Monomeric Methyl Metaphosphate: Reactions with Carbonyl Groups[1]

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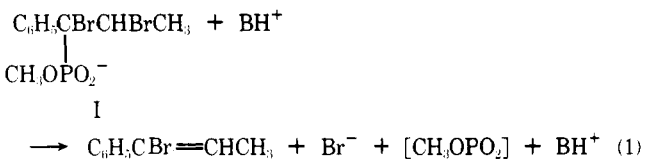
Abstract: Monomeric methyl metaphosphate, generated by fragmentation of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonate in the presence of 2,2,6,6-tetramethylpiperidine, can be identified by its electrophilic attack on the aromatic rings of substituted anilines. The aromatic substitution reactions are quenched by pyridine, acetonitrile, dioxane, and dimethoxyethane but not by chloroform. Monomeric methyl metaphosphate attacks acetophenone to yield an enol phosphate; it converts a mixture of *o*-trifluoromethylaniline and acetophenone into *N*-(1-methylbenzylidene)-2-aminobenzotrifluoride and also converts aniline and ethyl benzoate into *O*-ethyl-*N*-phenylbenzimidate. These latter reactions mimic enzymatic reactions that require ATP. These facts introduce the possibility that ATP plays a kinetic role as well as a thermodynamic one in metabolic processes.

Introduction

In previous research both a gas phase[2] and solution source[1,3] were developed for monomeric methyl metaphosphate. In solution, methyl hydrogen *erythro*- and *threo*-1-phenyl-1,2-dibromopropylphosphonates in the presence of 2,2,6,6-tetramethylpiperidine (here designated as "base") undergo Conant-Swan fragmentation.[4] The fragmentation yields 1-bromo-2-methylstyrene and a reactive intermediate that is capable of attack upon the ring of aromatic amines (eq 1). In



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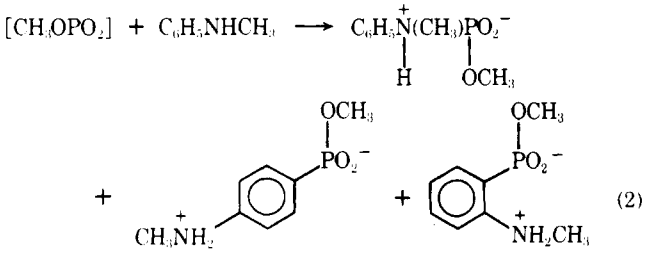

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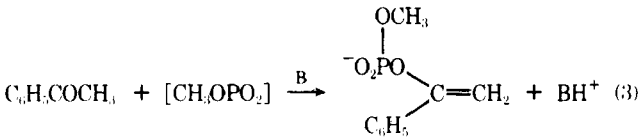
particular, in the presence of *N*-methylaniline, the reaction products include the monomethyl esters of *o*- and *p*-*N*-methylaminobenzenephosphonic acids,[1] and, in the presence of *N,N*-diethylaniline, the reaction products include the methyl ester of *p*-*N,N*-diethylaminobenzenephosphonic acid (eq 2).[2,3] We suggest that this attack upon an aromatic ring is diagnostic for monomeric methyl metaphosphate.

Although the phosphorylation of the ring was offered as a diagnostic test for monomeric methyl metaphosphate, the yields of aromatic substitution previously reported[3] were only 5%. Now we have found that the low yields resulted from the presence of an equal volume of acetonitrile as cosolvent and that 35–40% yields of aromatic substitution can be obtained in "neat" solutions of *N*-methylaniline and of *N,N*-diethylaniline. Other unusual solvent effects were observed and are discussed. Further, in the presence of acetophenone, monomeric methyl metaphosphate yields an enol phosphate (eq 3). In the presence of acetophenone and aniline, monomeric methyl metaphosphate yields a Schiff base (eq 4); in the presence of monomeric methyl metaphosphate, ethyl benzoate and aniline yield *O*-ethyl-*N*-phenylbenzimidate (eq 5).

This paper presents the evidence for these equations and a



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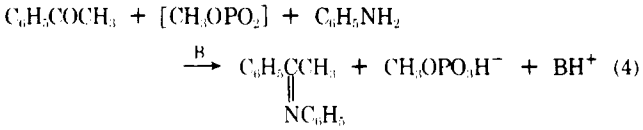

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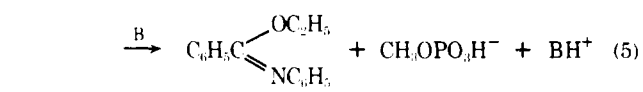

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discussion of the possible analogies between these processes and metabolic reactions that involve ATP.

Experimental Section

General. Ethanol-free chloroform, acetonitrile, dioxane, and 1,2-dimethyoxyethane from Burdick and Jackson were of high quality and were used without purification. Other commercial solvents were dried over calcium hydride and distilled. All solvents were stored over 4-Å molecular sieves. Commercial chemicals were crystallized before use. NMR spectra were obtained on Varian XL-100 or C80 spectrometers in Fourier transform mode; the phosphorus spectra were obtained with proton decoupling. Phosphorus chemical shifts are reported in parts per million from 85% phosphoric acid. As in previous papers[2,3,5] from this laboratory, upfield shifts are assigned a negative sign. Proton chemical shifts are relative to tetramethylsilane except where noted. *o*-Trifluoromethylaniline and 2,2,6,6-tetramethylpiperidine were obtained from Aldrich. Analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Materials. Methyl hydrogen *erythro*-1,2-Dibromo-1-phenylpropylphosphonate (I) was prepared as previously described;[3] a higher melting point was obtained (mp 166–167 °C). **Dimethyl *p*-*N,N*-diethylaminobenzenephosphonate** and **barium methyl *p*-*N,N*-diethylaminobenzenephosphonic acid** were prepared previously.[2] **Disodium methyl phosphate** was a gift of Dr. Jorge Goldstein. **Methyl dihydrogen phosphate** was prepared by passing an aqueous solution of the salt through Bio-Rad AG 50W-8X in the protonated form: ^{31}P NMR (D_2O, pD 1.28) δ −1.21 (s). **Tetramethylammonium dimethylphosphate** was prepared according to Chabrier and Selim:[6] ^{1}H NMR (D_2O) δ 3.18 (s, 12 H), 3.56 (d, J_{H-P} = 10.9 Hz, 6 H); ^{31}P NMR (D_2O) δ −2.42 (s). **Dimethyl bromophosphate** was made and distilled according to Goldwhite and Saunders:[7] ^{1}H NMR ($CDCl_3$) δ 3.87 (d, J_{H-P} = 14.3 Hz); ^{31}P NMR (CD_3CN–CH_3CN, 1:2 v/v) δ 4.66 (s). **Trimethyl phosphate** was synthesized by the method of Becher:[8] ^{1}H NMR ($CDCl_3$) δ 3.77 (d, J_{H-P} = 11.0 Hz); ^{31}P NMR ($CDCl_3$) δ −3.04 (s). **Barium *sym*-dimethylpyrophosphate** was prepared as

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Table I. Solvent Effect on the Product Distribution from Elimination of Methyl Hydrogen *erythro*-1-Phenyl-1,2-dibromopropylphosphonate in *N,N*-Diethylaniline–4% Methanol at 70 °C[a]

	product distribution, % yield ± 2%								
	neat			1 vol. of added solvent			4 vol. of added solvent		
solvent	arom product[b]	diMeP[c]	other[d]	arom product	diMeP	other[d]	arom product	diMeP	other[d]
neat	38	55	7[e]						
chloroform				38	56	6[f]	26	68	6
carbon tetrachloride[g]				18	75	7[h]	7	88	5[h]
pyridine				8	92	0	0	100	0
acetonitrile				5	95	0	0	100	0
dioxane				4	96	0	0	100	0
1,2-dimethoxyethane				6	94	0	0	100	0

[a] Reaction times are given in the Experimental Section. [b] Refers to methyl *p*-*N,N*-diethylaminobenzenephosphonate. [c] Refers to dimethyl phosphate. [d] Product is *sym*-dimethyl pyrophosphate unless otherwise stated. [e] 4% *sym*-dimethyl pyrophosphate, 3% unidentified product with ^{31}P NMR singlet at −19.01 ppm. [f] 3% *sym*-dimethyl pyrophosphate, 3% unidentified product with ^{31}P NMR singlet at −19.01 ppm. [g] When the reaction mixture in 4 vol. of carbon tetrachloride was heated for 9 days at 70 °C, secondary reactions occurred. [h] Unidentified product with ^{31}P NMR singlet at −24.4 ppm.

previously described.[3] ***sym*-Dihydrogen dimethylpyrophosphate** was prepared by passing the salt through Bio-Rad AG 50W-8X in the protonated form: ^{31}P NMR (D_2O, pD 1.65) δ 9.78 (s). **Acetophenone anil**[9] had mp 39–40 °C; ^1H NMR ($CDCl_3$) δ 2.21 (s, 3 H), 6.72–8.03 (br m, 10 H). ***N*-Phenyl-*O*-ethylbenzimidate** was prepared according to Landler:[10] ^1H NMR ($CDCl_3$) δ 1.41 (t, J = 7.0 Hz, 3 H), 4.39 (q, J = 7.0 Hz, 2 H), 6.64–7.36 (br m, 10 H).

