3,3- and 4,4-Dimethyl-*N*-methylpiperidine-2,2,5,5-d_4 (**5** and **7**) were prepared as above using a 20% methylamine–methanol solution.[11f] The compounds were identified by comparison of vpc retention times with authentic samples (prepared below). The *N*-methylpiperidines were preparatively separated by vpc as above.

3,3- and 4,4-Dimethylpiperidines. Authentic samples of these compounds and their *N*-methyl analogs were separately prepared by lithium aluminum hydride reduction of commercially available 2,2- and 3,3-dimethylglutaric methyl esters, followed by bromination and cyclization as for the above deuterated compounds. At each step, these materials were used to identify isomers prepared by the above routes.

Nmr Sample Preparations. The piperidines were weighed into an nmr sample tube and the appropriate amount of solvent was added to give a 15% solution (w/v). Protonated piperidines were prepared in CD_3OD by flushing the nmr sample tubes with HCl gas in successive portions until no further shift of the β-methylene protons was observed.

Ring Openings of Trimetaphosphoric Acid and Its Bismethylene Analog. Syntheses of Adenosine 5′-Bis(dihydroxyphosphinylmethyl)phosphinate and 5′-Amino-5′-deoxyadenosine 5′-Triphosphate

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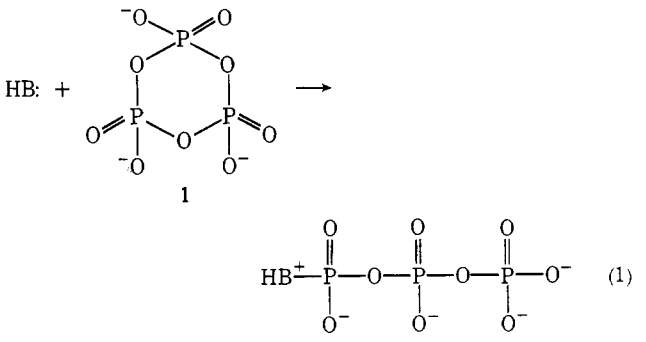
Abstract: The reactions of some alcohols with salts of trimetaphosphoric acid in nonaqueous solutions were investigated. Both the ease of ring opening and product distributions were found to be strongly dependent upon whether the solutions were acidic or basic. With acid catalysis, anhydrous methanol reacted with tricyclohexylammonium trimetaphosphate to give methyl phosphate as the only detectable esterified product, even in the early stages of the reaction; in basic media the products were identified as methyl mono-, di-, and triphosphate and inorganic mono-, di-, and triphosphate. The yield of the simple ring-opening product, methyl triphosphate, never exceeded approximately 10%. When 5′-amino-5′-deoxyadenosine was treated with trisodium trimetaphosphate in aqueous solution, a relatively rapid reaction occurred to form 5′-amino-5′-deoxyadenosine 5′-triphosphate, a new analog of adenosine 5′-triphosphate. The cyclic bismethylene analog of trimetaphosphate was synthesized and characterized, and acid-catalyzed ring opening reactions with this compound were performed with anhydrous methanol and anhydrous ethanol to generate the monomethyl and monoethyl esters of bis(dihydroxyphosphinylmethyl)phosphinate, respectively. Moreover, using 2′,3′-isopropylideneadenosine as the nucleophile in an analogous reaction, the synthesis of adenosine 5′-bis(dihydroxyphosphinylmethyl)phosphinate, the $\alpha,\beta:\beta,\gamma$-bismethylene analog of adenosine 5′-triphosphate, was achieved.

Ever since the early recognition of the fundamental importance of adenosine 5′-triphosphate (ATP) and other related nucleoside triphosphates in biological systems, considerable research has been directed toward developing methods for the synthesis of monoesters of triphosphoric acid.[2-6] The simplest and most direct of these is the ring opening of the trimetaphosphate anion **1** by appropriate nucleophilic reagents, as shown in eq 1. The trimetaphosphate anion is unique among the condensed inorganic phosphates in its degree of relative susceptibility to nucleophilic attack in basic media.[7] Several research groups have exploited this fact and have investigated the ring opening of salts of trimetaphosphate by various nucleophiles in aqueous solutions.[7-15] Thus, reactions



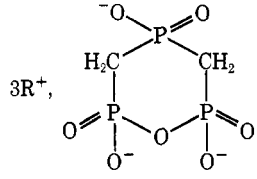
(1)

with hydroxide,[7] ammonia,[8,11] methylamine,[10] ethylamine,[10] dimethylamine,[13] and fluoride ion,[10] for example, have all led to isolable, stable salts of simple ring-opened products. In 1967, Feldmann[15] reported that trisodium trimetaphosphate reacted with strongly

(1) National Institutes of Health Predoctoral Fellow, 1967–1969.
(2) M. Smith and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 1141 (1958).
(3) J. G. Moffatt, *Can. J. Chem.*, **42**, 599 (1964).
(4) M. Ikehara and E. Ohtsuka, *Chem. Pharm. Bull.*, **11**, 961 (1963).
(5) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 649 (1961).
(6) D. Hoard and D. Ott, *ibid.*, **87**, 1785 (1965).
(7) E. Thilo, *Angew. Chem., Int. Ed. Engl.*, **4**, 1061 (1965).
(8) O. T. Quimby and T. J. Flautt, *Z. Anorg. Allg. Chem.*, **296**, 221 (1958).
(9) P. W. Schenk and K. Dommain, *ibid.*, **326**, 139 (1963).
(10) W. Feldmann and E. Thilo, *ibid.*, **327**, 158 (1964).
(11) W. Feldmann and E. Thilo, *ibid.*, **328**, 113 (1964).
(12) W. Feldmann, *ibid.*, **338**, 235 (1965).
(13) W. Feldmann, *Z. Chem.*, **5**, 26 (1965).
(13a) NOTE ADDED IN PROOF. After submission of this paper, R. L. Letsinger, J. S. Wilkes, and L. B. Dumas [*J. Amer. Chem. Soc.*, **94**, 292 (1972)] have reported on the synthesis of the related 5′-amino-5′-deoxythymidine 5′-triphosphate by an analogous procedure and have demonstrated its use as a substrate for DNA polymerase I of *Escherichia coli* in the synthesis of polydeoxyribonucleotides.
(14) W. Feldmann, *Chem. Ber.*, **99**, 3251 (1966).
(15) W. Feldmann, *ibid.*, **100**, 3850 (1967).

basic aqueous methanol to give a 39% isolated yield of methyl triphosphate after 3 weeks at room temperature. An attempt to prepare ethyl triphosphate by an analogous procedure gave only a 4% yield of the desired product after 7 weeks at room temperature. None of the corresponding alkyl triphosphates from normal alcohols of C_3 or larger could be prepared by this approach.[15] This is not unexpected since no alkoxide ion which is a very much stronger base than hydroxide ion can exist in any appreciable concentration in aqueous media.[16] In the hope of eventually extending this reaction to more complex alcohols than methanol and ethanol, we report here on some investigations of ring openings of trimetaphosphate salts by methanol in nonaqueous media.

We also report the synthesis and characterization of salts of the bismethylene analog of trimetaphosphate (**2**).[17] Because of the relative strength of the phosphorus–carbon bonds,[18] we felt that the simple ring-opened products from **2** with alcohols would form bis-



2a, $R^+ = Na^+$
b, $R^+ = C_6H_{11}NH_3^+$
c, $R^+ = [CH_3(CH_2)_3]_3NH^+$

methylene analogs of monoalkyl triphosphates of relatively great hydrolytic stability. Portions of this work have been presented as a communication.[19]

Results and Discussion

When tricyclohexylammonium trimetaphosphate was dissolved in anhydrous methanol and the solutions were heated in sealed tubes at 100°, the trimetaphosphate ring gradually solvolyzed. Since 1 equiv of acid was released each time a P–O–P bond was solvolyzed, the medium became progressively more acidic, and, eventually, complete methanolysis occurred leading to an essentially quantitative yield of methyl phosphate. Indeed, methyl phosphate was the only product detectable along with unreacted trimetaphosphate when the reaction was stopped at various stages and the products were investigated by both nmr spectroscopy and polyethylenimine cellulose thin-layer chromatography. Benzyl alcohol behaved similarly when heated with tricyclohexylammonium trimetaphosphate at 205° for 5 hr, but, in addition to giving a 50% isolated yield of dicyclohexylammonium benzyl phosphate, gave a 17% isolated yield of cyclohexylammonium dibenzyl phosphate.

