products were shown to be the exo and endo isomers of 5-phenyl-5-(trimethylsiloxy)bicyclo[2.2.1]hept-2-ene by their conversion to 2-phenylbicyclo[2.2.1]hepta-2,5-diene[23] by reaction with a mixture of CF_3CO_2H in CH_3CO_2H at room temperature for 3.5 h.

DCA-Sensitized Photolysis of 1-(Trimethylsiloxy)-1-phenylethene with CHpD. The irradiation was carried out as described above: a benzene solution of the enol ether (0.61 M) and CHpD (0.34 M) was photolyzed in the Rayonet for 17 h. Analysis of the crude reaction mixture by GC/MS showed four 1:1 adducts of CHpD to the enol ether. The crude reaction mixture was hydrolyzed and dehydrated as above to give 6-phenylbicyclo[3.2.2]nona-6,8-diene as the minor product, which was identified by comparison with an authentic sample prepared from 8-(trimethylsiloxy)-8-phenylbicyclo[3.2.2]non-6-ene: ^1H NMR (200 MHz, CD_2Cl_2) δ 0.06 (s, 9 H), 1.4–1.9, 2.1–2.2, 2.46–2.85 (m, 10 H), 5.57–5.68 (m, 1 H), 5.9–6.0 (m, 1 H), 7.10–7.64 (m, 5 H); GC/MS *m/e* 286, 192, 177, 153, 135, 91, 75, 73. The major product of this reaction is 9-phenylbibyclo[5.2.0]nona-2,8-diene.

Preparation of Authentic 2-Phenylbicyclo[2.2.2]octa-2,5-diene. 2-Phenylbicyclo[2.2.2]octan-5-en-2-ol was prepared in the standard way by reaction of bicyclo[2.2.2]oct-5-en-2-one (0.95 mol) with phenylmagnesium bromide in 2 mL of ether: GC/MS (EI) *m/e* (rel abundance) 200 (2, m^+), 182 (10), 154 (100). The alcohol was converted to its tosylate in the usual way and then heated at 80 °C for 12 h to complete elimination to the diene. The crude product was purified by chromatography on silica gel with 20% ethyl acetate in hexane: ^1H NMR ($CDCl_3$) δ 7.2–7.4 (m, 5 H), 6.53 (dd, J_1 = 6.3, J_2 = 2.1, 1 H), 6.46 (m, 2 H), 4.15 (m, 1 H), 3.7 (m, 1 H), 1.6–1.3 (m, 4 H); GC/MS (EI) *m/e* (rel abundance) 182 (3, m^+), 154 (100).

TCA-Sensitized Photolysis of DMBD and Phenylpropyne. A saturated dioxane solution of TCA, phenylpropyne (0.4 M), and DMBD (0.2M) was irradiated in the usual way. Only one crossed cycloadduct was detected by GC/MS. The product was isolated by fractional distillation under vacuum to give pale yellow oil: ^1H NMR (C_6D_6) δ 7.38–6.97 (m, 5 H), 4.985 (d, J = 3, 1 H), 4.865 (d, J = 3, 1 H), 2.315 (d, J = 15, 1 H), 2.065 (d, J = 15, 1 H), 1.74 (s, 3 H), 1.69 (s, 3 H), 1.47 (s, 3 H); ^{13}C NMR (C_6D_6) δ 149.7, 143.8, 136.3, 135.5, 126.9, 125.9, 109.8, 48.1, 44.6, 22.1, 19.2, 15.4; $C_{15}H_{18}$ MS *m/e* calcd 198.1409, found 198.1408; GC/MS *m/e* (rel abundance) 198.10 (36), 183.10 (100), 168.10 (38).

Synthesis of *trans*-2-Methyl-3-phenylbicyclo[2.2.2]octane. A portion of previously prepared *endo-trans*-5-methyl-6-phenylbicyclo[2.2.2]oct-2-ene (206 mg, 1.0 mmol) was dissolved in 20 mL of ethyl acetate in a thick-walled glass vessel. A catalytic amount of Pd/C was added to the solution, and the solution was agitated under 50 psi of H_2. After 30 min of reaction, the pressure was released, the catalyst was removed by filtration, the solvent was removed, and the resulting oil was distilled and filtered through silica gel with pentane. The product (30 mg) is a colorless oil: GC/HS *m/e* (rel abund) 200 (100), 118 (52), 117 (51), 115 (36), 109 (90), 91 (65), 67 (44). ^1H NMR δ 0.97 (d, 3 H, J = 6.6), 1.2–1.8 (m, 10 H), 2.04–2.09 (m, 1 H), 2.36 (d, 1 H, J = 8.1), 7.15–7.35 (m, 5 H). Anal. Calcd for $C_{15}H_{20}$: C, 89.94; H, 10.06. Found: C, 90.01; H, 10.03.

