to determine its rate constants. Accordingly, the observed rate constant, k_2, for hydrolysis may be expressed by eq 2, where the partial rate constants are those

$$k_2 = \frac{(k_d/K_a)[H^+]^2 + (k_e/K_a)[H^+] + k_f + k_g(K_w/[H^+])}{1 + [H^+]/K_a} \quad (2)$$

indicated in Scheme II and K_w is the ionic product of water under the experimental conditions.[22] Although the experimental evidence for the uncatalyzed hydrolysis of either the neutral or monoanionic phosphodiester bond is rather scarce, the rate constants, k_e and k_f, referring to these partial reactions are included in eq 2 since they contribute significantly to hydrolysis of the phenyl ester of *cis*-4-hydroxytetrahydrofuran 3-phosphate.[17] However, inclusion of the term $(k_e/K_a)/[H^+]$ did not improve the fit between experimental and calculated rate constants, and the values obtained for k_f must be regarded as rough estimations.

The rate of depurination of **1** and **2** is linearly related to hydronium ion concentration at pH < 2 (Figure 6, eq 3), i.e. under conditions were it competes with phosphate

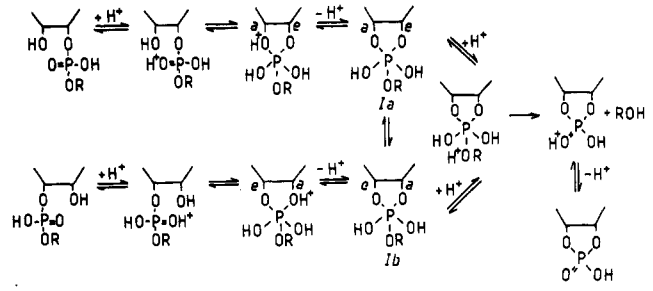
$$k_3 = k_h[H^+] \quad (3)$$

migration. The values obtained by least-squares fitting for the partial rate constants included in eqs 2 and 3 are listed in Table I.

Mechanisms of Phosphate Migration and Phosphodiester Hydrolysis. The close similarity of the pH–rate profiles of phosphate migration (Figure 5) and phosphodiester hydrolysis (Figure 6) at pH < 2 suggests that both reactions proceed under acidic conditions via the same pentacoordinated intermediate, as also proposed by Anslyn and Breslow[2] for the corresponding imidazole-

(22) (a) Clever, H. L. *J. Chem. Educ.* **1968**, *45*, 231. (b) Harned, H. S.; Owen, B. B. *Physical Chemistry of Electrolyte Solutions*; Reinold: New York, 1943; p 752.

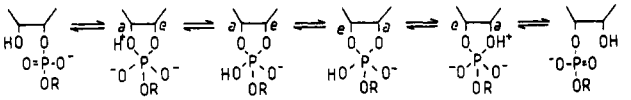
Scheme III



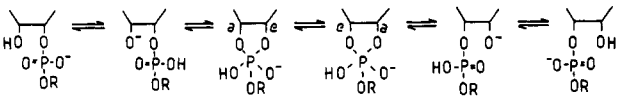
catalyzed reactions of uridylyl(3',5')uridine. Phosphodiester hydrolysis without intramolecular participation of the neighboring hydroxyl group may be excluded, since the reaction is more than 2 orders of magnitude faster than that of dimethyl phosphate.[20] Scheme III describes the assumed mechanism for the isomerization and hydrolysis of the 2',5'-diester. A rapid initial protonation of the neutral phosphodiester followed by a nucleophilic attack of the neighboring 3'-hydroxyl group results in formation of a pentacoordinated intermediate, Ia, where the 3'-oxygen is apical and 2'-oxygen equatorial. Since the electronegativity difference of an alkoxy and hydroxy ligand is small, the alkoxy group, OR, may adopt either an apical or equatorial position. Pseudorotation of the intermediate[23] will place the 2'-oxygen into apical and 3'-oxygen into equatorial position (Ib). Accordingly, the 2'-oxygen may be protonated and leave as a hydroxyl group, resulting in phosphate migration. Alternatively, protonation and subsequent departure of an apical alkoxy group, OR, leads to formation of protonated 2',3'-cyclic monophosphate, which is rapidly hydrolyzed to a mixture of 2'- and 3'-AMP by a mechanism previously[15] described. The estimated first and second pK_a's of the pentacoordinate intermediate are 9 and 13, respectively.[24] Structures Ia and Ib thus represent the nonionic form of the intermediate, which is by far the most stable at pH < 2. The suggested mechanism is in principle analogous to that of the acid-catalyzed hydrolysis of carboxylic acid esters, proceeding by protonation of the carbonyl oxygen and formation of a tetrahedral intermediate.[25]

