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The Mechanism of the Hydrolysis of μ-Monothiopyrophosphate

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Abstract: The hydrolysis of μ-monothiopyrophosphate (MTP) is described as a function of pH, temperature, and solvent composition. The hydrolysis of its precursor, tetramethyl μ-monothiopyrophosphate (Me_4MTP), is also examined. The profile of log k_{obs} versus pH for the hydrolysis of MTP exhibits two breaks corresponding to pK_a values of 4.73 and 8.49, which are assigned to the third and fourth dissociation constants of MTP, respectively. The rate constants for hydrolysis of the tetraanion and trianion of MTP at 25 °C are 0.0032 and 1.39 min^{-1}, and at 5 °C the trianion and dianion undergo hydrolysis with rate constants of 0.103 and 1.93 min^{-1}, respectively. The enthalpy of activation at pH 7.0 is 19.7 kcal·mol^{-1}, and the entropy of activation is 0.2 eu; the latter observation indicates that the mechanism involves a dissociative transition state. In contrast, Me_4MTP undergoes hydrolysis with k_{obs} = 1.21 min^{-1} at 25.3 °C, an enthalpy of activation of 13.0 kcal·mol^{-1}, and an entropy of activation of −22.7 eu. These values suggest that the hydrolytic mechanism for Me_4MTP involves an associative transition state. These mechanisms are strongly supported by comparisons of the hydrolytic rates for MTP and Me_4MTP with those for pyrophosphate (PP_i) and tetraalkyl pyrophosphate. The rate ratios for MTP/PP_i are 5.8×10^7 for the dianions, 1.7×10^7 for the trianions, and between 0.6×10^7 and 2.4×10^7 for the tetraanions. The large rate enhancements induced by the replacement of oxygen by sulfur are attributed to the weakness of the P–S bond relative to the P–O bond. This weakness will be expressed in a dissociative transition state, which involves a high degree of bond cleavage between P and the leaving group. In contrast, the rate ratio for Me_4MTP/Me_4PPi (tetramethyl pyrophosphate) is 48, which is indicative of little cleavage of the bond between P and the leaving group in the transition state, that is, an associative transition state.

Phosphoryl group transfer is of considerable interest, in part owing to the great number of biological phosphoryl group transfer reactions, but also because of the rich variety of mechanisms that phosphorus-containing compounds display.[1] Despite the importance of these reactions, the chemical and biological mechanisms of phosphoryl group transfer remain somewhat elusive.[2] The transition states for nonenzymatic phosphoryl group transfer by phosphate monoesters are dissociative but do not appear to involve the formation of a diffusible monomeric metaphosphate (PO_3^-) as an intermediate in aqueous solutions.[3] In the enzymatic catalysis of these reactions, it is not certain whether the transition state remains dissociative or becomes more associative. Metal complexation of the phosphate ester or anhydride might promote a more associative transition state.[4] Moreover, it has been pointed out that it is not obvious how an enzyme might catalyze a phosphoryl transfer through a dissociative transition state.[2]

Knowledge of the chemistry of μ-monothiopyrophosphate (MTP[5]), an analogue of pyrophosphate (PP_i), should aid the understanding of the mechanisms of phosphoryl group transfer. This compound has been synthesized by Loewus and Eckstein, who demonstrated its extreme lability and found it to be a substrate of inorganic pyrophosphatase.[6] In order to assess the enzymatic reactivity of MTP in quantitative terms, especially in comparison with PP_i, we have characterized its rate of hydrolysis as a function of temperature, pH, and solvent polarity. We have also evaluated the activation parameters for its hydrolysis. To compare the reactions of phosphate monoesters with phosphate triesters, we have also studied the hydrolysis of tetramethyl μ-monothiopyrophosphate (Me_4MTP).

We here report the results of our studies, which show that MTP is millions of times more reactive than PP_i toward water. All evidence is consistent with the postulate that the transition states for the hydrolysis of MTP and PP_i are dissociative, whereas those for the hydrolysis of the tetramethyl esters are associative.