In order to stop the reaction at the product of simple ring opening, the monoalkyl triphosphate, it is apparent that the presence of strong base is required. Thus, Quimby, Narath, and Lohman[20] have studied the effect of pH on the stabilities of a number of imido- and oxy-linked polyphosphates at 60° in aqueous solution, including inorganic triphosphate and trimetaphosphate. Both of these latter two compounds were found to be progressively more stable toward hydrolysis as the pH values of the solutions studied were varied from 1 to 8. At pH 11 the linear triphosphate was even more stable than at pH 8, but the trimetaphosphate, in contrast, was less stable than it was at pH 8, and considerably less stable at pH 11 than the linear triphosphate. Hence, at very high pH values (*ca.* pH 11 or greater) trimetaphosphate slowly reacts with hydroxide ion to give good yields of inorganic triphosphate,[7] relatively uncontaminated by further breakdown products.

An analogous situation obtains in nonaqueous media; that is, we were able to demonstrate the production of small amounts of methyl triphosphate from the reaction of methanol with trimetaphosphate in nonaqueous media in the presence of relatively strong bases. Thus, Feldmann's contention that water is required for the formation of methyl triphosphate from trimetaphosphate[15] is unwarranted. The methyl triphosphate and the other contaminating products, inorganic phosphate, inorganic pyrophosphate, inorganic tripolyphosphate, methyl phosphate, and methyl diphosphate, were detected by PEI cellulose thin-layer chromatography. The same mixture of products was reported by Feldmann by aqueous acid treatment of methyl triphosphate.[15] We obtained authentic samples of methyl triphosphate from both Feldmann and Brintzinger[21] and were able to show that the methyl triphosphate which we have generated has identical chromatographic properties and, moreover, has an identical nmr chemical shift in D_2O solution with those of the authentic samples. The doublets ($J_{POCH} \cong 11$ Hz) corresponding to the methyl groups of methyl phosphate, methyl diphosphate, and methyl triphosphate all were observed to absorb at different chemical shifts in D_2O solution.

It occurred to us that if two of the P–O–P oxygens of trimetaphosphate were replaced by methylene groups, then ring openings by solvolysis should lead to products uncontaminated by further breakdown products since phosphorus–carbon bonds are normally so strong.[22] Moreover, if successful, this would lead to a convenient synthesis of bismethylene analogs of monoalkyl triphosphates. The synthesis of the bismethylene analog of trimetaphosphate (**2**), patterned after the known conversion with condensing agents of inorganic tripolyphosphate to inorganic trimetaphosphate,[23] has now been achieved.

The identity of the bismethylene analog of trimetaphosphate (**2**) is now reasonably secure. Tricyclohexylammonium and trisodium salts giving correct microanalyses were prepared. The most compelling evidence for its structure, however, came from the potentiometric titration of the free acid, which showed the presence of only relatively strongly acidic protons[24]

(16) J. M. T. Caudri, *Recl. Trav. Chim., Pays-Bas*, **48**, 589 (1929).
(17) Two possible names have been suggested for the free acid by Dr. Kurt L. Loening, Nomenclature Director, Chemical Abstracts Service: (phosphinicodimethylene)diphosphoric acid cyclic *P,P'*-anhydride or 2,4,6-trihydroxy-1,2,4,6-oxatriphosphorinane 2,4,6-trioxide.
(18) T. Myers, K. Nakamura, and J. Flesher, *J. Amer. Chem. Soc.*, **85**, 3292 (1963).
(19) D. B. Trowbridge and G. L. Kenyon, *ibid.*, **92**, 2181 (1970).
(20) O. T. Quimby, A. Narath, and F. H. Lohman, *ibid.*, **82**, 1099 (1960).
(21) P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).
(22) G. M. Kosolopoff, "Organophosphorus Compounds," Wiley, New York, N. Y., 1950.
(23) E. Thilo, I. Grunze, and H. Grunze, *Monatsber. Deut. Akad. Wiss. Berlin*, **1**, 40 (1959).
(24) The observed titration curve is in accord with expectation based on known pK_a values for analogous compounds. For example, M. I. Kabachnik, T. A. Mastrukova, A. E. Shipov, and T. A. Melentyeva

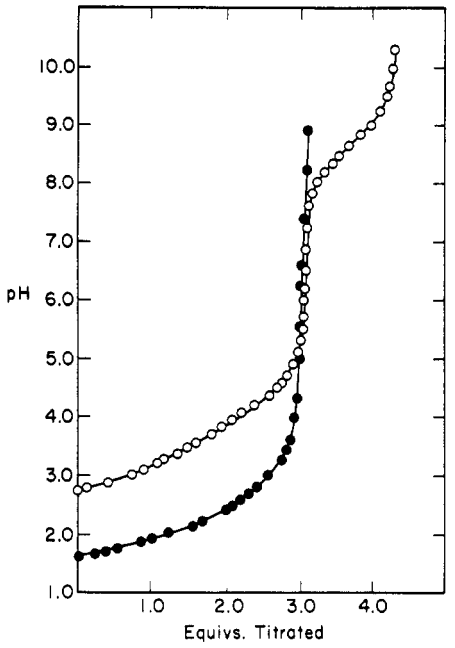


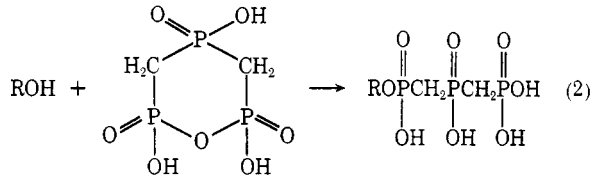
Figure 1. Potentiometric titration curves for the bismethylene analog of trimetaphosphoric acid (solid circles) and adenosine 5′-bis(dihydroxyphosphinylmethyl)phosphinic acid (open circles).

(see Figure 1). Also, when heated with either methanol or ethanol for long periods of time, quantitative yields, as determined by nmr spectroscopy, of the monomethyl and monoethyl esters, respectively, of bis(dihydroxyphosphinylmethyl)phosphinic acid were generated.

Both of these monoalkyl esters were characterized as their tetrasodium salts which could be recrystallized from aqueous ethanol. No evidence for further acid-catalyzed esterification could be observed, even after extended periods of heating in these solvents.

The behavior of **2** on PEI cellulose thin-layer chromatography was also in accord with the proposed structure. The observed R_f value of 0.70 with 0.5 *N* LiCl as eluent is considerably greater than that for the bis (dihydroxyphosphinylmethyl)phosphinic acid under the same conditions (R_f = 0.20).

When solvolyzed with methanol or ethanol, as mentioned above, the bismethylene analog of trimetaphosphate underwent ring opening to form the corresponding monoalkyl ester of bis(dihydroxyphosphinylmethyl)phosphinate; *i.e.*, eq 2. When this solvolysis



(2)

R = Me, Et, 2′,3′-isopropylideneadenosyl

was carried out using 2′,3′-isopropylideneadenosine, the product was hydrolyzed in strong acid, purified by column chromatography, and then neutralized with sodium hydroxide solution, the tetrasodium salt of the ATP analog adenosine 5′-bis(dihydroxyphosphinylmethyl)phosphinate (**3**) was isolated. In addition to chromatographic and microanalytical evidence for its composition and purity, evidence for the given structure is based on analysis of the uv spectrum, analysis of the nmr spectrum, and on the results of a potentiometric titration.

[*Tetrahedron*, **9**, 10 (1960)] have measured pK_a values for many compounds of the type RR′P(O)OH and observed a value of pK = 3.1 for the case where R = R′ = CH_3 and a value of pK_1 = 2.3 for the case where R = CH_3, R′ = OH.