***N*-(1-Methylbenzylidene)-2-aminobenzotrifluoride** was synthesized by refluxing acetophenone (10.2 g) and *o*-trifluoromethylaniline (16.1 g) in 20 mL of benzene under argon with 36 g of flame-dried 4-Å molecular sieves[9] for 38 h to give a 30% conversion into ketimine; prolonged heating did not improve the yield. Benzene, acetophenone, and 2-aminobenzotrifluoride were removed by distillation through a short column [bath temperature 55 °C (0.1 mm)], and the ketimine distilled at 108 °C (0.14 mm). It was then redistilled in a molecular still at 62 °C (10^{-4} mm). The product was a pale yellow liquid: ^1H NMR ($CDCl_3$) δ 2.18 (s, 3 H), 6.70–8.03 (br m, 9 H). Anal. Calcd for $C_{15}H_{12}NF_3$: C, 68.43; H, 4.59; N, 5.32; F, 21.65. Found: C, 68.92, 68.36; H, 4.58, 4.81; N, 5.21, F, 19.61, 20.53, 20.46, 20.41.

α-Phenylvinyl dimethylphosphate[11] had bp 124 °C (0.15 mm); ^1H NMR ($CDCl_3$) δ 3.83 (d, J_{H-P} = 11.2 Hz, 6 H), 5.18–5.33 (m, 2 H), 7.28–7.65 (br m, 5 H).

Cyclohexylammonium α-phenylvinyl methylphosphate was prepared by demethylating α-phenylvinyl dimethylphosphate (5.0 g) with anhydrous trimethylamine (3.7 g) in 20 mL of absolute ethanol under argon at room temperature overnight. Other methods of demethylation including heating with sodium iodide in acetonitrile and treatment with thiourea in refluxing acetonitrile proved less satisfactory. The solvent and excess reagents were removed by rotary evaporation, and the hygroscopic residue was dissolved in 20 mL of acetonitrile and then diluted with 225 mL of methylene chloride. It was then mixed with an equal volume of 0.1 M hydrochloric acid, and the solvents were rapidly separated; the methylene chloride was immediately drained into 2.5 mL of cyclohexylamine in 20 mL of methylene chloride. Solvent was removed by rotoevaporation and the solid dissolved in 5 mL of methanol; byproducts with some enol phosphate were crystallized at 0 °C with 100 mL of ether. The supernatant was rotoevaporated and the remaining salt dissolved in 200 mL of boiling acetonitrile and crystallized at ice temperature. The first crop (9% yield) was twice recrystallized from 40 mL of acetonitile. White needles, mp 127–130 °C dec, were obtained: ^1H NMR (D_2O) δ 0.9–2.10 (m, 11 H), 3.65 (d, J_{H-P} = 10.9 Hz, 3 H), 5.04 (d of d, $J_{H-P} = J_{H-H}$ = 2.4 Hz, 1 H), 5.33 (d of d, $J_{H-P} = J_{H-H}$ = 2.4 Hz, 1 H), 7.37–7.72 (br m, 5 H). Anal. Calcd for $C_{15}H_{24}NPO_4$: C, 57.69; H, 7.72; N, 4.49; P, 9.92. Found: C, 57.52, 57.47; H, 7.82, 7.90; N, 4.44; P, 9.94.

Dimethyl *N*-phenylphosphoramidate[12] had mp 86.5–88 °C (lit.[12] 88–88.5 °C); ^1H NMR ($CDCl_3$) δ 3.77 (d, J_{H-P} = 11.4 Hz, 6 H), 6.36–7.35 (br m, 5 H).

Barium methyl *N*-phenylphosphoramidate was synthesized in analogy to the earlier preparation of barium methyl *N*-methyl-*N*-phenylphosphoramidate.[2] Dimethyl *N*-phenylphosphoramidate (1 g) was refluxed for 4 h with 15 mL of a barium hydroxide solution which had been saturated at room temperature. The aqueous layer was decanted from residual solid; barium carbonate was precipitated with carbon dioxide and removed by centrifugation. Water was rotoevaporated, and the remaining solid partially dissolved in 300 mL of methanol and filtered. When the volume of methanol had been reduced to 100 mL and the solution cooled in ice, a flocculent precipitate was obtained: ^1H NMR (D_2O) δ 3.52 (d, J_{H-P} = 11.2 Hz, 3 H), 6.92–7.39 (br m, 5 H); ^{31}P NMR (D_2O–CH_3OH, 1:1 v/v) δ −1.55 (s). Anal. Calcd for $C_{14}H_{18}N_2O_6P_2Ba$: C, 33.00; H, 3.56; N, 5.52; P, 12.15. Found: C, 33.40, 33.33; H, 4.02, 3.97; N, 5.43; P, 12.15.

***N*-Octylammonium methyl *N*-phenylphosphoramidate** was prepared by passing a solution of the barium salt in methanol–water (1:1 v/v) through a small column of Bio-Rad AG 50W-8X in the *n*-octylammonium form, collecting the UV-absorbing fraction, and removing the solvent by rotary evaporation: ^{31}P NMR (D_2O–CH_3OH, 1:1 v/v) δ −1.54 (s).

Reactions with Aromatic Amines. Reactions between methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonate (I) and various reagents were carried out in closed 12 × 180 mm NMR tubes or sealed glass tubes at 70 °C and the products were identified and analyzed by ^{31}P NMR spectroscopy. In most cases, as indicated below, authentic samples of reaction products were added back to the NMR tubes to verify the identification. Product yields were estimated by integrating ^{31}P NMR spectra and in critical cases were confirmed by comparing with standards. In some instances (cited below) the products were purified and further identified by comparison of their ^1H NMR spectra with those of authentic materials.

N,N-Diethylaniline (3.0 mL) (diluted with solvent where so indicated) was added to the dibromopropylphosphonate (25 mg) (I), together with 50 μL of 2,2,6,6-tetramethylpiperidine and 120 μL of methanol. Methanol was always present unless otherwise stated. Most NMR tubes were stoppered with rubber serum caps and heated at 70 °C for 3–4 days (diluted mixtures) or 7 days (undiluted); however, the tubes with pyridine, carbon tetrachloride, and chloroform were sealed and heated for 3.5 days (pyridine), 4.5 days (carbon tetrachloride), and 7 days (chloroform). Almost all reactions were 95–100% complete; however, the reaction in 4 vol. of carbon tetrachloride was only ~80% complete. Following the heating, the solvent was evaporated, acetonitrile-d_3–water–triethylamine (1.5:0.05:0.3 mL) (an additional 0.5 mL of methanol to products formed in carbon tetrachloride) was added, and the products were analyzed by ^{31}P NMR spectroscopy. The data so obtained are summarized in Table I.