TCA Sensitized Photolysis of Phenylallene and CHD. The reaction was carried out as described above for the general case with a solution of phenylallene[24] (0.17 M) and CHD (0.24 M) in dioxane with hexadecane included as an internal standard. Following photolysis, the reaction mixture was hydrogenated with Pd/C as the catalyst, and yields of the adducts were determined by gas chromatography by comparison with authentic samples.

Acknowledgment. This work was supported by the National Science Foundation. D.H. is the recipient of a University of Illinois Fellowship. N.A. is thankful for a Fellowship from Ataturk University, Ezurum, Turkey.

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Kinetics and Mechanisms for Reactions of Adenosine 2′- and 3′-Monophosphates in Aqueous Acid: Competition between Phosphate Migration, Dephosphorylation, and Depurination

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Received November 17, 1988

First-order rate constants for mutual isomerization, hydrolytic dephosphorylation, and depurination of adenosine 2′- and 3′-monophosphates have been determined by HPLC over a wide pH range. The interconversion of 2′- and 3′-AMP is first order with respect to hydronium ion at pH < 1, approaches a second-order dependence at 1 < pH < 2, and becomes pH independent at 3 < pH < 6. The resulting equilibrium mixture contains 30% of 2′-AMP and 70% of 3′-AMP. Hydrolysis of cyclic 2′,3′-AMP has been observed to give the same product distribution. Hydrolytic dephosphorylation competes with the phosphate migration at 2 < pH < 6 and acidic depurination at pH < 3. The reactive ionic forms have been deduced from the shapes of the pH–rate profiles obtained. Incorporation of ^{18}O from solvent water into 2′- and 3′-AMP has been shown to be considerably slower than their acid-catalyzed interconversion, which excludes the reaction via cyclic 2′,3′-monophosphate as the main pathway.

Introduction

It has been known since the early 1950s that nucleoside 2′- and 3′-monophosphates undergo a mutual isomerization in aqueous acid.[1,2] However, surprisingly little attention has been paid to the kinetics and mechanism of this reaction. In fact, the available kinetic data are limited to the semiquantitative observation of Abrash et al.,[3] according to which uridine cyclic-2′,3′-monophosphate is hydrolyzed to a mixture of uridine 2′- and 3′-monophosphates about 10 times faster than the latter nucleo-

(1) Brown, D. M.; Todd, A. R. *J. Chem. Soc.* **1952**, 52.
(2) Cohn, W. E. *J. Am. Chem. Soc.* **1950**, *72*, 2811.
(3) Abrash, H. I.; Cheung, C.-C. S.; Davis, J. C. *Biochemistry* **1967**, *6*, 1298.

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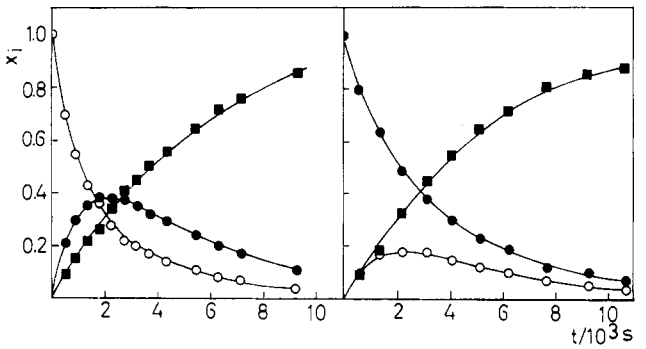

Figure 1. Time-dependent product distributions for reactions of 2'- and 3'-AMP in aqueous hydrogen chloride (0.10 mol dm^{-3}) at 363.2 K. Notation: 2'-AMP (open circles), 3'-AMP (filled circles), adenine (filled squares).