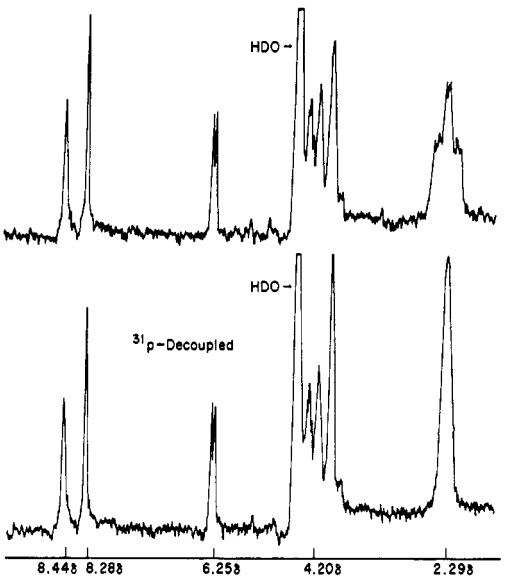
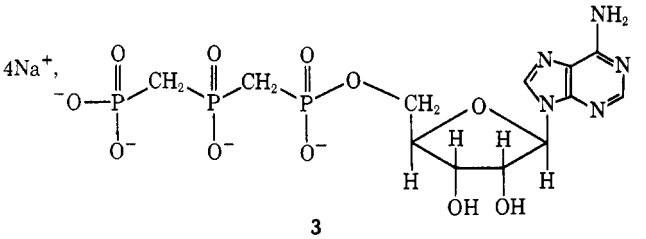


Figure 2. Proton nmr spectrum at 100 MHz (D_2O) of the tetrasodium salt of adenosine 5′-bis(dihydroxyphosphinylmethyl)phosphinic acid (above) and the same spectrum with ^{31}P nuclei decoupled (below).



3

The uv spectrum showed a λ_{max} at 259 nm (ϵ = 1.54 × 10^4). This is the same as that reported for ATP itself[25] and provides compelling evidence that the adenine ring is monosubstituted on the 1 position[26,27] as is the case for ATP.

The nmr spectrum taken at 100 MHz is shown in the upper part of Figure 2. The spectrum is very similar to that for ATP itself except, of course, for the multiplet centered at δ 2.29 which corresponds to the four protons of the methylene groups between the phosphorus atoms. The nmr spectrum taken on the same sample after decoupling the phosphorus atoms is shown in the lower part of Figure 2. As expected, the multiplet centered at δ 2.29 collapsed. Instead of the expected pair of singlets, one for each of the two methylene groups in different environments, the new peak was observed to be a broad singlet. Apparently, the chemical-shift difference at 100 MHz is not sufficiently large for these singlets to be resolved. Also, a broad peak which constitutes the major component of the multiplet centered at δ 4.20 partially collapsed as well when

(25) M. Boch, C. Nan-Sing Ling, R. Moell, and S. Lipton, *Arch. Biochem. Biophys.*, **62**, 253 (1956).

(26) N. J. Leonard and J. A. Deyrup, *J. Amer. Chem. Soc.*, **84**, 2148 (1962).

(27) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Amer. Chem. Soc.*, **86**, 5320 (1964).

the phosphorus was decoupled (Figure 2). This is also consistent with the proposed structure. This peak presumably arises from the two methylene protons on the 5′-carbon of the ribose moiety and should be coupled to phosphorus with $J_{POCH} \cong 7$–12 Hz.[28]

The potentiometric titration curve, shown in Figure 1, is in complete accord with the structure proposed; that is, the molecule is shown to have three rather strongly acidic protons and one rather weakly acidic proton. A comparison of the pK_a values for the least acidic titratable proton for ADP, ATP, and some of their known methylene analogs is made in Table I. The observed pK_a

Table I. pK_a Values for the Least Acidic Titratable Proton of Adenosine 5′-Diphosphate, Adenosine 5′-Triphosphate, and Some of Their Monomethylene and Bismethylene Analogs

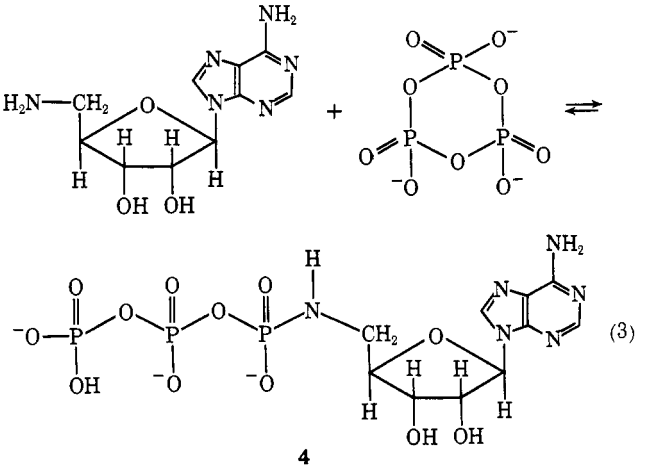
Compound	pK_a of least acidic proton
Adenosine 5′-diphosphate (ADP)	7.0[a]
Adenosine 5′-methylenediphosphonate (α,β-methylene analog of ADP)	8.0[a]
Adenosine 5′-triphosphate (ATP)	7.1[b]
5′-Adenylyl methylenediphosphonate (β,γ-methylene analog of ATP)	8.4–8.5[b]
Adenosine 5′-bis(dihydroxyphosphinylmethyl)-phosphinate (α,β:β,γ-bismethylene analog of ATP)	8.7[c]

[a] T. C. Myers, K. Nakamura, and A. Danielzadeh, *J. Org. Chem.*, **30**, 1517 (1965). [b] T. C. Myers, K. Nakamura, and J. Flesher, *J. Amer. Chem. Soc.*, **85**, 3292 (1963). [c] Determined by potentiometric titration (see Experimental Section; see also Figure 1).

value of 8.7 for the least acidic proton in **3** is seen to be in accord with the similar analogs. It is clear from this result that if an enzymatic system requires fully ionized anionic groups on the triphosphate moiety of ATP, these reactions must be performed at rather high pH for the bismethylene analog **3** to resemble and thereby replace the ATP at its binding site. Of course, the pK_a of this group could be altered by the presence of metal ions or in a special microenvironment within a given enzyme.[29]

One aspect of the isolation of the ATP analog **3** deserves comment, the chromatography on a Dowex-50 (H^+) cationic exchange resin (cross-linked polystyrene with sulfonic acid groups) column. The ATP analog was held up on the column sufficiently to allow separation from some contaminating bis(dihydroxyphosphinylmethyl)phosphinic acid which in turn emerged from the column relatively quickly. The most likely reason for the delay of the ATP analog on the highly acidic column is protonation of the molecule. The compound is not strongly basic, but even if only a small portion of the molecules at equilibrium is protonated by the sulfonic acid groups on the column at any given time, the ATP analog would still act as a cation and be delayed on the column. Chromatography of this general type is not unknown and has been used to separate and purify a wide variety of organic compounds including organic bases.[30] Moreover, Cohn,[31] and, more recently, Busch[32] have successfully used Dowex-50 (H^+) ion exchange column chromatography for the separations of some nucleoside and deoxynucleoside monophosphates.

The reaction of 5′-amino-5′-deoxyadenosine with trisodium trimetaphosphate to form 5′-amino-5′-deoxyadenosine 5′-triphosphate (**4**) is completely analogous to the known reactions with trimetaphosphate of ammonia,[8,11] methylamine,[10] ethylamine,[10] and dimethylamine.[13] The reaction was observed to be reversible, *i.e.*, eq 3. That is, when the ATP analog was



(3)

4

dissolved in water the slow formation of 5′-amino-5′-deoxyadenosine and trimetaphosphate could be followed by polyethylenimine cellulose thin-layer chromatography. Significantly, these were the only detectable products of the breakdown of **4**. As expected, the ATP analog chromatographed as a single spot, visualizable by both a uv lamp and by the Hanes–Isherwood molybdate spray reagent, with approximately the same R_f value as ATP itself on polyethylenimine cellulose thin-layer chromatography using 1.2 *N* LiCl as eluent. Further evidence for the structure shown came from a quantitative analysis of the inorganic phosphate released on hydrolysis of **4** in strong acid using the colorimetric determination of "labile" phosphate of Fiske and Subbarow.[33] Using the assumption that **4** has the same molar extinction coefficient as that for ATP (λ_{max} 259 nm, $\epsilon = 1.54 \times 10^4$), the results showed that 2.96 mol of phosphate was released per mole of **4**. Finally, the 5′-amino-5′-deoxyadenosine 5′-triphosphate has been shown in preliminary experiments to replace ATP as a substrate in the creatine kinase reaction. Since there is no reasonable possible source of contamination by ATP itself in the synthesis of **4**, and since creatine kinase from rabbit muscle requires a nucleoside triphosphate as the phosphorylating agent for the creatine,[34] this provides further substantiating evidence for the structure proposed for **4**. Further details about the activity of this new analog of ATP in biological systems will be published in due course.