The products shown in Table I were identified in the following ways. Methyl *p*-*N,N*-diethylaminobenzenephosphonate was observed by ^{31}P NMR spectroscopy at −14.0 ppm (−15.4 ppm in the carbon tetrachloride experiments) and confirmed in each case by adding back an equivalent amount of authentic product dissolved in water–methanol–acetonitrile-d_3 (0.025:0.30:1.00 mL) to the product mixture and repeating the spectrum. From the increase in signal size relative to that for dimethyl phosphate the yield could be calculated and compared with the integrated value; in neat diethylaniline the calculated yield was 35%. In addition, the aromatic substitution product was partially purified by TLC from reactions run in neat diethylaniline and diethylaniline diluted with four volumes of chloroform and identified by ^1H NMR as previously described.[2] The ^{31}P NMR signal for dimethyl phosphate, observed at −0.42 ppm (−0.86 ppm in the carbon tetrachloride experiments), was identified by adding tetramethylammonium dimethylphosphate (25 mg) in methanol (0.5 mL) to the product mixture; this showed an increase in the product peak

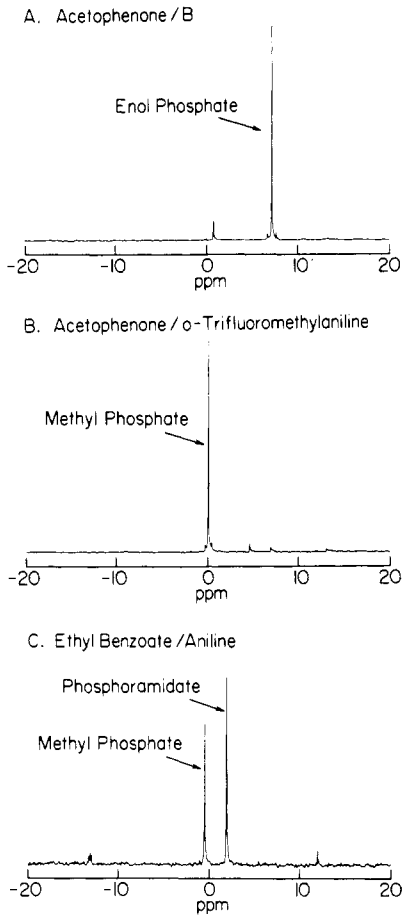


Figure 1. The 40.5-MHz ^{31}P NMR spectra of products formed from the fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonic acid (I) at 70 °C in (A) 3.0 mL of acetophenone mixed with 50 μL of 2,2,6,6-tetramethylpiperidine, (B) 3.0 mL of acetophenone mixed with 0.30 mL of *o*-trifluoromethylaniline and 50 μL of 2,2,6,6-tetramethylpiperidine, and (C) 3.0 mL of ethyl benzoate mixed with 0.30 mL of aniline and 50 μL of 2,2,6,6-tetramethylpiperidine. Chemical shifts are relative to an external standard of 85% phosphoric acid; upfield shifts are assigned a negative sign. The products were identified as described in the Experimental Section.

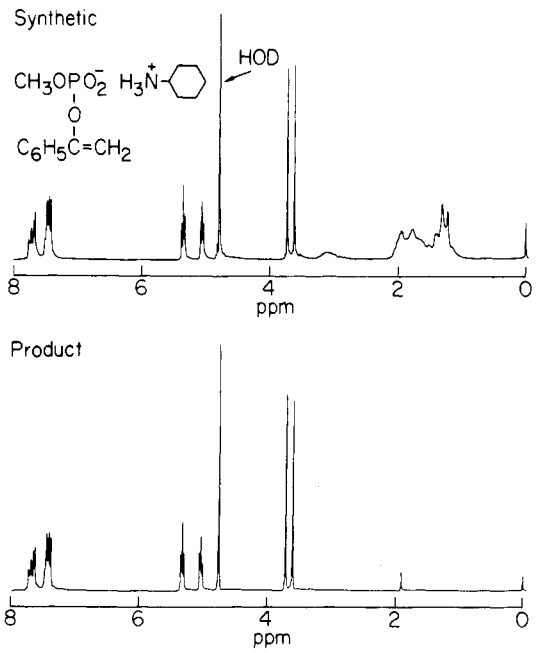


Figure 2. The 100-MHz ^1H NMR spectrum of two samples of α-phenylvinyl methylphosphate in D_2O; the chemical shifts are given relative to that for the signal for sodium 2,2-dimethyl-2-silapentane-5-sulfonate which appears at 0 ppm. The upper spectrum is that for the cyclohexylammonium salt of authentic α-phenylvinyl methyl phosphate, while the lower spectrum is that for product purified from fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonic acid (I) at 70 °C in 3.0 mL of acetophenone mixed with 50 μL of 2,2,6,6-tetramethylpiperidine.

relative to that of added trimethyl phosphate (10 μL). *sym*-Dimethyl pyrophosphate was identified by its ^{31}P NMR signal at 11.54 ppm by adding back an equivalent amount of *sym*-dimethylpyrophosphoric acid to the reactions run in neat diethylaniline; presumably the peak observed at 11.54 ppm in other experiments is also *sym*-dimethyl pyrophosphate. Two products were identified only by their ^{31}P NMR chemical shifts and are indicated in the footnotes to Table I.

A control experiment was designed to detect possible reaction by way of methyl bromophosphate (see Results and Discussion for route to this compound). A premixed solution of diethylaniline (3.00 mL), methanol (0.12 mL), and tetramethylpiperidine (0.05 mL) was added to dimethyl bromophosphate (0.05 mL) and heated for 10 min at 70 °C. Acetonitrile-d_3 (1.5 mL) was added as a lock for ^{31}P NMR spectroscopy; one major (−1.86 ppm, 86%) and one minor product (11.06 ppm, 14%) were detected. The major peak was identified as trimethyl phosphate by addition of an authentic sample. The minor product was not identified, but is not one of the known products of aromatic substitution; addition of dimethyl *p*-*N*,*N*-diethylaminobenzenephosphonate gave a new signal at −23.82 ppm.

Reaction with Acetophenone. The reaction of dibromopropylphosphonate (25 mg), I, in acetophenone (3.0 mL) mixed with tetramethylpiperidine (50 μL) required 1 day at 70 °C. After addition of 1 mL of acetonitrile-d_3, the reaction mixture was analyzed. The ^{31}P spectrum (Figure 1a) showed two products, methyl phosphate (10%, 1.27 ppm) and enol phosphate (90%, 7.52 ppm); the yields are based on the dibromopropylphosphonate used. Although crystals slowly formed in the product mixture at room temperature, they could be dissolved by adding acetonitrile-d_3–water–triethylamine (1.0:0.05:0.3 mL). When the spectrum was repeated with the homogeneous solution, no change in the product distribution was observed. The identification of the major product was confirmed by adding authentic cyclohexylammonium α-phenylvinyl methyl phosphate (20 mg dissolved in water–acetonitrile-d_3, 0.075:1.00 mL) to the NMR tubes; the identification of the minor product was confirmed by adding 1 equiv of methyl dihydrogen phosphate in triethylamine–acetonitrile-d_3 (0.3:0.5 mL). Additionally, α-phenylvinyl methyl phosphate was purified by partitioning the reaction mixture between 10 mL of methylene chloride and 10 mL of 0.1 N sodium hydroxide, rotoevaporating the aqueous layer, and chromatographing the residue on a 20 × 20 × 0.25 cm precoated silica gel plate with methanol–acetonitrile (1:1 v/v). The major band (R_F 0.54–0.66) was eluted and the product was identified by comparing its ^1H NMR spectrum with that of synthetic cyclohexylammonium α-phenylvinyl methyl phosphate (Figure 2).

Control experiments were run to determine whether phosphorylation had taken place by way of the enol of acetophenone (see Results and Discussion for the rationale behind these experiments). Elimination in acetophenone–2,2,6,6-tetramethylpiperidine was carried out in the presence of 5% (molar, relative to acetophenone) phenol (0.11 mL) or 5% *sec*-phenethyl alcohol (0.15 mL) or a mixture (5% each alcohol). ^{31}P NMR spectra were run after adding acetonitrile-d_3–water–triethylamine (2.0:0.05:0.3 mL) to the product mixture. The presence of phenol and/or alcohol led to a reduction in enol phosphate from 90% (neat) to 66% (phenol), 35% (*sec*-phenethyl alcohol), and 32% (mixture). This was accompanied by an increase in methyl phosphate and the appearance of minor amounts of additional products in phenol (~5%, 6.60 ppm), in *sec*-phenethyl alcohol (6%, 3.00 ppm), and in the mixture (5%, 3.00 ppm to 5%, 6.60 ppm). These experiments argue against phosphorylation of enol.