tides are interconverted. More extensive studies have been carried out with simple acyclic model compounds. Bailly reported already in 1942 that glycerol 2-monophosphate is readily rearranged in aqueous acid to the corresponding 1-monophosphate,[4] and Harrison et al. observed this reaction to be accompanied with incorporation of ^{18}O from solvent water into the phosphate group.[5] Later the ^{18}O incorporation was attributed to an intermediary formation of a cyclic phosphodiester, with which a direct intramolecular displacement competes.[6] The existence of these two routes has recently been verified by using 1,2-propanediol 2-monophosphate as a model compound.[7] Both reactions were suggested to proceed by way of a trigonal-bipyramidal transition state. According to Westheimer's concept of a pseudorotating pentacoordinated intermediate the entry and departure of the ligands is possible in an apical position only.[8] The transition state leading to the cyclic ester may adopt this kind of stereochemical arrangement without preceding pseudorotation, and hence the reaction is called as an "in-line associative" process. In the direct displacement reaction the pentacoordinated intermediate must pseudorotate before the leaving group may occupy an apical position, and the reaction is called as an "adjacent associative" process. Buchwald et al.[7] has shown that the latter reaction proceeds with retention of configuration at phosphorus, in consistence with the predicted behavior of a pseudorotating pentacoordinated intermediate.

Detailed understanding of the reactions that nucleoside 3'-monophosphates may undergo is desirable in attempting to evaluate the significance of secondary reactions occurring during the treatments of nucleic acids or their fragments under acidic conditions. Moreover, this kind of information may have some relevance to the studies aimed at clarifying the 2'-hydroxyl group assisted reactions that phosphotriester intermediates have been shown[9,10] to undergo during the chemical synthesis of oligoribonucleotides. For these reasons the interconversion of adenosine 2'- and 3'-monophosphates (2'- and 3'-AMP) and their dephosphorylation and depurination reactions were studied kinetically over a wide pH range. Comparative kinetic studies with adenosine cyclic 2',3'-monophosphate (2',3'-cAMP) were included to elucidate the role of a cyclic phosphodiester as a potential reaction intermediate.

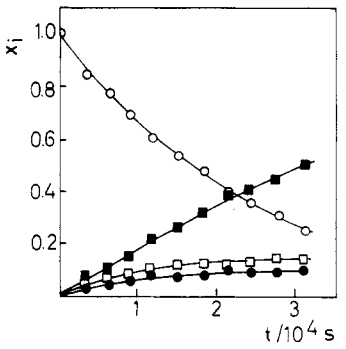

Figure 2. Time-dependent product distribution for reaction of 2'-AMP in aqueous hydrogen chloride (5×10^{-3} mol dm^{-3}, I = 0.10 mol dm^{-3} with NaCl) at 363.2 K. Notation: 2'-AMP (open circles), 3'-AMP (filled circles), adenosine (open squares), adenine (filled squares).

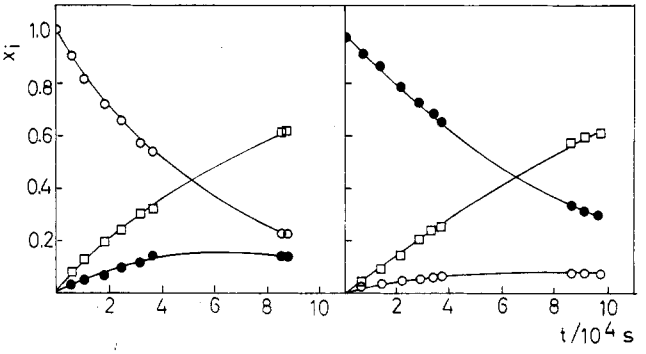

Figure 3. Time-dependent product distribution for reactions of 2'- and 3'-AMP in an acetic acid buffer ([HOAc]/[NaOAc] = 0.1/0.1 mol dm^{-3}) at 363.2 K. Notation: 2'-AMP (open circles), 3'-AMP (filled circles), adenosine (open squares).

Results and Discussion

Product Distributions. Figure 1 shows the time-dependent product distribution obtained by HPLC for the reactions of 2'- and 3'-AMP in 0.10 mol dm^{-3} aqueous hydrogen chloride at 363.2 K. As seen, these compounds undergo a relatively rapid interconversion and are simultaneously hydrolyzed to adenine and the appropriate ribose phosphates. The latter reaction must be approximately as rapid with both 2'- and 3'-AMP, because the release of adenine strictly obeyed first-order kinetics in spite of the concurrent phosphate migration. Furthermore, the rate constants obtained by using either 2'- or 3'-AMP as a strating material were equal within the limits of experimental errors, viz. $(2.06 \pm 0.03) \times 10^{-4}$ s^{-1} and $(2.11 \pm 0.03) \times 10^{-4}$ s^{-1}, respectively. No sign of an intermediary appearance of adenosine could be detected, which excludes hydrolytic dephosphorylation as a significant reaction pathway at high concentrations of hydronium ion. The hydrolysis of adenosine is under the experimental conditions only 2 times faster than the disappearance of 2'- and 3'-AMP,[11] and hence its formation should result in a detectable accumulation.