As seen from Table I, the phosphate migration and phosphodiester hydrolysis proceed at comparable rates under acidic conditions. In other words, the pentacoordinated intermediate is collapsed to a cyclic 2',3'-monophosphate approximately as readily as to acyclic phosphodiesters, in spite of the fact that the cyclic ester is undoubtedly more strained than the acyclic ones. Moreover, the product distribution is similar with the methyl and isopropyl esters; the methyl esters (**1a**, **2a**) undergo both migration and hydrolysis 3 times as fast as the isopropyl esters (**1b**, **2b**), most likely owing to the fact that replacing the methyl group with a more bulky and electropositive isopropyl group retards the nucleophilic attack of a neighboring hydroxyl group. Under acidic conditions the P–O bond rupture is preceeded by protonation of the oxygen atom (Scheme III). Since the inductive effects of the leaving alkoxy group on the protonation and heterolysis steps are opposite, the rate of the bond cleavage may be expected to be rather insensitive to the polar nature of the departing alcohol. Increasing electropositivity, for example, facilitates protonation but

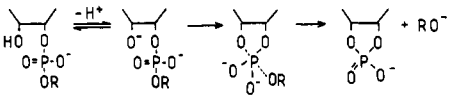
Scheme IV



Scheme V



Scheme VI



retards the departure of the protonated alkoxy group, leaving the product distribution practically unchanged.

For the pH-independent migration two kinetically indistinguishable mechanisms may be described: (i) attack of unionized hydroxyl group on phosphodiester monoanion (Scheme IV) or (ii) attack of ionized hydroxyl group on neutral phosphodiester (Scheme V). For the following reason the former alternative appears more attractive. The dimethyl ester of uridine 3'-monophosphate has been shown to undergo a base-catalyzed hydrolysis.[26] The reaction most likely proceeds by a rapid initial ionization of the 2'-hydroxyl group followed by rate-limiting attack of the resulting oxyanion on phosphorus. The first-order rate constant for this reaction has been estimated to be of the order of 10^{-3} s^{-1} at pH 9 at 310 K.[26] Under these conditions the mole fraction of the 2'-ionized starting material falls in the range 10^{-5}–10^{-4}, and hence the rate constant for the intramolecular attack of the 2'-oxyanion is smaller than 100 s^{-1}. This means that the upper limit for the corresponding value at 363 K is 10^4 s^{-1}. This value appears to be too small to support the mechanism depicted in Scheme V. Since the pK_a values of the monomethyl phosphate and 2'-hydroxyl groups of **2a** differ by at least 12 orders of magnitude, the mole fraction of the species containing both a neutral methylphosphate group and an ionized 2'-hydroxyl group is only of the order of 10^{-12} under neutral conditions. Accordingly, the first-order rate constant for phosphate migration via this species would be 10^{-8} s^{-1} at 363 K, assuming that the attack of 2'-oxyanion on neutral monomethyl phosphate group of **2a** is approximately as rapid as the attack on dimethyl phosphate group. As seen from Figure 1, the observed rate constant is more than 2 orders of magnitude higher.

As mentioned above, the phosphate migration is under neutral conditions at least 1 order of magnitude faster than the phosphodiester hydrolysis, in striking contrast to the situation in strongly acidic solutions. A tentative explanation for this difference is that the initial product of phosphodiester hydrolysis is under acidic conditions a fully protonated 2',3'-monophosphate and under basic conditions its monoanionion. If the latter species is more strained than the former, the observed difference in the product distribution is expected.