Reaction with Acetophenone and Amines. Dibromopropylphosphonate (25 mg), I, acetophenone (3.0 mL), *o*-trifluoromethylaniline (0.30 mL), and 2,2,6,6-tetramethylpiperidine (50 μL) were heated for 12 h at 70 °C. Acetonitrile-d_3 (1 mL) was added as a deuterium lock for NMR spectroscopy; the ^{31}P NMR spectrum (Figure 1b) showed four products with signals at −0.42 (90%, methyl phosphate), 7.22 (3%, enol phosphate), and 4.94 and 13.74 ppm (7% total, unidentified). Methyl phosphate was identified and its yield confirmed by adding back an equivalent amount of methyl phosphoric acid dis-

solved in acetonitrile-d_3–triethylamine (0.5:0.3 mL) and comparing the increase in signal size to that for added trimethyl phosphate (10 μL): the yield was 80% by this method. Enol phosphate was identified by adding an equivalent amount of authentic sample to the product mixture as described above. Although some product(s) slowly crystallized from the NMR tube at room temperatures, when the solid was dissolved as described above for the acetophenone experiment, no change in the product distribution was observed by ^{31}P NMR spectroscopy.

N-(1-Methylbenzylidene)-2-aminobenzotrifluoride was purified from the reaction mixture and its yield determined by partitioning the mixture between 10 mL of 0.1 M borate buffer (pH 9.0) and 10 mL of carbon tetrachloride. The carbon tetrachloride was evaporated, and the excess acetophenone and *o*-trifluoromethylaniline were removed by vacuum distillation through a short-path still at 55 °C (bath temperature) (0.1 mm) with care taken to discontinue the distillation immediately following removal of the last drops. To determine the yield of ketimine, the residue was dissolved in chloroform-d_1 for ^1H NMR spectroscopy. Residual acetophenone and amine as well as ketimine were all present. The signals for the methyl group of acetophenone (δ 2.52) and of the ketimine (δ 2.18) are sufficiently separated to permit quantitative integration of the spectra. A second integrated spectrum after the addition of 10 μL of acetophenone provided a standard for determining the yield. The average yield from three experiments was 6.6×10^{-5} mol. In reconstruction experiments 84–90% of added ketimine could be recovered from a mixture of acetophenone, *o*-trifluoromethylaniline, and tetramethylpiperidine. The yield corrected for loss during purification was $7.3–7.9 \times 10^{-5}$ mol. This compares with the yield by integration of 6.0×10^{-5} mol of methyl phosphate. Furthermore, the ketimine was purified by molecular distillation (using an apparatus similar to Ace Glass Sublimator No. 8021) at 55 °C (0.1 mm) onto a surface warmed at 45 °C; the ^1H NMR spectrum was identical with that of an authentic sample.

Several types of controls were performed. An experiment was carried out to see if methyl phosphate came from a reaction of enol phosphate with amine; it does not, since enol phosphate is stable under the reaction conditions. An experiment was performed (see Kinetics, below) where the reaction mixture was worked up promptly after mixing. The yield of ketimine under these conditions was negligible; the formation of ketimine, then, does not occur during workup.

A control was run to explore the possibility of phosphorylation of the carbinolamine intermediate in equilibrium with acetophenone and *o*-trifluoromethylaniline. The reaction in acetophenone–*o*-trifluoromethylaniline was carried out in the presence of added 5% (molar, relative to acetophenone) *sec*-phenethyl alcohol (0.15 mL), a model for the intermediate. The presence of alcohol caused only a minor drop in the yield of methyl phosphate (90 to 85%), an equally small drop in the yield of ketimine, and the appearance of a new product at 2.95 ppm (5% yield). These experiments argue against phosphorylation of a carbinolamine intermediate (see Results and Discussion).

Experiments parallel to those described above for *o*-trifluoromethylaniline were performed with aniline itself. These experiments yielded 90% methyl phosphate and 10% phosphoramidate; no enol phosphate was detected. However, four times as much acetophenone anil as methyl phosphate was formed, so the experiment was not pursued further.

Reaction with Ethyl Benzoate and Aniline. The dibromopropylphosphonate (25 mg), I, ethyl benzoate (3.0 mL), aniline (0.3 mL), and tetramethylpiperidine (50 μL) were heated at 70 °C for 3–5 days. Acetonitrile-d_3 (2.00 mL), water (0.05 mL), and triethylamine (0.3 mL) were added. ^{31}P NMR spectroscopy (Figure 1c) showed the presence of methyl phosphate (40%, δ −0.56 ppm), methyl *N*-phenylphosphoramidate (50%, δ 1.85 ppm), and several minor products. The major products were identified by adding authentic samples to the NMR tubes, and repeating the spectra. By adding an equivalent amount of methyl hydrogen phosphate, the yield of methyl phosphate could be confirmed by comparing the ratio of areas of signals for methyl phosphate and phosphoramidate before and after addition; the yield was 32% by this method. The yield of *O*-ethyl-*N*-phenylbenzimidate was determined by first partially purifying it as described for the trifluoroketimine, dissolving it in chloroform-d_1, and adding acetophenone (10 μL); the product was identified and quantitated by ^1H NMR spectroscopy. The signal from the methyl group of added acetophenone (a standard for integrations) was clearly separated from the others, but the signals from the methylene groups of ethyl benzoate and of ethylbenzimidate partially overlapped. Computer expansion of the signals allowed determination of the area for the benzimidate protons and comparison with that for the methyl signal for acetophenone gave the yield. In control experiments, 77–88% of benzimidate that was added to a reaction mixture could be recovered. After correcting for losses in accord with these controls, the average yield of benzimidate from four experiments ($2.5–2.8 \times 10^{-5}$ mol) is identical with that for methyl phosphate (2.7×10^{-5} mol) by integration.

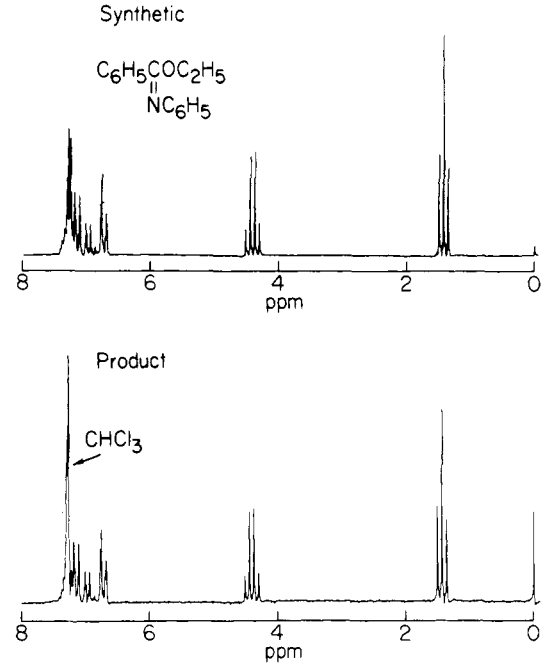


Figure 3. The 100-MHz ^1H NMR spectrum of two samples of *O*-ethyl *N*-phenylbenzimidate in chloroform-d_1; Me_4Si is at 0 ppm. The upper spectrum is that of an authentic sample of *O*-ethyl *N*-phenylbenzimidate. The lower spectrum is that of product purified from fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonic acid (I) in 3.00 mL of ethyl benzoate mixed with 0.30 mL of aniline and 50 μL of 2,2,6,6-tetramethylpiperidine; the purification procedure is described in the Experimental Section.

Additionally, *O*-ethyl-*N*-phenylbenzimidate was isolated from the reaction mixture by the same method used to isolate the ketimine. The purified product was identified by comparing its ^1H NMR spectrum with that of an authentic sample (Figure 3).