In contrast, the hydrolytic dephosphorylation competes efficiently with the phosphate migration and depurination in the pH range from 2 to 3 (Figure 2), as indicated by the intermediary appearance of adenosine under these conditions. On going to still lower concentrations of hydronium ion, depurination is retarded so much that dephos-

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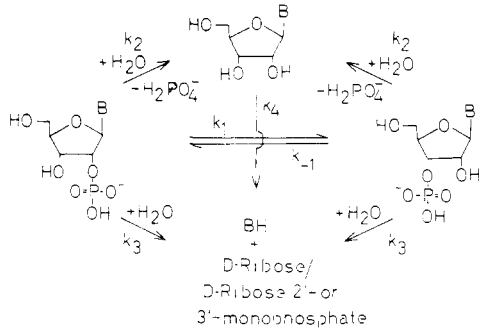
(5) Harrison, W.; Boyer, P. D.; Falcone, A. *J. Biol. Chem.* **1955**, *215*, 303.
(6) Fordham, W. D.; Wang, J. H. *J. Am. Chem. Soc.* **1967**, *89*, 4197.
(7) Buchwald, S. L.; Pliura, D. H.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4916.
(8) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.
(9) Reese, C. B.; Skone, P. A. *Nucleic Acids Res.* **1985**, *13*, 5215.
(10) Pathak, T.; Chattopadhyaya, J. *Acta. Chem. Scand.* **1985**, *B39*, 799.
(11) Lönnberg, H.; Lehikoinen, P. *Nucleic Acids Res.* **1982**, *10*, 4339.

Scheme I



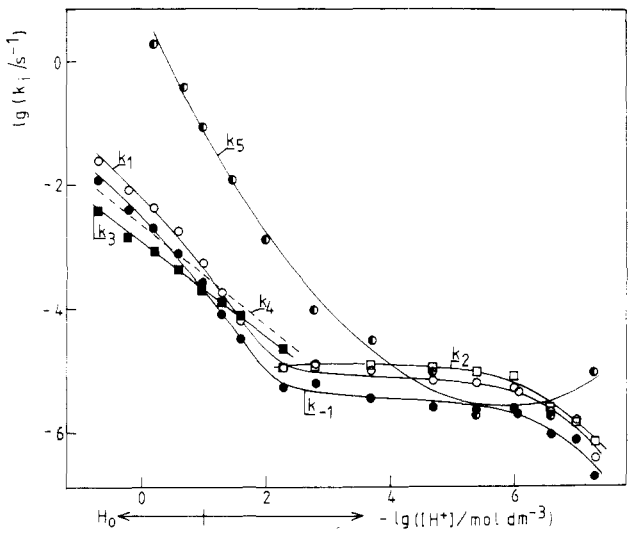


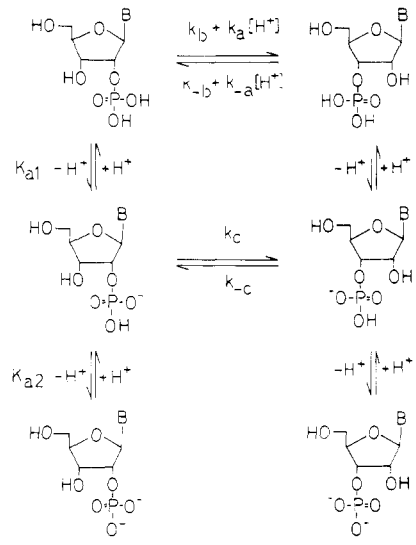
Figure 4. Rate profiles at 363.2 K for the reactions indicated in Scheme I. Ionic strength adjusted to 0.10 mol dm^{-3} with sodium chloride at $[H^+] < 0.10$ mol dm^{-3}.

phorylation becomes the only reaction detected in addition to phosphate migration. The time-dependent product distributions in Figure 3 also show that 2′- and 3′-AMP must be dephosphorylated at about the same rate, since the rate of adenosine formation is independent of the isomeric nature of the starting nucleotide.