Experimental Section

Materials and Methods. Infrared spectra were measured on a Perkin-Elmer infrared spectrometer (Model 137). Ultraviolet

(28) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 2, Pergamon Press, Oxford, England, 1966, p 1063.
(29) D. E. Schmidt, Jr., and F. H. Westheimer, *Biochemistry*, **10**, 1249 (1971).
(30) M. M. Melandri and A. Brittini, *Farmaco, Ed. Prat.*, **23**, 144 (1968).
(31) W. E. Cohn, *Science*, **109**, 376 (1949).
(32) E. W. Busch, *J. Chromatogr.*, **37**, 518 (1968).
(33) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).
(34) W. J. O'Sullivan and M. Cohn, *ibid.*, **243**, 2737 (1968).

spectra were measured using a Cary Model 14 recording spectrophotometer. Proton nmr spectra were determined on either a Varian Model T-60, A-60, or HA-100 spectrometer using tetramethylsilane (for nonaqueous solutions) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (for aqueous solutions) as internal standards, unless otherwise specified. Microanalyses and Karl-Fischer titrations[35] for water content were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. Melting points are uncorrected. All potentiometric titrations were carried out using a Sargent recording pH stat, Model S-30240, in conjunction with a Corning Model 12 Research pH meter.

Methanol,[36] *N,N*-dimethylformamide,[37] tri-*n*-butylamine,[38] pyridine,[39] and *p*-toluenesulfonyl chloride[40] were carefully dried and distilled before use as described by specific procedures in the literature references cited. By Karl-Fischer titration the water contents of the methanol and *N,N*-dimethylformamide, for example, were found to be <0.005 mg of H_2O/ml. Ethanol and benzyl alcohol were dried by storage for a minimum of 1 week over Linde type 4A molecular sieves (Union Carbide Corp., Tonawanda, N.Y.). The benzyl alcohol dried in this manner, for example, was found to have a water content of 0.20 mg of H_2O/ml.

Diethylaminoethyl (DEAE) cellulose (Bio-Rad Cellex-D) was purified by the method of Peterson and Sober[41] before use. Similarly, Bio-Rad AG-50WX8 (Dowex-50) cation exchange resin was purified before use by the method of Stein and Moore.[42] Anion exchange thin-layer chromatography was carried out on microscope slides (2.5 × 7.5 cm) coated with polyethylenimine (PEI)-impregnated cellulose as described previously.[43] Unless otherwise stated, spots were visualized using Hanes–Isherwood molybdate spray.[44]

The trisodium and tricyclohexylammonium salts of trimetaphosphoric acid were prepared as described previously.[45] The tricyclohexylammonium trimetaphosphate obtained gave a single spot on PEI cellulose thin-layer chromatography using 1.0 *M* LiCl as the eluent (R_f = 0.70). It also gave a single spot (R_f = 0.27) on paper chromatography using Whatman no. 1 paper and the eluting solvent described by Ebel,[46] 2-propanol–water–trichloroacetic acid–concentrated ammonia (150:50:5:0.4, v/v). The infrared spectrum (KBr) of this salt was found to be similar to but substantially different from that of the previously described material.[45] Thus, major peaks appeared at 3.10–4.00 (br), 4.91, 6.25, 6.50, 6.64, 6.81, 6.83, 7.22, 7.28, 7.78, 8.10, 8.61, 8.88, 9.04, 10.25 (br), 13.03, and 13.52 μ. It was later found that on standing over a period of weeks the solid-state infrared spectrum of these crystals progressively became more similar to that of the previously described material.[45] Since both materials gave correct microanalyses for a nonhydrated salt, this is presumably a case of polymorphism.

Tris(tri-*n*-butylammonium) trimetaphosphate was prepared by ion exchange from the tricyclohexylammonium salt. Thus, pure tricyclohexylammonium trimetaphosphate was dissolved in the minimal amount of water and passed through a Dowex-50 (H^+) column. The resulting dilute aqueous solution of the free acid was neutralized with freshly distilled tri-*n*-butylamine. The solvent was removed at reduced pressure and the salt was dried over P_2O_5 *in vacuo*. The salt was initially a viscous oil which slowly set up to an amorphous glass which proved to be intractable to recrystallization. The sample gave a single spot on PEI cellulose thin-layer chromatography (R_f = 0.70, 1.0 *M* LiCl eluent) which corresponded exactly to that for the tricyclohexylammonium salt (see above). As a further check on its identity and also as a check on the ability of the free acid to survive these manipulations, a 1.240-g portion of the tris(tri-*n*-butylammonium) trimetaphosphate was dissolved in water and the resulting solution was converted to the free acid by the use of the Dowex-50 column. This acid was neutralized with cyclohexylamine and the resulting tricyclohexylammonium salt was recrystallized. The ir spectrum of this product was identical with that of an authentic sample. The amount recovered was 0.621 g (83%).

Trilithium trimetaphosphate was prepared by neutralization of the free acid with LiOH and was dried over P_2O_5 *in vacuo* for 24 hr. It also gave a single spot with the expected R_f value for trimetaphosphate when chromatographed on a PEI cellulose thin layer. As expected,[47] aqueous solutions of the trisodium, tricyclohexylammonium, trilithium, or tris(tri-*n*-butylammonium) salts of trimetaphosphoric acid gave no precipitate with Ag^+, Pb^{2+}, or Ca^{2+}.

S-Methylthiuronium dimethyl phosphate[48] was used as an authentic sample for comparison purposes in both nmr and chromatographic studies.

S-Methylthiuronium dihydrogen phosphate was also prepared for comparison purposes. Thus, an aqueous solution which contained 4.85 g of 85% H_3PO_4 was neutralized to pH 4 with saturated $Ba(OH)_2$ solution and 6.05 g of 2-methyl-2-thiopseudourea sulfate (Aldrich Chemical Co.) was added. The $BaSO_4$ which formed was removed by filtration. After removal of the water at reduced pressure, the product was recrystallized from a mixture of ethanol and acetonitrile. The yield was 3.80 g (47%), mp 202.5–204.5° dec.

Anal. Calcd for $C_2H_9N_2O_4PS$: C, 12.77; H, 4.82; P, 16.46; S, 17.04. Found: C, 12.82; H, 4.87; P, 16.37; S, 16.64.

The ir spectrum (KBr) showed major bands at 2.70–4.90 (br), 6.00, 6.98, 8.16, 8.81, 9.11–9.46 (br), 9.75, 10.58, 11.50, 13.71, and 14.33 μ. The nmr spectrum (D_2O) showed a sharp singlet at δ 2.58.

S-Methylthiuronium methyl hydrogen phosphate, also prepared for use as an authentic sample, was generated by reaction of thiourea with dimethyl 1-phenylvinyl phosphate. Thus, dimethyl 1-phenylvinyl phosphate was prepared by the method of Chopard, *et al.*[49] The product mixture was distilled and a fraction was collected at 105–107° (1.5 mm) (lit.[49] bp 106–109° (0.5 mm)). A 2.29-g (0.010 mol) portion of this ester was added to a solution of 0.76 g (0.010 mol) of thiourea in 2-propanol, and the solution was heated at reflux for 4 hr. After about 90 min, a solid began to precipitate. The solvent was removed at reduced pressure, and the solid residue was recrystallized from ethanol–acetonitrile. The yield of purified *S*-methylthiuronium methyl hydrogen phosphate was 1.2 g (58%), mp 151–152° dec.

Anal. Calcd for $C_3H_{11}N_2O_4PS$: C, 17.82; H, 5.48; N, 13.86; P, 15.35. Found: C, 18.06; H, 5.81; N, 13.86; P, 15.32.

The ir spectrum (KBr) showed major bands at 2.75–4.80 (br), 5.99, 7.00, 8.24, 8.48, 8.62, 8.88, 9.30, 9.60, 10.67, 12.75, 13.59, and 14.22 μ. The nmr spectrum (D_2O) showed peaks at δ 2.60 (3 H, s), 3.59 (3 H, d, J_{POCH} = 11 Hz).