A control was run to rule out the very unlikely possibility that the products resulted from phosphorylation of a minute amount of tetrahedral intermediate in equilibrium with ethyl benzoate and aniline. An additional 5% (molar, relative to ethyl benzoate) *sec*-phenethyl alcohol (0.13 mL) was added to the reaction mixture. This led to a small decrease in the yields of methyl phosphate (40 to 34%) and phosphoramidate (50 to 46%) and the appearance of a new product at 1.51 ppm (10% yield). The yield of *O*-ethyl *N*-phenylbenzimidate showed little if any change. Again, a possible phosphorylation of an intermediate is extremely improbable (see Results and Discussion).

Kinetics. The rates of fragmentation of dibromopropylphosphonate, I, and subsequent methyl phosphate formation in acetophenone–*o*-trifluoromethylaniline and ethyl benzoate–aniline (standard reaction mixtures were used; see above) at 70 °C were followed by cooling the reaction mixture at specified times, adding solvents (described above) for ^{31}P NMR spectroscopy, and obtaining integrated ^{31}P NMR spectra. The loss of dibromopropylphosphonate, I, and appearance of methyl phosphate could then be followed on a percentage basis, the percentage of either component being determined from its signal size relative to the total for all of the observed signals. The observed rate constants for fragmentation and methyl phosphate formation were identical in both reactions. The pseudo-first-order rate plot for the reaction in acetophenone–*o*-trifluoromethylaniline is based on ten points and is linear for at least 2–3 half times; the half time is 1 h. The pseudo-first-order rate plot for the reaction in ethyl benzoate–aniline is based on seven points and is linear for at least four half times; the

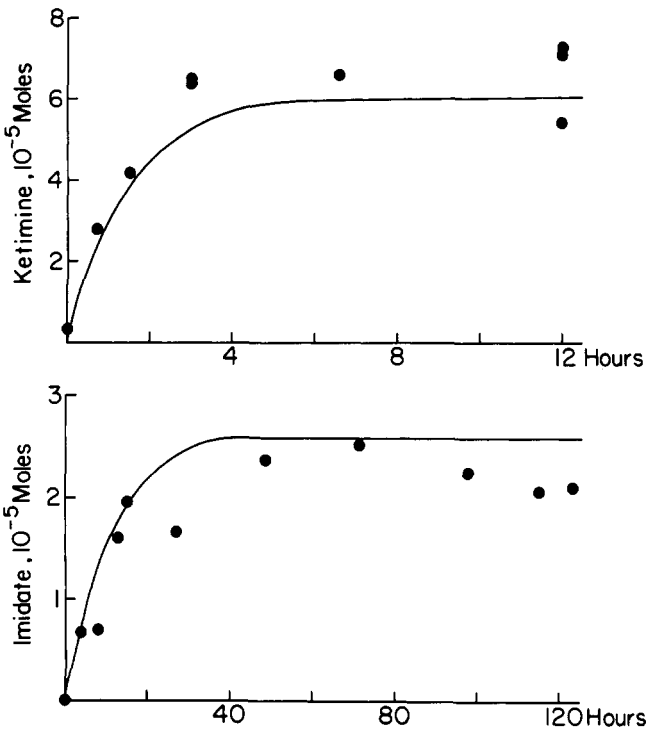


Figure 4. The upper plot compares the rates of formation of methyl phosphate (calculated solid line) and *N*-(1-methylbenzylidene)-2-aminobenzotrifloride (●) from fragmentation of 25 mg of methyl hydrogen *erythro*-1,2-dibromopropylphosphonate (I) at 70 °C in 3.0 mL of acetophenone mixed with 0.30 mL of *o*-trifluoromethylaniline and 50 μL of 2,2,6,6-tetramethylpiperidine. The lower plot compares the rates of formation of methyl phosphate (calculated solid line) and *O*-ethyl *N*-phenylbenzimidate (●) from fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonate at 70 °C in 3.0 mL of ethyl benzoate mixed with 0.30 mL of aniline and 50 μL of 2,2,6,6-tetramethylpiperidine. The calculated lines are based on half times of methyl phosphate formation and the final yields. These values as well as the yields of *N*-(1-methylbenzylidene)-2-aminobenzotrifloride and *O*-ethyl *N*-phenylbenzimidate were determined as described in the Experimental Section.

half time is 8 h. In addition, the rates of formation of *N*-(1-methylbenzylidene)-2-aminobenzotrifluoride (in acetophenone) and *O*-ethyl *N*-phenylbenzimidate (in ethyl benzoate) were followed by determining yields at the indicated times by partially purifying these products, adding an acetophenone standard, and integrating the ^1H NMR spectra as described above. The rates of ketimine and methyl phosphate formation (in acetophenone) are compared in Figure 4 (upper panel); they are identical. Likewise, the rates of benzimidate and methyl phosphate formation (in ethyl benzoate) are compared, also in Figure 4 (bottom panel), and they too are identical.

Results and Discussion

Aromatic Substitution. The data for the attack of monomeric methyl metaphosphate on *N,N*-diethylaniline in the presence of 4% methanol with and without additional solvents are shown in Table I.

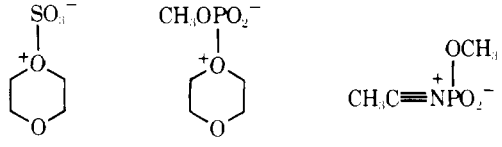
In *N,N*-diethylaniline mixed with methanol alone, aromatic substitution occurs to the extent of ~38%, while 55% of the monomeric methyl metaphosphate reacts with methanol to yield dimethyl phosphate. Presumably methanol competes effectively with dibromopropylphosphonate, I, for monomeric methyl metaphosphate, since without methanol a complex polymer mixture is formed.[3] *sym*-Dimethylpyrophosphate (7%) is also a product. It is unlikely that the pyrophosphate is formed by dimerization of monomeric methyl metaphosphate since this species should be in low concentration and is nonselective. An alternative explanation is that monomeric methyl metaphosphate adds to the nitrogen of *N,N*-diethylaniline to yield a phosphorylating agent which reacts either with methanol or with dimethyl phosphate. The latter reaction would give trimethyl pyrophosphate which could be demethylated by amines.[13] In any event, when monomeric methyl metaphosphate is generated in *N*-methylaniline which is 4% in methanol,[14] more phosphoramide (33%) than dimethyl phosphate (8%) is formed but no pyrophosphate was detected; the other products are mainly those from aromatic substitution.

When additional solvents are added to the reaction system mixed with 4% methanol, the yield of aromatic substitution is reduced, and that of dimethyl phosphate increased. The effect of chloroform is modest; an equal volume of this diluent has no effect on the yield of aromatic substitution, whereas a fourfold dilution reduces it from 38 to 26%. Other solvents cause severe decreases in the precentage of aromatic substitution. Four volumes of pyridine, acetonitrile, dioxane, or 1,2-dimethoxyethane reduce the extent of aromatic substitution below detection limits; dimethyl phosphate is then the only product that can be seen by NMR. The reaction in carbon tetrachloride proceeds with reduced amounts of aromatic substitution.

Similar experiments with *N*-methylaniline, rather then *N,N*-diethylaniline as the aromatic reactant, were cited in preliminary reports;[1] methanol, however, was excluded since the aniline itself limits polymerization. In *N*-methylaniline mixed with 2,2,6,6-tetramethylpiperidine, monomeric methyl metaphosphate partitions between aromatic substitution (20% para, 15% ortho) and phosphoramidate (46%). Again the aromatic substitution is not greatly reduced by chloroform, even in tenfold excess, whereas dioxane or acetonitrile as solvent all but eliminates the electrophilic substitution.

The partitioning of monomeric methyl metaphosphate in *N*-methylaniline is particularly striking. There is almost as much reaction with the aromatic ring as there is with the free electron pair on nitrogen.[1] Monomeric methyl metaphosphate must then rank among the least selective reagents known. It is probable that reactions with *N*-methylaniline are occurring at near the diffusion-controlled rate.[15a] In fact, the monomeric methyl metaphosphate need never be free; it might, for example, participate in transfer reactions by a "preassociation mechanism".[15b] Such fine details of the phosphorylations have not yet been elucidated.