Scheme I summarizes the reactions that 2′- and 3′-AMP undergo under acidic conditions. The rate profile depicted in Figure 4 describe the competition between these reactions at different concentrations of hydronium ion. Kinetic data for the hydrolysis of 2′,3′-cAMP, a potential intermediate of phosphate migration, is also included. Kinetics and mechanisms of different reactions are discussed consecutively.

Phosphate Migration and Hydrolysis of 2′,3′-cAMP. The rate profiles for the interconversion of 2′- and 3′-AMP consist of four distinct regions (Figure 4). The reaction is of first-order with respect to hydronium ion at pH < 1, i.e. on the acidic side of the pK_{a_1} value of the phosphate group,[12] approaches a second-order dependence between pH 1 and 2, becomes pH independent at 3 < pH < 6, and shows again a first-order dependence on acidity at pH > 7. The isomerization of 2′-AMP to 3′-AMP is over the whole acidity range faster than its reverse reaction, the ratio of the rate constants, k_1/k_{-1}, varying from 2.1 to 2.6. This kind of rate profile is consistent with a set of reactions indicated in Scheme II. The broad plateau between pH 3 and 6 refers to intramolecular nucleophilic attack of neighboring hydroxyl group on the 2′- and 3′-mono-

Scheme II



phosphate monoanion (k_c and k_{-c} in Scheme II). In the vicinity of pH 7 the phosphate monoanions are dissociated to dianions,[12] and hence nucleophilic attack of hydroxyl group is considerably retarded. The ascending part of the rate profile at pH < 3 in turn refers to the reactions of the uncharged phosphate group. An intramolecular nucleophilic attack on the phosphorus atom of the neutral phosphate group results in a phosphate migration that may be either uncatalyzed (k_b and k_{-b}) or catalyzed by hydronium ion (k_a and k_{-a}). The latter process must be quantitatively more important, since the reaction order with respect to hydronium ion is considerably larger than unity on the basic side of pK_{a_1} of the phosphate group. Accordingly, in this region two protons are needed for the reaction of the predominant ionic form, i.e. phosphate monoanion. At pH < 1 the prevailing ionic form is neutral monophosphate, and hence the observed first-order dependence on acidity may be attributed to a hydronium ion catalyzed reaction of this species. In this respect, the situation is analogous to the hydrolysis of monoalkyl monophosphates, where the hydronium ion catalyzed reaction is under all conditions more important than the spontaneous heterolysis of the uncharged ester.[13]

On the bases of the preceding discussion, the observed first-order rate constant, k_1, for the conversion of 2′-AMP to 3′-AMP may be expressed by eq 1, where it is further assumed that the influence of the protonation state of the base moiety on the reactions of the phosphate moiety is negligible. An analogous equation is valid for the rate

$$k_1 = \frac{\frac{k_a}{K_{a_1}}[H^+]^2 + \frac{k_b}{k_{a_1}}[H^+] + k_c}{\frac{k_{a_2}}{[H^+]} + 1 + \frac{[H^+]}{K_{a_1}}} \quad (1)$$

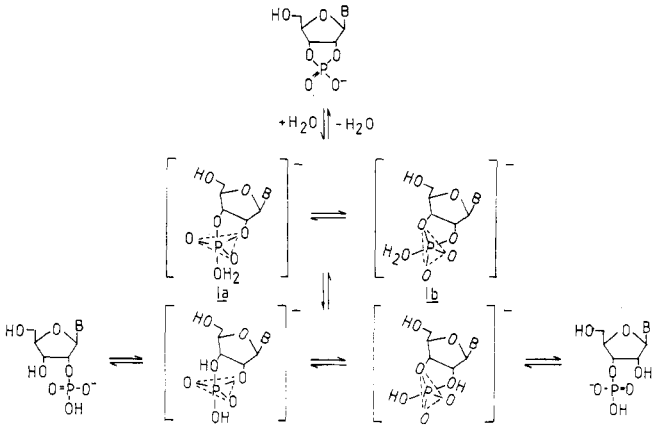
constant, k_{-1}, of the reverse reaction. Least-squares fitting[14] gave the following values for the partial rate and equilibrium constants: $K_{a_1} = 5.2 \times 10^{-2}$ mol dm^{-3}, $K_{a_2} = 5.9 \times 10^{-7}$ mol dm^{-3}, $k_a = 6.4 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}, $k_b = 4.6 \times 10^{-5}$ s^{-1}, and $k_c = 7.8 \times 10^{-6}$ s^{-1}. The corresponding values for the reverse process were: $k_{a_1} = 5.1 \times 10^{-2}$ mol dm^{-3}, $K_{a_2} = 5.8 \times 10^{-7}$ mol dm^{-3}, $k_{-a} = 3.0 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}, $k_{-b} = 2.2 \times 10^{-5}$ s^{-1}, and $k_{-c} = 3.4 \times 10^{-6}$ s^{-1}.