Disodium dihydrogen pyrophosphate was purchased from J. T. Baker Chemical Co. Chromatographically pure pentasodium tripolyphosphate was isolated as large crystalline plates from the reaction of trisodium trimetaphosphate and NaOH in aqueous methanol as described by Feldmann.[15] An authentic sample of the trisilver salt of methyl pyrophosphate was prepared from disodium dihydrogen hypophosphate[50] by the method of Schülke.[51] Authentic samples of methyl triphosphate were kindly supplied by Dr. W. Feldmann,[15] Institut für Anorganische Chemie der Deutschen Akademie der Wissenshaften zu Berlin, Berlin-Aldershof, Germany, and by Dr. R. Yount, Washington State University, Pullman, Wash. The latter sample had been originally prepared by Dr. H. Brintzinger[21] at the Institut für Anorganische Chemie, University of Basel, Basel, Switzerland.

Maier's method[52,53] was used for the synthesis of pentaethyl bis(dihydroxyphosphinylmethyl)phosphinate, bp 153–157° (1 × 10^{-3} mm) (lit.[52] bp 146–153° (1 × 10^{-3} mm)). Since the analysis reported by Maier showed a carbon content which was nearly 1% low, this compound was submitted for microanalysis.

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Anal. Calcd for $C_{12}H_{29}O_8P_3$: C, 36.56; H, 7.41; P, 23.57. Found: C, 36.24; H, 7.51; P, 23.22.

The nmr spectrum corresponded to that reported by Maier.[52]

Bis(dihydroxyphosphinylmethyl)phosphinic acid was prepared from the pentaethyl ester by a slight modification of Maier's procedure.[52] Thus, a 2.0-g sample of this ester was heated on a steam bath in 30 ml of 48% HBr for 24 hr. Water and excess HBr were removed *in vacuo* to leave a nearly quantitative yield of the free acid as a highly viscous, hygroscopic oil. The nmr spectrum (D_2O) corresponded to that reported by Maier[52] and confirmed the complete absence of detectable ethyl ester groups. An aqueous solution of a portion of this acid was treated with 3 equiv of cyclohexylamine to yield a crystalline tricyclohexylammonium salt, mp 200–202° (lit.[52] mp 205°). The ir spectrum of this salt showed principal bands at 6.08, 8.45, 8.74, 9.30, 9.82, 10.45, 10.90 (br), and 12.40 μ. On PEI–cellulose thin-layer chromatography using 0.3 *M* LiCl as eluent this material gave a single spot (R_f = 0.20), which was visualized as a colorless spot against a light yellow background with the Hanes–Isherwood molybdate spray reagent.[54]

A portion of the bis(dihydroxyphosphinylmethyl)phosphinic acid was converted to the bismethylene analog of trimetaphosphate as follows. A 0.560-g (2.18 mmol) sample of the pentaacid, 2.5 g (13.5 mmol) of anhydrous tri-*n*-butylamine, and 2.0 g (10 mmol) of dicyclohexylcarbodiimide (Aldrich Chemical Co.) were dissolved in 10 ml of anhydrous pyridine. Dissolution of the pentaacid was slow and required a great deal of shaking. Before complete dissolution of the pentaacid was accomplished, the pyridine solution began to appear cloudy. The solution was allowed to stand at room temperature for 3 days. The dicyclohexylurea which had precipitated was removed by filtration, and the urea was washed successively with a portion of water and a portion of ether. The combined filtrate and washings were taken to dryness *in vacuo*. A small amount of water was then used to redissolve the product. This aqueous solution was extracted several times with ether to remove any traces of dicyclohexylcarbodiimide.

The aqueous solution was then passed through a Dowex-50 (H^+, 1.5 × 45 cm) column. The effluent acid was immediately neutralized with freshly distilled cyclohexylamine. Water was removed from the solution at reduced pressure to leave a glassy yellow solid. This was recrystallized from methanol–acetonitrile to give 0.65 g (50% yield) of crystals of a monohydrate of the tricyclohexylammonium salt, mp 220–230° dec.

Anal. Calcd for $C_{20}H_{46}N_3O_9P_3 \cdot H_2O$: C, 43.55; H, 8.77; N, 7.62; P, 16.85. Found: C, 43.90; H, 9.10; N, 7.58; P, 17.12.

The ir spectrum (Nujol) showed principal bands at 3.00, 6.12, 6.50, 8.35 (br), 9.40, 9.73, 10.75 (br), 12.08, and 12.50 μ.

A single spot (R_f = 0.70), again visualized as a colorless spot on a light yellow background with molybdate spray, was observed for this compound on PEI cellulose thin-layer chromatography using 0.3 *M* LiCl as eluent. As expected, on the same plate the bis(dihydroxyphosphinylmethyl)phosphinic acid traveled much more slowly (R_f = 0.20). For purposes of comparison a similar plate was spotted with samples of trisodium trimetaphosphate and trisodium tripolyphosphate, the analogous substances. Upon elution with 1.0 *M* LiCl R_f values of 0.70 and 0.10, respectively, were observed for these latter two samples.

A portion of the tricyclohexylammonium salt was dissolved in water and the solution was passed through a 1.5 × 45 cm Dowex-50 (H^+) column. The free acid was obtained as an aqueous solution and quickly neutralized with freshly distilled tri-*n*-butylamine. After removal of the water, the tris(tri-*n*-butylammonium) salt was obtained as a viscous oil. It gave a single spot with the expected R_f value on PEI cellulose thin-layer chromatography.

A portion of the tris(tri-*n*-butylammonium) salt was dissolved in water and converted to the trisodium salt by passage through a Dowex-50 (Na^+) column. The solvent was removed *in vacuo*, and the nmr spectrum was taken in D_2O. The spectrum showed peaks centered at δ 2.22 (apparent t, J = 18 Hz). The hygroscopic salt, isolated by recrystallization from EtOH–H_2O, analyzed for a trihydrate.

Anal. Calcd for $C_2H_4O_7P_3Na_3 \cdot 3H_2O$: C, 6.8; H, 2.8; P, 26.1. Found: C, 7.1; H, 2.5; P, 26.3.

The ir spectrum (Nujol) showed major bands at 2.70–3.91 (br), 8.55 (br), 9.28, 9.77, 11.10 (br), 12.72 (br), and 13.78 μ.

The bismethylene analog of trimetaphosphate was also examined by potentiometric titration. Thus, 0.0264 g (0.0496 mmol) of the anhydrous tricyclohexylammonium salt (dried thoroughly over P_2O_5 *in vacuo*) was dissolved in 1 ml of H_2O and passed through a Dowex-50 (H^+, 0.5 × 12 cm) column by elution with water. The acid was immediately titrated as described above. The titration curve (Figure 1) showed the presence of only strongly acidic protons, and 1.47 ml of 0.100 *N* NaOH was required. If one assumes that three acidic protons are present per molecule, the experimental molecular weight determined by this titration is 539 (calcd for anhydrous salt 533).

5′-Amino-5′-deoxyadenosine was prepared from 2′,3′-isopropylideneadenosine following the multistep procedure described by Jahn.[56] In order to facilitate future repetition of this procedure, we report here some spectral information for the product and the intermediates. Thus, 2′,3′-isopropylideneadenosine (Aldrich Chemical Co.) was treated with dry *p*-toluenesulfonyl chloride following the procedure of Kuhn and Jahn.[57] The resulting product, 5′-tosyl-2′,3′-isopropylideneadenosine, was found to cyclize to the tosylate salt of 2′,3′-isopropylidene-3,5′-cycloadenosine[58] with great facility on attempted recrystallization from methanol. The desired product, 5′-tosyl-2′,3′-isopropylideneadenosine, has a characteristic bright yellow color.[58] The nmr spectral information is listed in Table II. In contrast, the tosylate salt of the 2′,3′-isopropylidene-3,5′-cycloadenosine was colorless.[58] The nmr data are recorded in Table II. A crude preparation of 5′-tosyl-2′,3′-isopropylideneadenosine (2.01 g, 4.40 mmol, contaminated with some of the tosylate salt of 2′,3′-isopropylidene-3,5′-cycloadenosine) was formylated using formic–acetic anhydride[59,60] according to Jahn's procedure.[56] Recrystallization from methanol–chloroform afforded 2.0 g (90% yield) of *N*-formyl-5′-tosyl-2′,3′-isopropylideneadenosine. The nmr spectral data are included in Table II. A 3.00-g (6.14 mmol) portion of this N-formylated product was treated with NaN_3 in dimethyl sulfoxide and worked up by Jahn's procedure.[56] The 5′-azido-5′-deoxyadenosine, purified by recrystallization from water, gave satisfactory microanalyses for carbon, hydrogen, and nitrogen. The ir band at 4.80 μ, corresponding to the expected azido absorption, was observed. Upon Raney nickel reduction[56] a 0.48-g portion of the azido intermediate was converted to 0.40 g (93%) of the desired 5′-amino-5′-deoxyadenosine, which was isolated as a pale green solid. This product was used without further purification in the synthesis of 5′-amino-5′-deoxyadenosine 5′-triphosphate. The uv (H_2O) showed λ_{max} 258 nm and no longer showed the azido absorption band in the ir spectrum. The nmr spectrum (D_2O) showed peaks at δ 3.12 (2 H, br s), 4.34 (3 H, m), 6.06 (1 H, d, J = 6 Hz), 8.39 (1 H, s), 8.55 (1 H, s).