A reasonable explanation for the solvent effects rests on the assumption that monomeric methyl metaphosphate combines with the unshared electron pairs of pyridine, acetonitrile or ether, to yield mild and selective phosphorylating agents which react preferentially with methanol. In analogy, the reactivity of SO_3, isoelectronic with monomeric metaphosphate monoanion, is moderated by forming isolable crystalline adducts with pyridine, dioxane, or *N,N*-dimethylaniline.[16,17] Also,



reactions of SO_3 with acetonitrile and ethers have been observed.[16] Presumably, monomeric methyl metaphosphate is reacting in a similar fashion.

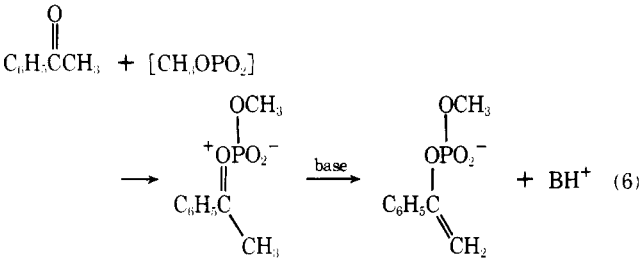
These dilution experiments suggest that two different phosphorylating agents are formed, one of which is capable of electrophilic substitution in the aromatic ring, whereas the other, formed in the presence of pyridine (or the weak bases), is only capable of reacting with more active nucleophiles. If the milder phosphorylating agent is indeed the metaphosphate adduct, then the former is almost certainly monomeric methyl metaphosphate.

Previous observations might be reconsidered in light of the nonselectivity of monomeric methyl metaphosphate. Monomeric alkyl metaphosphates have been proposed as active

phosphorylating agent formed in pyridine from a nucleoside 5′-phosphate and a condensing agent, triisopropylbenzene sulfonyl chloride, and characterized it as monomeric metaphosphate; perhaps this reagent is a pyridine–metaphosphate adduct. Gerrard and Hamer[20] reported that the alkaline solvolysis of optically active methyl *N*-cyclohexylphosphoramidothioic chloride in dimethoxyethane–water (1:1 v/v) gave a racemic product and proposed a free metaphosphate intermediate. However, when the reaction was repeated under the same conditions in methanol–water (1:1 v/v), only partial racemization was observed. The solvent effect on the stereochemical outcome is puzzling. A tentative explanation is that racemization in dimethoxyethane is achieved by equilibration of the phosphorylating agent between the ether oxygens. Kirby and Varvoglis[21] reported similar second-order rate constants for reactions of dioxane (0.42 M^{-1} min^{-1}) and amines, including imidazole (0.80 M^{-1} min^{-1}), with 2,4-dinitrophenyl phosphate. These results and others were discussed in terms of a "borderline" mechanism with metaphosphate character; the involvement of dioxane as a nucleophile seems reasonable. Rebek's three-phase test[22] for monomeric metaphosphates in either neat dioxane or acetonitrile or in these solvents mixed with proton sponge could involve any or all of these solvents as monomeric metaphosphate carriers.

A possible alternative to monomeric methyl metaphosphate as the phosphorylating agent has been suggested.[22] Conceivably, CH_3OPO_2 and Br^-, produced by fragmentation, might be trapped in a cage and react to form $CH_3OP(Br)O_2^-$; this anion might then be the effective electrophile. This possibility, however, is extremely remote. Although we have not succeeded in preparing the methyl bromophosphate anion, dimethyl bromophosphate,[12] which would presumably be more effective as an electrophile than the corresponding monoanion,[23] does not affect aromatic substitution on *N,N*-diethylaniline under standard reaction conditions.

Reaction with Acetophenone. In acetophenone mixed with a small amount of 2,2,6,6-tetramethylpiperidine, the principal reaction product from I is the enol phosphate (eq 6). The



(6)

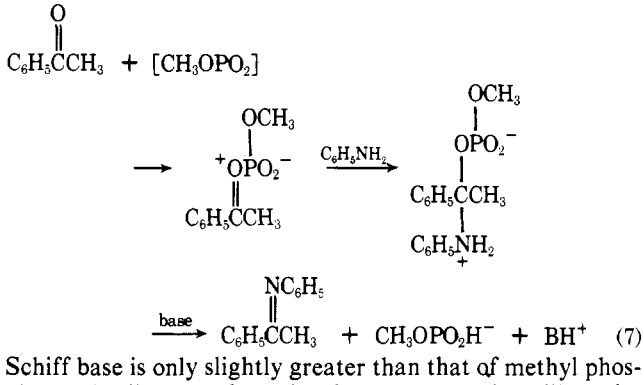
product has been isolated and is identical with that produced by way of the Perkow reaction.[11] The structure of the enol phosphate is fully established by its ^1H NMR spectrum (Figure 2), where the chemical shifts and splittings of the signals from the vinylic protons are diagnostic. Presumably monomeric methyl metaphosphate attacks the carbonyl group of acetophenone, which then loses a proton to base to yield the enol phosphate.

Alternatively, the reaction could conceivably proceed by phosphorylation of enol present in equilibrium with the ketone. However, the amount of enol in acetophenone is very small, ≤0.035%.[24] Monomeric methyl metaphosphate could react with enol in preference to the large concentration of acetophenone present only if the metaphosphate were highly selective.

However, this is precisely what monomeric metaphosphates are not. Monomeric methyl metaphosphate ranks among the least selective reagents known. In particular, many studies have established that metaphosphates generated from many different sources react nonselectively with alcohols.[25] If monomeric methyl metaphosphate were reacting with enol, then it should be quenched in the presence of model alcohols. When a control reaction was carried out in the presence of 5% (molar, relative to acetophenone) phenol or *sec*-phenethyl alcohol, only a minor amount of additional product (≤6%) was formed; the yield of enol phosphate decreased from 90 (neat) to 66 (phenol) and 35% (*sec*-phenethyl alcohol), while more methyl phosphate was formed. While the increase in methyl phosphate requires explanation (see below), the important observation here is that, when phenol or an alcohol is in great excess over enol, the yields of phosphorylated alcohols are at the least 7–10 times below that for enol phosphate. This is the opposite of what is predicted for reaction of monomeric metaphosphate with enol and argues for a direct attack on acetophenone (eq 3).

Attempts were made to trap the acetophenone–metaphosphate adduct with nucleophiles. When the fragmentation of I is carried out in the presence of aniline and acetophenone, the reaction products are methyl phosphate and the Schiff base of acetophenone; no enol phosphate is detected. Since the carbonyl group is the only source of oxygen, it appeared that methyl phosphate was a product from reaction of monomeric methyl metaphosphate with acetophenone. However, since the yield of Schiff base is four times that of methyl phosphate, a direct condensation of ketone with amine might account for it all. Since water is also a product of condensation, monomeric methyl metaphosphate could react with the water to give methyl phosphate.

When, however, *o*-trifluoromethylaniline is used instead of aniline, the ketimine that is produced must arise from the action of monomeric methyl metaphosphate (eq 7). The yield of



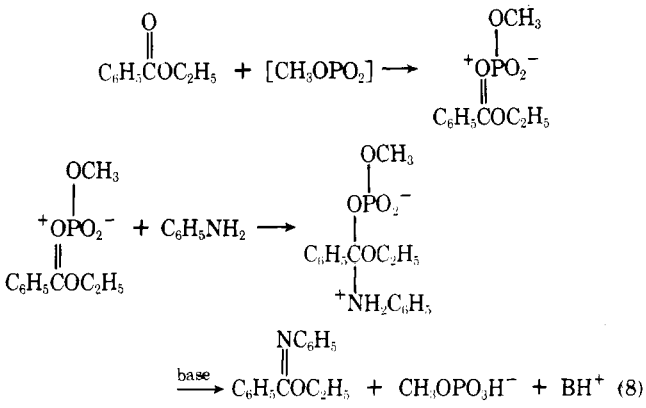
(7)

Schiff base is only slightly greater than that of methyl phosphate. The direct condensation does not proceed readily under the reaction conditions and only with prolonged heating does the yield slowly increase. Of critical importance, moreover, is the observation that the rates of Schiff base and methyl phosphate formation are identical (Figure 4). These facts together indicate that both products are formed from a common intermediate. Monomeric methyl metaphosphate must activate the acetophenone toward formation of the Schiff base, presumably as shown in eq 7. In this reaction, then, the monomeric methyl metaphosphate presumably attacks the carbonyl group of acetophenone. The intermediate is the same one suggested for the reaction to yield enol phosphate. It resembles a protonated ketone; the positive charge will activate both the carbonyl group toward nucleophilic attack and the methyl group toward loss of a proton.