Experimental Section

Materials. All chemicals were obtained from commercial suppliers. KCl was dried at 120 °C for 8 h, and tetramethylammonium chloride was dried in vacuo at 55 °C for 8 h. The latter had been washed with $CHCl_3$ and recrystallized from alcohol. All hydroxide solutions were standardized against potassium phthalate (Mallinckrodt), which had been dried overnight at 120 °C. Lithium hydroxide was synthesized under argon from lithium metal (Aldrich) and water and was filtered through sintered glass. HCl was distilled, and the constant-boiling fraction was taken. Potassium dihydrogen phosphate (Mallinckrodt) and dibasic potassium phosphate (Fisher) were dried at 120 °C for several hours. Dioxane was freed of peroxides by passage through a column of basic alumina and then distilled from anhydrous $SnCl_2$.

Methods. The pH values of solutions were measured with a Radiometer Copenhagen Model PHM 26 pH meter equipped with a Beckman combination electrode. For kinetic experiments, the pH measurements were taken at the end of the run; they were either taken at the same temperature as the run or a correction was applied to the pH on the basis of the temperature difference (<5 °C) between the run and the measurement. The pH meter was always standardized at the same temperature at which it was used. No correction was applied to the pH meter readings for the presence of organic solvents; the error so introduced is probably negligible.[7] The pH meter reading was corrected for the presence of 82% D_2O.[8]

Me_4MTP and MTP were synthesized by modifications of the procedures described by Michalski et al.[9] and Loewus and Eckstein,[6] respectively. The hydrolysis of MTP was measured by spectrophotometrically monitoring the formation of thiophosphate at wavelengths between 232 and 215 nm. A Cary 118C recording spectrophotometer equipped with a Forma Model 2160 circulating bath and jacketed cells was used.[10] Reactions were started by the addition of a small volume of a stock MTP solution into a 1-mL cuvette containing a temperature equilibrated solution of buffer and other components. The concentration of MTP was 30–200 μM, and reactions were carried out in triplicate, unless otherwise noted. Studies were carried out at $I = 0.1$ with KCl, at a buffer concentration of 25 mM, and at 25.0 ± 0.5 °C, unless otherwise specified. The cuvettes were covered with Teflon covers or stoppers, and the cell compartment was flushed continuously with N_2. The K^+ salts of the following buffers were used for the pH-rate studies: succinate, pH 3.7–5.7; MES, pH 5.7–6.8; MOPS, pH 6.9–7.2; BES, pH 7.23; HEPES,

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[5] Abbreviations: MTP, μ-monothiopyrophosphate; PP_i, pyrophosphate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(2-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; NMR, nuclear magnetic resonance; *I*, ionic strength; E_a, Arrhenius activation energy; ΔS^*, entropy of activation; ΔH^*, enthalpy of activation, MES, 4-morpholinoethanesulfonic acid; MOPS, 4-morpholinopropanesulfonic acid; BES, *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazinoethanesulfonic acid; TAPS, 3-[[tris(hydroxymethyl]amino]-1-propanesulfonic acid; CHES, 2-(cyclohexylamino)ethanesulfonic acid; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid.

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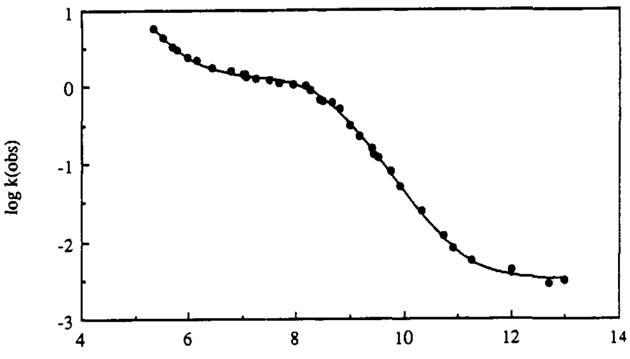

Figure 1. Logarithm of the first-order rate constants for the hydrolysis of MTP as a function of pH at 25 °C and ionic strength 0.1. The symbols represent averages of the rate constants and pH values over three experiments. The solid line is a least-squares fit to the data; below pH 7.0 the line derives from the fit to eq 2. At pH 7 and above, the line derives from the fit to eq 1.

pH 7.5–7.9; TAPS, pH 7.9–8.8, CHES, pH 8.9–9.8; CAPS, pH 9.9–11.3. At pH values of 12 and above, potassium hydroxide was used except as noted.