Reaction of the Bismethylene Analog of Trimetaphosphate with Methanol. In several experiments samples of the tricyclohexylammonium salt of the bismethylene analog of trimetaphosphate were sealed in tubes with dry methanol in the presence of small amounts of freshly distilled, anhydrous methanesulfonic acid as a catalyst. The tubes were heated at 100° for varying periods of time. After the desired reaction time, the tubes were cooled and opened and the contents were passed through a Dowex-50 (H^+) column, water being used as the eluent. After removal of the solvent, the nmr spectra of the products were taken in D_2O. By electronic integration the size of the doublet (J_{POCH} = 11 Hz) due to the CH_3OP protons was compared to the size of the quartet of peaks due to the PCH_2PCH_2P protons. This gave an approximate measurement of the degree of esterification that had occurred; for example, after ring opening was complete, the ratio of the integrated area for the methyl doublet to that of the methylene group quartet was 3:4. The experiments[61] showed that even after prolonged (18–24 hr) heating at 100° using methanesulfonic acid as catalyst, only the monomethyl ester, the product of simple ring opening, was formed. The effect of the added methanesulfonic acid as a catalyst was demonstrated by a reaction in which a portion

(54) We and others[55] have observed that phosphonates and phosphinates may be visualized on chromatograms using the Hanes–Isherwood molybdate spray reagent.

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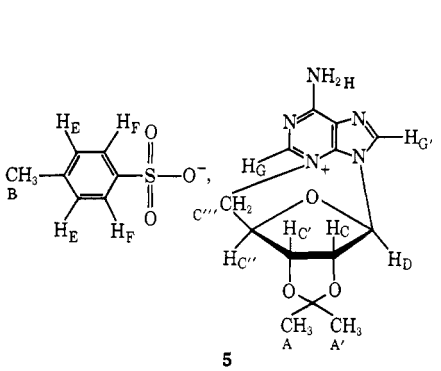
Table II. Nmr Parameters for Some Closely Related Nucleoside Derivatives[a]



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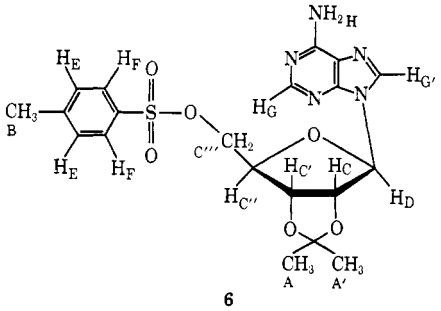

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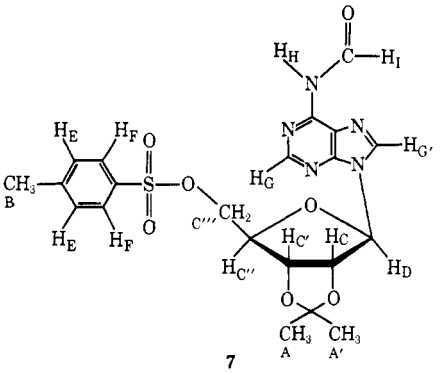

7

Compd	$H_A + H_{A'}$	H_B	$H_C + H_{C'} + H_{C''} + H_{C'''}$	H_D	H_E	H_F	$H_G + H_{G'}$	H_H	H_I
5	s, 1.24; s, 1.49	s, 2.24	m, 4.68	s, 6.75	d, 7.05 (J = 8 Hz)	d, 7.45 (J = 8 Hz)	s, 8.63; s, 8.83	d, 9.40 (J = 6 Hz)	
6	s, 1.35; s, 1.57	s, 2.18	m, 5.04; d, 4.38[b]	d, 6.22 (J = 2 Hz)	d, 7.25 (J = 8 Hz)	d, 7.60 (J = 8 Hz)	s, 8.06; s, 8.22	d, 9.42 (J = 6 Hz)	
7	s, 1.30; s, 1.53	s, 2.32	m, 4.30;[b] m, 5.20	s, 6.23	d, 7.11 (J = 8 Hz)	d, 7.50 (J = 8 Hz)	s, 8.44; s, 8.49	d, 11.30 (J = 10 Hz)	d, 9.90 (J = 10 Hz)

[a] All measurements were made in Me_2SO-d_6 solutions at 60 MHz. [b] Partially obscured by H_2O peak.

of the tricyclohexylammonium salt was heated at 100° for 16 hr in a sealed tube in the absence of the catalyst. Integration of the nmr spectrum of the product mixture obtained showed only approximately 12% conversion to the monomethyl ester. In another case the free acid (0.3 g) of the trimetaphosphate bismethylene analog was generated by passing a methanolic solution of the tris(tri-*n*-butylammonium) salt through a *methanolic* Dowex-50 (H^+) resin column. The acid was heated at reflux in 50 ml of dry methanol for 72 hr. After removal of the solvent, the nmr spectrum (D_2O) showed that a quantitative conversion to the monomethyl ester had occurred. A portion of this product was converted to the tetrasodium salt by the addition of 4 equiv of NaOH solution, and an analytical sample was prepared by recrystallizing several times from ethanol–water. A hygroscopic product was obtained.

Anal. Calcd for $C_3H_7O_9P_3Na_4 \cdot 2.5H_2O$: C, 9.0; H, 3.0; P, 23.2. Found: C, 9.1; H, 3.3; P, 23.5.

The ir spectrum of this tetrasodium salt (Nujol) showed major bands at 2.80–3.21 (br), 8.43, 8.65, 9.14, 9.50 (br), 10.11 (br), and 12.50 μ (br).

The nmr spectrum (D_2O) showed peaks at δ 2.08 (4 H, apparent t, J = 18 Hz), 3.58 (3 H, d, J = 11 Hz).

Reaction of the Bismethylene Analog of Trimetaphosphate with Ethanol. A completely analogous set of experiments to those described above was performed with ethanol replacing the methanol, including the use of an *ethanolic* Dowex-50 (H^+) column. Once again only monoesterification was achieved, even under prolonged heating at 100° in sealed tubes, and a quantitative conversion to the monoethyl ester occurred. An analytical sample of the hygroscopic tetrasodium salt trihydrate was prepared exactly as described for the monomethyl ester.

Anal. Calcd for $C_4H_9O_8P_3Na_4 \cdot 3H_2O$: C, 11.3; H, 3.5; P, 21.9. Found: C, 11.5; H, 3.8; P, 22.0.

The ir spectrum (Nujol) showed major peaks at 2.78–3.30 (br), 8.23, 8.40, 8.80, 8.99 (br), 9.46 (br), 10.68 (br), and 12.83 μ (br).

The nmr spectrum (D_2O) showed peaks at δ 1.30 (3 H, t, J = 7 Hz), 2.05 (4 H, apparent t, J = 18 Hz), 3.93 (2 H, m).