An hypothesis may now be offered to explain the increased yields of methyl phosphate observed when fragmentation was carried out in acetophenone–base mixed with 5% phenol or alcohol. In a similar manner to that for amines, the phenol or alcohol might trap the metaphosphate–carbonyl adduct to give a phosphorylated hemiketal; base might then eliminate methyl phosphate from this latter intermediate.

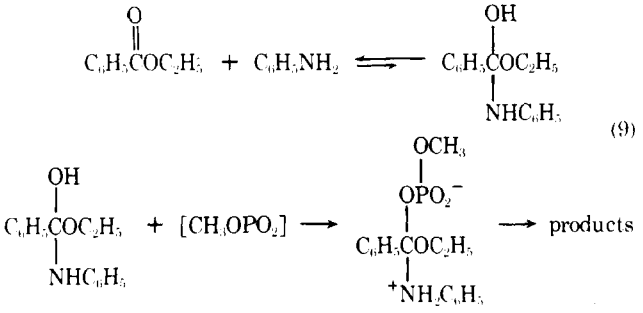
Reaction with Ethyl Benzoate. When monomeric methyl metaphosphate is generated in the presence of ethyl benzoate and aniline, equal amounts of methyl phosphate and *O*-ethyl

N-phenylbenzimidate are formed (eq 5). The rates of formation of methyl phosphate and benzimidate are identical (Figure 4), indicating a common intermediate. The benzimidate was identified by comparison of its ^{1}H NMR spectrum with that for synthetic material (Figure 3). The simplest pathway for the process consistent with the products is shown in eq 8. The



direct reaction of ethyl benzoate and aniline yields benzanilide, not *O*-ethyl *N*-phenylbenzimidate.

For both Schiff base formation and for imidate formation, an alternative pathway must be considered. This is the path where a carbinol intermediate, or a tetrahedral intermediate, is formed from the carbonyl compound and amine, and is then trapped by the monomeric methyl metaphosphate. The resulting phosphorylated tetrahedral intermediates also occur along the pathways of eq 7 and 8. The alternative is illustrated for the benzimidate synthesis in eq 9.



(9)

Although this pathway formally leads to the same products as that of eq 8, it is intrinsically unlikely. The concentration of the tetrahedral intermediate in equilibrium with ester and aniline, even if this equilibrium is achieved, is minute.[26] Guthrie[27] estimates that the equilibrium constant for the addition of dimethylamine to methyl acetate is 9.1×10^{-11} M^{-1}. The equilibrium constant for the addition of aniline to ethyl benzoate is probably even lower. This is because, while the addition of amines to carbonyl compounds show little dependence on amine basicity,[28] the substitution of phenyl and ethoxy groups on the intermediate should destabilize it;[29] the solvent effect is probably small since all species involved are electrically neutral. It is quite unlikely that monomeric methyl metaphosphate would react selectively with such an extremely low concentration of intermediate.

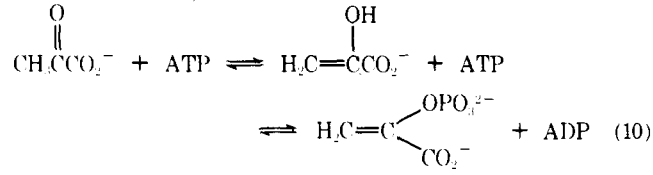
The argument for the reaction pathway for the formation of Schiff base (eq 7), although weaker, is similar. The difficulty arises in determining an equilibrium constant for carbinolamine formation. A rather severe extrapolation must be made[30] and the value obtained, 10^{-4} M^{-1}, should only be considered an order of magnitude estimate at best. Nonetheless, the concentration of carbinolamine should be quite low compared with that for acetophenone.

If a nonselective reagent like monomeric methyl metaphosphate were reacting with low concentrations of intermediates, then it should be quenched in the presence of much higher concentrations of added model alcohols. However, when the fragmentation of dibromopropylphosphonate, I, was carried out in either acetophenone–*o*-trifluoromethylaniline or ethyl benzoate–aniline in the presence of 5% (molar, relative to carbonyl compound) *sec*-phenethyl alcohol, only minor differences in the product distribution were observed. Since little if any of the alcohol was phosphorylated, it is quite unlikely that the much lower concentrations of the intermediates were phosphorylated.

Enzymology. The work cited above strongly supports the idea that monomeric methyl metaphosphate adds readily to carbonyl groups and activates them for enol phosphate formation or nucleophilic attack. Since the chemical hydrolysis of phosphate monoesters, including ATP[31] and other "high energy" phosphates,[32] proceeds in solution by a monomeric metaphosphate[33] or a closely related "borderline" mechanism,[15b,20,34] a strong electrophile or its near equivalent is presumably available for biochemical transformations. Although the analogy between the reactions here cited and metabolic ones is tempting, the pathways of the enzymic processes are unproved. Enzyme catalyzed phosphoryl transfers could proceed by a monomeric metaphosphate mechanism, some sort of "borderline" mechanism and/or a stepwise displacement mechanism involving a pentacoordinate intermediate.[35]

A great deal of attention has been given to kinases which catalyze phosphoryl transfer between ATP and alcohols, amines or acids. There remain, however, more complex reactions where phosphoryl transfer is coupled to additional chemical transformations; the mechanisms of these reactions have proved particularly challenging. Metaphosphate mechanisms provide an alternative to those which have been usually considered.

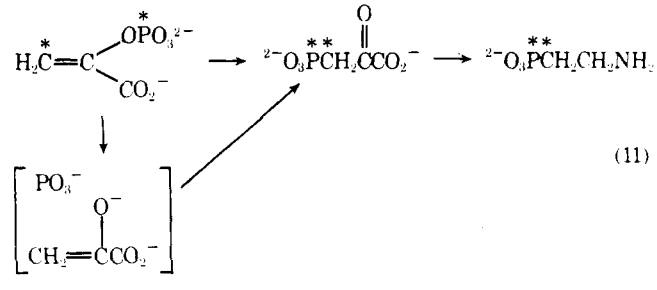
Rose and his collaborators[36] have adduced considerable evidence that the reaction catalyzed by pyruvate kinase proceeds is shown in eq 10. In this formulation, pyruvate is first



(10)

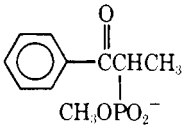
enolized and then trapped by ATP. This is not then the enzymic analog of the process suggested in eq 6 for the formation of enol phosphate from acetophenone. Other evidence must, however, be considered. Lowe and Sproat[37] have found that pyruvate kinase acts on ATP in the absence of pyruvate or phosphoenol pyruvate to scramble the oxygen atoms about the β phosphorus atom; they proposed that the enzyme splits off monomeric metaphosphate which then rejoins the β phosphorus following its rotation.[38] Knowles and co-workers[39] have, however, concluded that the reaction of pyruvate kinase as well as those of other kinases proceeds with inversion at the gamma phosphorus of ATP; this suggests a "borderline" or stepwise displacement mechanism rather than an S_N1 process that utilizes a free monomeric metaphosphate.