For all calculations the activity of the solvent was taken to be unity. Rate constants were calculated primarily by least-squares fitting of the integrated form of the first-order rate equation: $\ln (A_\infty - A_t) = \ln (A_\infty - A_0) - kt$.[11] The R^2 values were always above 0.99 and usually above 0.997. The end point was usually determined after at least 10 half-lives, and the rate constant was usually calculated from data comprising 3 half-lives. When the end point was difficult to determine (some studies at low pH at 5 °C), the Guggenheim method was employed.[11]

Above pH 10.5 the rate constants were calculated by taking the initial slopes of the progress curves. At high pH and high ionic strength, thiophosphate decomposes at a rate which increases with time in a process that appears to be independent of dissolved oxygen but depends on the concentration and type of counterion. This necessitated the use of initial rates to calculate rate constants at high pH, but it was not severe enough to compromise such measurements. Rate constants from initial rates required accurate values for MTP concentration and the molar absorptivity of thiophosphate. The concentration of MTP was determined by assays for total phosphate. The molar absorptivity of thiophosphate was determined spectrophotometrically from standard solutions for each pH and counterion. The values ranged from 2810 to 3760 M^{-1} cm^{-1}, and they were used to calculate the rate constant from the initial value of ΔA_{227}. The molar absorptivity of MTP was small and insensitive to pH at high pH values, and the value 460 M^{-1} cm^{-1} measured at pH 12 was used in the calculations. Agreement between the initial rate method and the Guggenheim method at pH 10.8 was satisfactory.

Two Fortran programs which fit data by a nonlinear least-squares algorithm were used to calculate pK_a values and limiting rate constants from the rate constants found at various pH values.[12] Data were fitted to eqs 1 and 2. In these equations $k_{\text{low pH}}$, $k_{\text{high pH}}$, and $t_{1/2}{}^*$ are pH-independent parameters.

$$\log k_{\text{obs}} = \log \frac{\{k_{\text{low pH}} + k_{\text{high pH}}(K_a/[\text{H}^+])\}}{(1 + K_a/[\text{H}^+])} \tag{1}$$

$$\log t_{1/2} = \log \frac{t_{1/2}{}^*(1 + K_{a2}/[\text{H}^+])}{(1 + [\text{H}^+]/K_{a1})} \tag{2}$$

Activation Parameters. The activation parameters for the hydrolysis of MTP at pH 7.0 in 25 mM MOPS (I = 0.1 M with KCl) were determined by measuring the rate constants at seven temperatures and calculating the Arrhenius activation energy (E_a) from the slope of the graph of ln k vs $1/T(\text{K}^{-1})$. The enthalpy of activation ($\Delta H^‡$) was calculated from the slope of the graph of $\ln (k/T)$ versus $1/T(\text{K}^{-1})$.[13] The entropy of reaction was calculated by inserting the average rate constant at 25 °C into the equation $\Delta S^‡/4.576 = (\log k_{\text{avg}}) - 10.753 - (\log T) + E_a/4.576T$, where T is the temperature in Kelvin and $\Delta S^‡$ is the entropy of activation.[13] The parameters were calculated from least-squares fits of the rate constants collected at various temperatures.

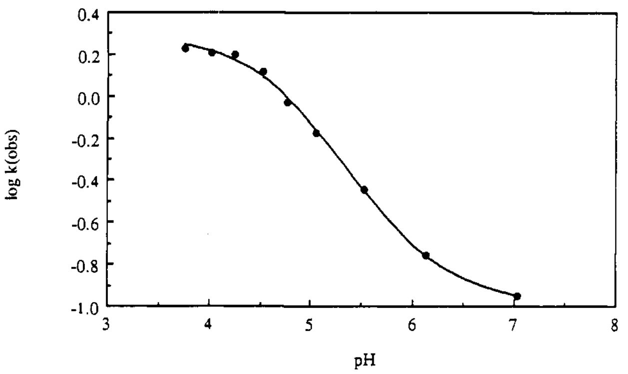

Figure 2. Logarithm of the first-order rate constants for MTP hydrolysis as a function of pH at 5 °C and ionic strength 0.1. The symbols represent the averages of the rate constants and pH values over three experiments. The data are fitted to eq 1.

Table I. pK_a Values for Pyrophosphates and Related Compounds

compd	pK_3	pK_4	conditions	ref
PP$_i$	6.12	8.95	Me_4N^+	14
PP$_i$	6.03	8.40	K^+	15
MTP	4.73[a]	–	K^+	this work
MTP	–	8.49[b]	K^+	this work
MTP	–	8.51[c]	K^+	this work
n-$BuSPO_3H_2^-$	5.50[d]		K^+[e]	16
n-$BuOPO_3H_2^-$	6.84[d]		K^+[e]	16

[a] T = 5 °C. [b] Determined by fitting rate constants to eq 1. [c] Determined by fitting rate constants to eq 2. [d] Second dissociation constant. [e] Ionic strength 1.0.