Identification of Products in the Methanolysis of Trimetaphosphate. In several reactions described in detail below, trimetaphosphate was treated with methanol under various relatively acidic and basic conditions. In addition to unreacted trimetaphosphate, six compounds were often encountered in varying amounts in the product mixtures obtained; these were inorganic orthophosphate, inorganic pyrophosphate, inorganic triphosphate, methyl phosphate, methyl pyrophosphate, and methyl triphosphate. Authentic samples of each of these compounds were available for comparison purposes (see Materials and Methods). Fortunately, all seven compounds were cleanly separable on a single plate by PEI cellulose thin-layer chromatography using 1.0 M LiCl as eluent. The observed R_f values for the seven compounds (in order of decreasing R_f value) were: methyl phosphate (0.96), inorganic orthophosphate (0.86), trimetaphosphate (0.70), methyl pyrophosphate (0.60), methyl triphosphate (0.25), inorganic pyrophosphate (0.15), and inorganic triphosphate (0.07). The same mixture of products had been previously resolved into its components by two-dimensional paper chromatography by Feldmann.[15] When our mixture of products was chromatographed using this procedure, the same pattern of spots (visualized with molybdate spray) appeared as that reported by Feldmann.[15]

It was also useful to investigate the nmr spectra of these product mixtures in D_2O. It was found that the doublet for the OCH_3 group differed for methyl phosphate, methyl pyrophosphate, and methyl triphosphate. For example, in strongly basic, relatively dilute solutions in D_2O (~1% solutions) the observed chemical shifts were: methyl phosphate, δ 3.58, methyl pyrophosphate, 3.68; methyl triphosphate, 3.73. The identity of each of these compounds was confirmed by the addition of authentic samples to the nmr tubes. In none of the experiments described below was dimethyl phosphate (OCH_3 doublet appearing at δ 3.64; R_f value at solvent front on PEI thin-layer chromatography with 1.0 N LiCl) detected as a product.

Reaction between Methanol and Tricyclohexylammonium Trimetaphosphate. Four 0.30-g samples of tricyclohexylammonium trimetaphosphate were each dissolved in 3-ml portions of anhydrous methanol; the solutions were sealed in tubes and heated at 115° for 4, 9, 21, and 63 hr, respectively. Each tube was opened and the solvent from each was removed *in vacuo*. A single doublet, corresponding to formation of methyl phosphate, appeared in the OCH_3 region of the nmr spectrum (D_2O) of each sample. Using the cyclohexyl absorption region as an internal nmr integration standard, the following approximate per cent conversions to methyl phosphate were found for the four samples: 4-hr sample, <1%; 9-hr sample, 11%; 21-hr sample, 28%; 63-hr sample, 90%. Investigation of the products using chromatography (see above) showed the presence of only methyl phosphate and starting material, trimetaphosphate.

Reaction between Benzyl Alcohol and Tricyclohexylammonium Trimetaphosphate. A sample of tricyclohexylammonium trimetaphosphate (0.40 g, 0.75 mmol) was placed in 100 ml of carefully dried benzyl alcohol, and the mixture was heated at reflux at 205° for 5 hr. A $CaCl_2$ drying tube was in the top of the condenser throughout this period. The excess benzyl alcohol was removed at reduced pressure, the resulting solid was triturated with ether, and the ether extracts were discarded. The solid residue was dissolved in water, excess cyclohexylamine was added, and the excess solvent and amine were removed at reduced pressure. The residual solid was recrystallized from water–acetone to give three fractions. The first two fractions were combined and recrystallized from methanol–acetonitrile to give 0.43 g (50% yield) of dicyclohexylammonium benzyl phosphate, mp 231–233° (lit.[62] mp 233°). The

nmr spectrum (D_2O) was consistent with this assigned structure, showing peaks at δ ~1.50 (20 H, m), ~3.10 (2 H, m), 4.82 (2 H, d, J_{POCH} = 6 Hz), 7.27 (5 H, s). The third fraction required no further recrystallization. The nmr spectrum (D_2O) was consistent with the structure of cyclohexylammonium dibenzyl phosphate, showing peaks at δ ~1.50 (10 H, m), ~3.10 (1 H, m), 4.87 (4 H, d, J_{POCH} = 6 Hz), 7.32 (10 H, s). The yield was 0.14 g (17%), mp 167–170° (lit.[63] mp 173°).

Reaction between Methanol and Trimetaphosphate under Basic Conditions. In several sets of experiments[61] methanol was treated with various salts of trimetaphosphate in nonaqueous solvents under scrupulously anhydrous conditions. The following sets of conditions all led to formation of the six products detected as described above and included formation of some methyl triphosphate (usually estimated to be *ca.* 10% of the product mixture or less): trilithium trimetaphosphate (260 mg, 1 mmol) and lithium methoxide (7 mg, 1 mmol) in methanol (3 ml) heated at 100° in a sealed tube for 1 hr; tris(tri-*n*-butylammonium) trimetaphosphate (840 mg, 1 mmol), methanol (33 mg, 1 mmol), and tri-*n*-butylamine (1.87 g, 10.1 mmol) in dry dimethylformamide (2 ml) in a sealed tube heated at 105 ± 5° for 72 hr; tris(tri-*n*-butylammonium) trimetaphosphate (312 mg, 0.392 mmol), methanol (31 mg, 0.97 mmol), and *N,N',N''*-tricyclohexylguanidine[64] (923 mg, 3.03 mmol) in dry dimethylformamide at 100° for 24 hr.

Reaction between 2',3'-Isopropylideneadenosine and the Bismethylene Analog of Trimetaphosphate. A portion of the tris(tri-*n*-butylammonium) salt of the bismethylene analog of trimetaphosphate (0.182 g, 0.230 mmol), carefully dried by heating at 60° for 8 hr (1 mm), was dissolved in 2 ml of dry *N,N*-dimethylformamide. 2',3'-Isopropylideneadenosine (Aldrich Chemical Co., 0.426 g, 1.40 mmol) and freshly distilled, anhydrous methanesulfonic acid (0.0173 g, 0.181 mmol) were added to the solution. The reaction flask was fitted with a reflux condenser which was capped with a $CaCl_2$ drying tube and the solution was heated at reflux for 4 hr. After removal of the solvent at reduced pressure, the residual solid was dissolved in 5 ml of H_2O. This aqueous solution was added to a DEAE cellulose column (2.5 × 42 cm, HCO_3^- form). Stepwise elution with 200-ml portions each of 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 *M* triethylammonium bicarbonate gave two major uv-absorbing fractions, each of which corresponded (assuming λ_{max} 259 nm, $\epsilon = 1.54 \times 10^4$, see below) to about 30% of the theoretical yield. These two fractions emerged with the 0.03, 0.04, and 0.05 *M* triethylammonium bicarbonate washings. Both fractions were separately taken to dryness *in vacuo*, which also removed the volatile triethylammonium bicarbonate. The second fraction, shown by nmr spectroscopy to contain the desired product, was then dissolved in a small amount of water and passed through a Dowex-50 (H^+) column. The product emerged slowly as an acidic, uv-absorbing material. This acid was allowed to stand in the aqueous eluent solution for 3 hr in order to ensure complete removal of the isopropylidene protective group. After adjustment of the solution to pH 7 with dilute NaOH solution, this sample was applied to a DEAE cellulose column (2.5 × 42 cm) in the bicarbonate form. Chromatography was accomplished using stepwise elution with 200-ml portions each of 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 *M* triethylammonium bicarbonate. A large uv-absorbing fraction emerged at the 0.05–0.06 *M* salt concentrations. After removal of the solvent and volatile salt *in vacuo*, the nmr spectrum (D_2O) showed considerable contamination by a PCH_2P containing substance, subsequently shown to be the bis(dihydroxyphosphinylmethyl)phosphinic acid. The solution was then concentrated to 1 ml and added to a Dowex-50 (H^+, 1.5 × 45 cm column) and chromatography was performed with water as the eluent. One-milliliter fractions were collected. Fractions 10 and 11 contained the bis(dihydroxyphosphinylmethyl)phosphinic acid, identified by its nmr spectrum and by its PEI cellulose thin-layer chromatographic behavior. Fractions 15–20 contained the ATP analog. After addition of the required amount of dilute NaOH solution, the solvent was removed *in vacuo* to leave the hydroscopic tetrasodium salt of adenosine 5'-bis(dihydroxyphosphinylmethyl)phosphinate. The total yield was 23 mg or 16% based on the amount of the tris(tri-*n*-butylammonium) salt of the bismethylene analog of trimetaphosphate.

Anal. Calcd for $C_{12}H_{16}N_5O_{11}P_3Na_4 \cdot H_2O$: C, 23.65; H, 2.98; N, 11.50. Found: C, 23.91; H, 3.01; N, 11.03.