The question of how enzymes catalyze P–C bond formation is an interesting one. Labeling studies[40] (eq 11) indicate that



(11)

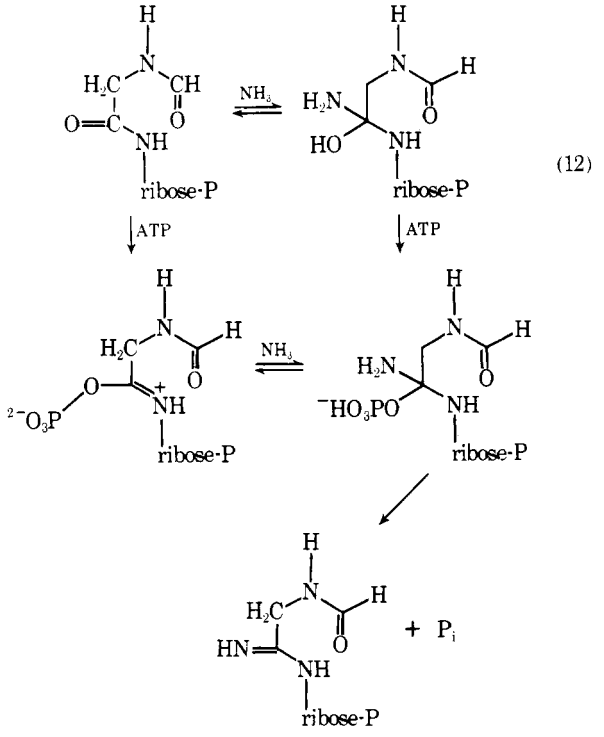
phosphoenol pyruvate serves as a precursor for pyruvylphosphonic acid and eventually 2-aminoethylphosphonic acid. We are unaware of any chemical examples of direct displacement reactions at phosphorus by carbanions or their equivalents; the reaction might be difficult since it requires considerable energy to place carbon in an apical position in a pentacoordinate intermediate,[41] the direction of attack for other nucleophiles. On the other hand, the methyl ester of 1-benzoylethylphosphonic acid (II) is a minor rearrangement product isolated from the



II

reaction of dibromopropylphosphonate, I, in *N*-methylaniline.[1] Although the enzyme catalyzed reaction may involve a direct displacement reaction of some sort, the transformation of I may serve to model a possible rearrangement (eq 11) of phosphoenol pyruvate to pyruvylphosphonic acid by a metaphosphate mechanism.[1]

A certain class of ATP-dependent amidotransferases converts the carbonyl group into C=N with stoichiometric cleavage of ATP to ADP and P_i.[42] Labeling experiments have demonstrated transfer of the carbonyl oxygen into inorganic phosphate.[42b] These processes formally resemble the preparation of ketimine (eq 7) and benzimidate (eq 8) as promoted by monomeric methyl metaphosphate. Two possible enzyme catalyzed pathways are shown in eq 12. In one ATP attacks



(12)

the carbonyl group to activate it for nucleophilic attack, whereas in the other a tetrahedral intermediate is formed that is subsequently trapped by ATP. In contrast to evidence for the chemical examples given above, no definitive argument can be advanced at present for or against either enzymic pathway. The concentration of the tetrahedral intermediate on the enzyme may well be substantial, and the proximity to ATP could favor a bimolecular reaction,[43] but from a mechanistic point of view the attack of ATP upon a carbonyl group is attractive, since it would activate the carbonyl group toward nucleophilic attack and give a thermodynamically stable phosphorylated intermediate. Phosphorylation of the carbonyl oxygen could occur by a monomeric metaphosphate, "borderline," or stepwise displacement mechanism.

Recently, it has been reported that certain amide hydrolases require ATP for activity. These include 5-oxoprolinase,[44] the Rec A gene product,[45] or a closely associated activity which requires ATP for the cleavage of a specific peptide linkage in the λ repressor, and an ATP-dependent proteolytic system from reticulocytes.[46] 5-Oxoprolinase is the best characterized system and catalyzes hydrolysis of a cyclic amide with stoichiometric cleavage of ATP to ADP and P_i. Although a direct reaction of ATP with the carbonyl oxygen has not yet been demonstrated, the amide group could be activated for reaction with water in the same manner as that proposed for the amidotransferases. Although the peptidases may require ATP for activation in the biological sense, i.e., as an allosteric effector,[47] the alternative chemical activation cannot to the best of our knowledge be ruled out at the present time.

Almost 4 decades ago, Lipmann[48] showed how ATP is thermodynamically important in pulling forward otherwise unfavorable reaction sequences. The present work introduces the possibility that ATP plays a kinetic role as well by activating the carbonyl group for attack by nucleophiles.

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Dioxygen Transfer from 4a-Hydroperoxyflavin Anion. 2. Oxygen Transfer to the 10 Position of 9-Hydroxyphenanthrene Anions and to 3,5-Di-*tert*-butylcatechol Anion

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Abstract: The reaction of the peroxy anion of N^5-ethyl-4a-hydroperoxy-3-methyllumiflavin (4a-$FlEtO_2^-$) with the anions of 3,5-di-*tert*-butylcatechol (VIII), 10-ethoxy-9-phenanthrol (Ia), and 10-methyl-9-phenanthrol (Ib) has been investigated. All products may be accountable through the transfer of O_2 from the 4a-$FlEtO_2^-$ reactant to the phenolate anions with the production of reduced flavin anion ($FlEt^-$) and a hydroperoxycyclohexadienone. From $VIII^-$(*t*-BuOH) there was obtained 3,5-di-*tert*-butyl-*o*-quinone (IX) and Ib^- yielded (*t*-BuOH or CH_2CN) 10-hydroxy-10-methyl-9,10-dihydro-9-phenanthrone (IIIb), while Ia^- provided both 9,10-phenanthrenequinone (V) and monoethyl 1,1′-diphenate (IVa) (the ratio of V:IVa being solvent dependent). The mechanisms for the decomposition of intermediate peroxide anions to products are discussed. The conversion of Ia^- to IVa by oxygen transfer from 4a-$FlEtO_2^-$ amounts to a catalysis by $FlEt^-$ of the reaction of $^3O_2$ with Ia and serves as a biomimetic reaction of flavoenzyme dioxygenase. The kinetics for the reaction of $VIII^-$ with 4a-$FlEtO_2^-$ require the formation of an intermediate. Since the rate constants for the reaction of both $VIII^-$ and 2,6-di-*tert*-butyl-4-methylphenolate anion with 4a-$FlEtO_2^-$ are identical under saturating conditions by these phenolate ions, it is concluded that the intermediate is formed in a unimolecular reaction from 4a-$FlEtO_2^-$ ($k = 0.36$ s^{-1}) as in eq 19. Dissociation of 4a-$FlEtO_2^-$ to $FlEt^-$ + O_2 and reaction of phenolate ions with O_2 may be discounted since the second-order rate constants for the reaction of phenolate ions with O_2 are less than required for the kinetic competency of this process. Dissociation of 4a-$FlEtO_2^-$ to yield neutral flavin radical ($FlEt\cdot$) + $O_2^{-}\cdot$ followed by reduction of $FlEt\cdot$ by phenolate ion to provide $FlEt^-$ and phenoxy radical with the coupling of the latter with $O_2^{-}\cdot$ is also improbable. Thus, though the second-order rate constants for $1e^-$ reduction of $FlEt\cdot$ by the various phenolate species are sufficiently large to allow the kinetic competency of this step, there exists no evidence that $O_2^{-}\cdot$ can couple with any radical species to provide a hydroperoxide. The oxygen-donating intermediate formed from 4a-$FlEtO_2^-$ is suggested to be the 4a,10-dioxetane (XII) or an oxygen molecule more loosely associated with $FlEt^-$. The equilibrium constant for the formation of such an intermediate may be as small as 10^{-5} if the rate of reaction of phenolate ion with this species approaches a diffusion-controlled process.

Of much present concern are the mechanisms by which the mono- and dioxygenase enzymes combine with triplet molecular oxygen to provide species capable of transferring one or both of the oxygen atoms to a substrate molecule. The only non-metal-requiring oxygenases require flavin molecules as cofactors. This laboratory has been engaged in mechanistic studies of the reaction of oxygen and oxygen species with flavins and the problem of oxygen activation by flavins.[1-8] The transfer of molecular oxygen in toto by metallodioxygenase enzymes was first recognized by Hayaishi.[9] The mechanisms of these reactions are at least partially understood and metal-centered biomimetic systems have been explored.[10]

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