(12) Izatt, R. M.; Christensen, J. J.; Rytting, J. H. *Chem. Rev.* **1971**, *71*, 439.

(13) Cox, J. R., Jr.; Ramsay, O. B. *Chem. Rev.* **1964**, *64*, 317.

(14) Ruckdeschel, F. R. *Basic Scientific Subroutines*; Byte/McGraw-Hill: Peterborough, NH, 1981; Vol. 2 p 75.

Scheme III



The rate profile obtained for the hydrolysis of 2′,3′-cAMP (k_5 in Figure 4) is very similar to that reported by Eftink and Biltonen[15] for the hydrolysis of uridine cyclic-2′,3′-monophosphate. As shown by them, the observed first-order rate constant may be expressed by eq 2, where

$$k_5 = k_d[H^+]^2 + k_e[H^+] + k_f + k_g[OH^-] \quad (2)$$

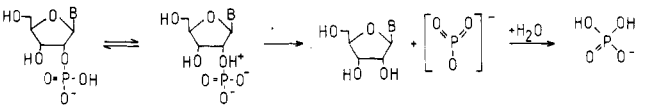
k_d, k_e, k_f, and k_g are the rate constants for the second- and first-order hydronium ion catalyzed, uncatalyzed, and hydroxide ion catalyzed reactions of the monoanion of 2′,3′-cAMP, respectively. The values obtained by least-squares fitting for these constants were: $k_d = 7.6$ dm^6 mol^{-2} s^{-1}, $k_e = 0.11$ dm^3 mol^{-1} s^{-1}, $k_f = 2.4 \times 10^{-6}$ s^{-1}, and $k_g = 0.7$ dm^3 mol^{-1} s^{-1}.

It is noteworthy that hydrolysis of 2′,3′-cAMP, which is at high concentrations of hydronium ion about 100 times faster than the phosphate migration, yields 2′- and 3′-AMP in exactly the same ratio as the interconversion of the latter compounds. Probably both reactions take place via the same pentacoordinated intermediate, which is decomposed to 2′- and 3′-AMP, and possibly also to 2′,3′-cAMP, as illustrated in Scheme III for the uncatalyzed processes. This intermediate may be obtained either by an intermolecular attack of water on 2′,3′-cAMP or by an intramolecular cyclization of 2′- and 3′-AMP. According to the concept of pseudorotating pentacoordinated intermediates, the entering nucleophile always occupies an apical position and five-membered rings adopt an exclusive apical–equatorial placement. Consequently, the cyclization of 2′-AMP must lead to an intermediate bearing the 3′-oxygen in an apical and 2′-oxygen in an equatorial position (Ia), while in the intermediate resulting from 3′-AMP (Ib) 2′-oxygen is apical and 3′-oxygen equatorial. An attack of water on 2′,3′-cAMP may give either of these structures. In all likelihood, Ia and Ib undergo a rapid equilibration at the elevated temperature employed, and thus the product distribution is independent of the nature of the starting material, and is determined by the relative rates of the reactions leading to 2′-AMP and 3′-AMP.

The available data strongly suggest that the equilibrium mixture of the pentacoordinated intermediates is converted to 2′,3′-cAMP much more slowly than to 2′- and 3′-AMP. When an equilibrium mixture of 2′- and 3′-AMP was treated with sulfuric acid (0.25 mol dm^{-3}) in ^{18}O-enriched water (^{18}O atom fraction > 96), and the isotopic composition of the enzymatically released phosphoric acid was determined by mass spectrometry, the ^{18}O isotope was detected to incorporate only slowly into the starting materials. The first-order rate constant for the incorporation

(15) Eftink, M. R.; Biltonen, R. L. *Biochemistry* **1983**, *22*, 5134.

Scheme IV



was 7.3×10^{-4} s^{-1}, while $k_1 + k_{-1}$ for the isomerization was 6.2×10^{-3} s^{-1}. Moreover, 2′,3′-cAMP was not accumulated during the isomerization of 2′- and 3′-AMP at pH 4–6, although its hydrolysis is under these conditions was even slower than the interconversion of 2′- and 3′-AMP. The preceding observations are consistent with the earlier results of Haake and Westheimer,[16] according to which the hydrolysis of ethylene phosphate is 5 times faster than exchange of oxygen with solvent. In summary, both the acid-catalyzed and uncatalyzed interconversions of 2′- and 3′-AMP appear to take place predominantly by an "adjacent associative" mechanism, i.e. without a marked intermediary appearance of 2′,3′-cAMP.