It has been suggested previously that the alkaline hydrolysis of dinucleoside 3',5'-monophosphates proceeds by a simple "in-line" mechanism without pseudorotation of the pentacoordinated intermediate, since no isomerization to a 2',5'-diester was detected.[4-6] Accordingly, the attacking hydroxyl group undergoes a rapid initial deprotonation,

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and the leaving alkoxy ligand adopts directly an apical position (Scheme VI). This mechanism is adequate to explain the results of the present investigation as well. As mentioned above, no sign of mutual isomerization of the methyl esters, **1a** and **2a**, was observed at pH > 10. The hydrolysis rates of **1a** and **2a** were equal within the limits of experimental errors. This does not mean, however, that the 2′- and 3′-oxyanions would necessarily attack exactly as rapidly; differences in the rate constants for the rate-limiting attack may be compensated by differences in preequilibrium ionization. It is also worth noting that the hydrolysis rate appears to be rather susceptible to the polar and/or steric nature of the leaving group. As mentioned above, the isopropyl esters, **1b** and **2b**, undergo degradation of the base moiety under alkaline conditions. The first-order rate constant for the disappearance of **1b** was observed to be 2.4×10^{-5} s^{-1} in 0.1 mol dm^{-3} sodium hydroxide at 363.2 K. Accordingly, the hydrolysis of **1b** to 2′- and 3′-AMP is at least 2 orders of magnitude slower than that of the methyl esters. The large reactivity difference may be of both steric and inductive origin. Since the isopropoxy group is larger and more electropositive than methoxy group, it may be expected to retard inductively the nucleophilic attack of the neighboring 3′-oxyanion on the phosphorus atom, to form a steric hindrance to this attack, and to depart as alkoxide ion less readily than the methoxy group. For comparison, the benzyl esters of uridine 2′- and 3′-monophosphates have been reported to hydrolyze 300 times as fast as their isopropyl counterparts.[27]

Experimental Section

Materials. Preparation of the methyl (**1a**, **2a**) and isopropyl esters (**1b**, **2b**) of adenosine 2′- and 3′-monophosphates used in kinetic measurements has been described elsewhere.[28] Adenosine 2′-, 3′-, and 2′,3′-cyclic monophosphates, adenosine, and adenine 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.[29] Chromatographic separations were carried out on a Hypersil ODS5 column (4 × 250 mm) by using the following mixtures as eluants: acetic acid/sodium acetate buffer (pH 4.3) containing 0.2 mol dm^{-3} ammonium chloride and 7% (v/v) acetonitrile (separation of 2′- and 3′-AMP, adenosine, and adenine from each other and the mixture of adenosine 2′- and 3′-methylphosphates; separation of adenosine 2′- and 3′-isopropylphosphates), 0.1 mol dm^{-3} ammonium acetate containing 11% (v/v) acetonitrile (separation of adenosine 2′- and 3′-methylphosphates from each other and the mixture of adenosine, adenine, and 2′- and 3′-AMP). Since the base moiety of all the compounds studied was adenine, signal areas were assumed to be proportional to concentrations.

The hydronium ion concentrations, adjusted with hydrogen chloride, and formate, acetate, maleate, triethanolamine, and glycine buffers, were calculated from the pK_a values of the buffer acids under the experimental conditions.[30-34]

Calculation of the Rate Constants. First-order rate constants, k_1 and k_{-1}, for the interconversion of adenosine 2′- and 3′-alkylphosphates, **1** and **2**, were calculated by eqs 4 and 5, where x stands for the mole fraction of the 2′-isomer in the isomeric mixture at moment t, and x_e is the corresponding quantity at equilibrium. It should be noted that both isomers were observed to be hydrolyzed to monophosphates at equal rates.

$$(k_1 + k_{-1})t = \ln [(1 - x_e)/(x - x_e)] \quad (4)$$

$$k_1/k_{-1} = (1 - x_e)/x_e \quad (5)$$

First-order rate constants, k_2, for hydrolysis of adenosine 2′- and 3′-alkylphosphates to a mixture of 2′- and 3′-AMP were obtained by eq 6, where [AMP] is the total concentration of 2′- and 3′-AMP at moment t and $[S]_o$ is the initial concentration of the starting material (either **1** or **2**). k_d is the first-order rate constant for disappearance of the starting material and k_4 the first-order rate constant for decomposition of 2′- and 3′-AMP by depurination and/or dephosphorylation (determined earlier[15]). First-order rate constant for depurination of adenosine 2′- and 3′-alkylphosphates were obtained as a difference of k_d and k_2.

$$\frac{[\mathrm{AMP}]}{[\mathrm{S}]_o} = \frac{k_2}{k_4 - k_d}(e^{-k_d t} - e^{-k_4 t}) \quad (6)$$

Registry No. **1a**, 74494-54-5; **1b**, 132803-62-4; **2a**, 69024-48-2; **2b**, 52278-64-5.

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