The nmr spectrum at 100 MHz (D_2O) showed peaks at δ 2.25 (2 H, apparent t, J_{PCH} = 20 Hz), 2.33 (2 H, apparent t, J_{PCH} = 20 Hz), 4.20 (5 H, m), 6.25 (1 H, d, J = 6 Hz), 8.28 (1 H, s), 8.44 (1 H, s) (see Figure 2). A ^{31}P-decoupled spectrum is also shown in Figure 2. The uv spectrum showed λ_{max} 259 nm ($\epsilon = 1.54 \times 10^4$).

A single spot, detected by a uv lamp, was observed on ascending paper chromatography in both an acidic solvent system[65] (dioxane–2-propanol–trichloroacetic acid–acetic acid–concentrated ammonia–water, 30:26.3:1.9:0.6:0.9:29.6, v/v) and a basic solvent system[66] (2-propanol–dimethylformamide–2-butanone–concentrated ammonia–water, 20:20:20:1:39, v/v). The observed R_f values were 0.44 and 0.32, respectively.

The ATP analog also showed a single uv-absorbing spot on PEI cellulose thin-layer chromatography. The R_f value was 0.35 with 0.5 *M* LiCl. The same spot could also be visualized by use of the Hanes–Isherwood molybdate spray reagent[54] which caused the spot to appear as a colorless spot against the light yellow background of the plate.

Finally, spots of the ATP analog could also be visualized using the periodate–benzidine spray reagent of Viscontini, *et al.*,[67] which is diagnostic for the presence of the *vic*-dihydroxyl group of the ribose ring.

A portion of the ATP analog was converted to the free acid by passage through a Dowex-50 (H^+) column and subjected to potentiometric titration using the methods described previously. The titration curve, consistent with the proposed structure, is shown in Figure 1.

Reaction between 5'-Amino-5'-deoxyadenosine and Trisodium Trimetaphosphate. 5'-Amino-5'-deoxyadenosine (107 mg, 0.4 mmol) and trisodium trimetaphosphate hexahydrate (168 mg, 0.4 mmol) were dissolved in 2 ml of water and allowed to react for 24 hr at room temperature. The water was removed at reduced pressure. Examination of the product mixture by PEI cellulose thin-layer chromatography (1.2 *N* LiCl) indicated the presence of three components: trimetaphosphate (R_f = 0.66), 5'-amino-5'-deoxyadenosine (R_f = 0.56), and a new spot (R_f = 0.18) which was visualized both by irradiation with a uv lamp and by the molybdate spray reagent. (Under the same chromatographic conditions adenosine 5'-triphosphate itself gave an R_f value = 0.12.) Isolation of the product was accomplished by chromatography on a 1.4 × 32.2 cm A-25 DEAE sephadex (Pharmacia Chemical Co.) column (HCO_3^- form) at 6°. The chromatographic conditions were patterned after those of Caldwell[68] for ATP except that he used triethylammonium acetate instead of triethylammonium bicarbonate. Fractions of 600 drops each were collected and each fraction was monitored (Isco Model UA2 uv analyzer) at 254 nm. Thus, the product mixture was dissolved in the minimum amount of 0.05 *M* triethylammonium bicarbonate and added to the column. Elution with 0.05 *N* triethylammonium bicarbonate to remove the unreacted trimetaphosphate and 5'-amino-5'-deoxyadenosine was followed by a linear gradient between 0.05 and 0.4 *N* triethylammonium bicarbonate to remove any 5'-amino-5'-deoxyadenosine mono- or diphosphate which may have formed. No uv absorbing material appeared. Finally, a linear gradient between 0.4 and 1.0 *N* triethylammonium bicarbonate was used and the product emerged over 35 fractions which were combined and lyophilized to leave the tris(triethylammonium) salt of 5'-amino-5'-deoxyadenosine 5'-triphosphate. The product was analyzed quantitatively using the labile phosphate determination of Fiske and Subbarow[33] as described by Leloir and Cardini.[69] The method was calibrated using an analytically pure sample of ATP obtained from Calbiochem Corp. Assuming a λ_{max} at 259 nm ($\epsilon = 1.54 \times 10^4$) the determination showed 2.96 μmol of labile phosphate per micromole of 5'-amino-5'-deoxyadenosine 5'-triphosphate.

The isolated product initially gave a single spot (R_f = 0.18) when chromatographed on a PEI cellulose thin layer using 1.2 *N* LiCl as eluent. This spot could be visualized both by a uv lamp and by the Hanes–Isherwood spray reagent. Gradually, the ATP analog decomposed in aqueous solution and the products of the break-

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down were shown by chromatography to be 5'-amino-5'-deoxyadenosine and trimetaphosphate.

The 5'-amino-5'-deoxyadenosine 5'-triphosphate was shown in preliminary experiments to replace ATP itself as a substrate in the rabbit muscle creatine kinase reaction. The coupled assay procedure of Tanzer and Gilvarg[70] as modified by Rotthauwe and Cerqueiro-Rodriguez[71] was used. Also, formation of phosphocreatine as well as the loss of the ATP analog and formation of what appears to be 5'-amino-5'-deoxyadenosine 5'-diphosphate in the enzymatic reaction were followed using PEI cellulose thin-layer chromatography as described elsewhere.[43] Further details concerning the isolation and biological activity of this ATP analog will be published at a later date.

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Mechanism of Ester Aminolyses in Aprotic Solvents

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Abstract: The reaction of pyrrolidine with esters in acetonitrile and chlorobenzene is much more sensitive to substituents on the leaving group of the esters (ρ = 4–6) than to substituents on the acyl portion of the esters (ρ = 1–2). This is the reverse order of sensitivity found in reactions of esters with hydroxide ion in water. We conclude from the ρ values (and from the effects on the aminolysis rates of tertiary amine, pyrrolidinium ion, azide ion, and pyrrolidine-*d*) that collapse of a tetrahedral intermediate is rate determining. Possible structures of the tetrahedral intermediate are considered, and the impact of our results on the conclusions of previous work in the area is discussed.

In 1834 Liebig[2] discovered that amines react with esters to form amides. About a century later Hinshelwood[3] and Day[4] became interested in the mechanism of this reaction in aprotic solvents, but they decided not to pursue the subject upon finding that simple alkyl esters react slowly in media lacking water or alcohol. Although a great deal of attention was subsequently given to ester aminolyses in aqueous solvents,[5–8] not until recently has the mechanism in aprotic solvents been explored in detail.[9–18] This latter work has generated an unusual number of conflicting conclusions. For example, the overall third-order aminolysis of esters in aprotic media has been considered both a cyclic concerted[9,10] and a general base[11] process. Aminolysis mechanisms have usually entailed a tetrahedral intermediate,[12] although a direct displacement without an intermediate may be preferable.[13] Overall second-order aminolyses have been discussed in terms of both four-membered cyclic transition states[14] and ionic processes.[11] It has been suggested[14] and denied[13] that primary and secondary amines react in aprotic solvents by different mechanisms. Tertiary amine catalysis has been ascribed to both nucleophilic[15] and general base[16] catalysis. Acceleration by the hydroxyl group in the aminolysis of salicylate systems in nonhydroxylic media has been viewed as a general acid catalysis[16] and as an ion-pair effect.[17] In the present paper we describe experiments that lead to a unifying mechanistic theory for ester aminolyses in aprotic solvents.[19]

Experimental Section

Materials. Acetonitrile was distilled twice over phosphorus pentoxide and once over anhydrous potassium carbonate using a 30-cm Vigreux column. Chlorobenzene was washed successively with concentrated sulfuric acid, aqueous sodium carbonate, and water. The chlorobenzene was then dried over anhydrous magnesium sulfate and distilled over calcium hydride using a 30-cm Vigreux column. Heptane was distilled over lithium aluminum hydride.

Pyrrolidine and *n*-butylamine were distilled over calcium hydride prior to use. Triethylenediamine (Dabco) was crystallized twice from heptane and sublimed twice at room temperature (0.1–0.2 mm) to afford colorless crystals, mp 158–159° (lit.[11] mp 157–159°).

We prepared tetraethylammonium azide as follows. A 10% aqueous solution of tetraethylammonium hydroxide was concentrated with the aid of a rotary evaporator. After diluting with absolute methanol, we neutralized the solution with a two–threefold excess of hydrazoic acid in ether.[20] The solvent was removed,

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