Dephosphorylation. Hydrolysis of 2′- and 3′-AMP to adenosine and hydrogen phosphate ion competes with the phosphate migration at pH > 3, the rate of this reaction remaining almost constant from pH 2 to 6, i.e. in the region where phosphate monoanion is the predominant ionic form. Since the hydrolysis is approximately as rapid as that of monoanions of simple monoalkyl phosphates,[13] it seems probable that neighboring hydroxyl groups do not participate intramolecularly in the reaction, and the mechanism presented for phosphomonoesters is utilized. Accordingly, a proton transfer from the phosphate hydroxyl ligand to the esterified oxygen, either directly or through water molecules, results in rupture of the PO bond with concomitant formation of a metaphosphate ion, either as a free species or preassociated with a molecule of water (Scheme IV).[17] Dianions of phosphomonoesters do not contain a proton that could be transferred to the leaving alkoxy group, and hence the reaction is retarded in the vicinity of pK_{a_2} of the 2′-/3′-phosphate group. With neutral phosphomonoesters the developing metaphosphate intermediate may be assumed to be less efficiently stabilized by resonance, and thus a deceleration takes place at high concentrations of hydronium ion. The observed first-order rate constant may be expressed by eq 3, and

$$k_2 = k_h/([H^+]K_{a_1}^{-1} + 1 + K_{a_2}[H^+]^{-1}) \quad (3)$$

the best fit with experimental data is obtained with values: $K_{a_1} = 7 \times 10^{-2}$ mol dm^{-3}, $K_{a_2} = 8 \times 10^{-7}$ mol dm^{-3}, and $k_h = 1.6 \times 10^{-5}$ s^{-1}.

Depurination. Hydrolysis of the N-glycosidic bond competes with interconversion of 2′- and 3′-AMP at pH < 3. The reaction is of first-order with respect to hydronium ion and is in all likelihood mechanistically analogous to the hydrolysis of adenosine[11] and its 5′-monophosphates.[18] Accordingly, a rapid initial protonation of the adenine moiety, giving an N^1-monocation or N^1,N^7-dication,[19] is followed by a unimolecular rate-limiting heterolysis to adenine and D-ribose 2′/3′-monophosphate. The first-order rate constants obtained with an equilibrium

(16) Haake, P. C.; Westheimer, F. H. *J. Am. Chem. Soc* **1961**, *83*, 1102.

(17) For a discussion on the role of metaphosphate intermediate see: (a) Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313. (b) Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4911. (c) Ramirez, F.; Marecek, J.; Minore, J.; Srivastava, S.; le Noble, W. J. *J. Am. Chem. Soc.* **1986**, *108*, 348. (d) Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 6126. (e) Burgess, J.; Blundell, N.; Cullis, P. M.; Hubbard, C. D.; Misra, R. *J. Am. Chem. Soc.* **1988**, *110*, 7900.

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(19) Remaud, G.; Zhou, X.-X.; Chattopadhyaya, J.; Oivanen, M.; Lönnberg, H. *Tetrahedron* **1987**, *43*, 4453.

mixture of 2′- and 3′-AMP in aqueous hydrogen chloride (0.10 mol dm^{-3}, 363.2 K), viz. 2.09×10^{-4} s^{-1}, is almost equal to that determined for 5′-AMP (1.82×10^{-4} s^{-1}),[18] and thus somewhat smaller than that determined for adenosine (4.50×10^{-4} s^{-1}).[11] For comparison, the relative rate constants for the acid-catalyzed hydrolysis of 2′-deoxycytidine and its 3′- and 5′-monophosphates have been reported to be 1, 0.40, and 0.24, respectively.[20]

As mentioned in the foregoing, 2′- and 3′-AMP are hydrolyzed at about same rate. This is rather surprising, since the 2′-phosphate group is α to the developing oxocarbenium ion center, and hence its inductive influence on the stability of the ionic intermediate would be expected to be larger than that of the 3′-phosphate group. To obtain further evidence for the comparable rates of depurination of 2′- and 3′-AMP, we studied the hydrolysis of 7-methylguanosine 2′- and 3′-monophosphates, with which the depurination at pH 4–5 is much faster than their interconversion. The rate constants obtained at pH 4.7 (acetic acid buffer, 363.2 K) were 1.45×10^{-4} s^{-1} and 1.37×10^{-4} s^{-1}, respectively. One may tentatively assume that the effects of phosphate groups on conformation of the ribofuranosyl ring play an equally important role as the inductive effects.

In summary, the present data give a quantitative description of the reactions that 2′- and 3′-AMP may undergo in acidic and neutral solutions and help to understand the mechanisms of these reactions. In basic solutions degradation of the adenine moiety becomes the major reaction, and most probably this process is kinetically and mechanistically similar to the alkaline decomposition of adenosine.[21]

Experimental Section

Materials. The adenine nucleotides used in the kinetic measurements were products of Sigma. They were used as received, after checking of purity by HPLC. Preparation of 7-methylguanosine 2′- and 3′-monophosphates has been described earlier.[22]

Kinetic Measurements. Reactions were followed by analyzing the compositions of the samples withdrawn at appropriate intervals by HPLC, as described previously.[23] The initial substrate concentration was about 7×10^{-4} mol dm^{-3}, and the temperature was maintained at 363.2 K within 0.1 K. Chromatographic separations were carried out on a commercial Hypersil ODS column (4 × 250 mm, 5 m) using an acetic acid buffer (pH 4.3) containing ammonium chloride (0.2 mol dm^{-3}) and acetonitrile (4% v/v) as an eluant. Mole fractions of the compounds in each aliquot were determined with aid of calibration solutions of known concentrations.

The hydronium ion concentrations, adjusted with hydrogen chloride and formate, acetate, maleate, and triethanolamine buffers, were calculated from the pK_a values of the buffer acids under the experimental conditions.[24-27] No catalysis by the buffer constituents was observed at the concentrations employed (<0.1 mol dm^{-3}).

First-order rate constants, k_1 and k_{-1}, for the interconversion of 2′- and 3′-AMP were calculated by eq 4 and 5, where x stands

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

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

for [2′-AMP]/([2′-AMP] + [3′-AMP]) at moment t, and x_e is the corresponding quantity at equilibrium. First-order rate constants for dephosphorylation (k_2) and depurination (k_3) were obtained by eq 6 and 7, where [Ado] is the concentration of adenosine at

$$[\text{Ado}] = ([2'\text{-AMP}] + [3'\text{-AMP}]) \left[\frac{k_2}{k_4 - k_d} \exp(-k_d t) + \frac{k_2}{k_d - k_4} \exp(-k_4 t) \right] \quad (6)$$

$$k_d = k_2 + k_3 \quad (7)$$

moment t. The values of k_4 were taken from ref 11. When no adenine was detected, k_2 was assumed to be equal to k_d, the rate constant for the disappearance of 2′- and 3′-AMP. Similarly, k_d was assumed to be equal to k_3 in runs where no adenosine was formed. Rate constants, k_5, for the hydrolysis of 2′,3′-cAMP were calculated by integrated first-order rate equation.

^{18}O Incorporation. Incorporation of ^{18}O into 2′- and 3′-AMP concurrent with their interconversion was studied as described earlier for 1,2-propanediol 2-monophosphate.[7] Accordingly, an equilibrium mixture of the starting materials (0.016 mmol of 2′-AMP and 0.027 mmol of 3′-AMP) was treated with sulfuric acid (0.28 mmol) in ^{18}O-enriched water (1 cm^3, Amersham, ^{18}O atom % > 96) at 363.2 K. Aliquots of 0.15 cm^3 were withdrawn, and isotopic composition of the phosphoric acd, released enzymatically from the starting materials,[7] was determined on a VG 7070E mass spectrometer with a direct inlet system. The ^{18}O contents (calculated from intensities of peaks 81, 83, 85, 98, 100, and 102) in samples withdrawn at 120, 220, 420, and 900 s were 0.10, 0.14, 0.28, and 0.46, respectively.

Acknowledgment. We wish to thank Pentti Oksman, M.Sc., and Kirsti Wiinamäki for their skillful assistance in the mass spectrometric analyses.

Registry No. 2′-AMP, 130-49-4; 3′-AMP, 84-21-9; 2′,3′-cAMP, 634-01-5.

Supplementary Material Available: Table of first-order rate constants k_i for 2′- and 3′-AMP and 2′,3′-cAMP (1 page). Ordering information is given on any current masthead page.

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