Hydrolysis of Me_4MTP. The hydrolysis of Me_4MTP was carried out at reactant concentrations of about 120–320 μM. The hydrolysis was observed spectrophotometrically at 218 nm in 10 mM MOPS buffer (pH 7.0) and at an ionic strength of 0.1 adjusted with KCl, unless otherwise noted. The solutions contained 1.0–2.5% acetone, which was used to dilute the Me_4MTP. Since Me_4MTP was diluted in acetone for hydrolytic studies, Me_4MTP in acetone was examined by ^{31}P NMR and found to be stable for several days. All other methods were the same as those used to study MTP hydrolysis. Activation parameters for the hydrolysis of Me_4MTP were evaluated from rate constants at several temperatures by the same procedure described above for MTP.

Results

The pH–Rate Profile for the Hydrolysis of MTP. The rate constants for MTP hydrolysis as a function of pH are shown in Figures 1 (25 °C) and 2 (5 °C). At 25 °C the reaction became too fast to measure by standard techniques at pH values below 5.3, but at 5 °C it was slow enough to study at pH ≥3.7. The kinetic values of pK_a resulting from fits of the rate constants to the rate equations in the Experimental Section are given in Table I and compared with literature values for PP$_i$, together with values for n-butyl phosphate and n-butyl phosphorothiolate. The value of pK_4 (8.49) for trianionic MTP is remarkably similar to the literature value for trianionic PP$_i$. The pK_a of 4.73 is assigned to the dianion/trianion equilibrium (pK_3), even though this is more than one pH unit below the corresponding value for PP$_i$. The pK_a values of pyrophosphate are not very sensitive to temperature,[14] so that the difference in pK_3 is likely due to electronic factors.

The rate constants for the hydrolysis of the tetraanionic, trianionic, and dianionic species of MTP are collected in Table II, along with comparable literature data for PP$_i$ and other compounds. The hydrolysis of MTP is independent of buffer concentration below 0.1 M and proceeds with no significant solvent deuterium isotope effect at pH 7.04 (data not shown). The rate constants for MTP hydrolysis at ionic strength 0.1 reach a minimum value near pH 13.

The pH–rate data in Figure 1 have been fitted to eq 1, in which a plateau value for the rate constant is implicit between pH values

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Table II. Hydrolysis Rate Constants for MTP and Related Compounds

compd	$k_{obs} \times 10^6$ (s^{-1})	T (°C)	I^a	ref
MTP^{4-}	5.37×10^1	25	0.1	this work
$HMTP^{3-}$	2.32×10^4	25		
$HMTP^{3-}$	1.72×10^3	5		
H_2MTP^{2-}	3.22×10^4	5		
$H_2PP_i^{2-}$	$5.6 \times 10^{-4\,b}$	5	0.15	17
PP_i (pH 4)	$4.1 \times 10^{-2\,b}$	30	0.65	18
PP_i (pH 7)	$1.4 \times 10^{-3\,b}$	25		
PP_i (pH 10)	$1.9 \times 10^{-6\,b}$	30		
PP_i (pH 13)	1.4	125		
$H_2PP_i^{2-}$	9.25	70	0.15	17
$H_2PP_i^{2-}$	3.1	60	0.44	19
$H_2PP_i^{2-}$	4.7	65.5		20
HPP_i^{3-}	1.2	70	0.15	17
$HPPi^{3-}$	1.0×10^{-1}	60	0.44	19
HPP_i^{3-}	8.8×10^{-1}	65.5		20
PP_i^{4-}	0	70	0.1	17
PP_i^{4-}	$7.8 \times 10^{-3\,c}$	65.5		20
$MeOPO_3H^-$	$2.5 \times 10^{-4\,b}$	25		21
n-$BuSPO_3H^-$	2.73×10^2	25.2	1.0	
p-$O_2NPhSPO_3^{2-}$	1.24×10^3	25	1.0	22

[a] I = ionic strength. [b] k_{obs} calculated from data at other temperatures using the Arrhenius equation. [c] Extrapolated value.

Table III. Effect of Solvent Polarity on the Hydrolysis of MTP

I^a	k_{obs} (min^{-1})	% dioxane (v/v)^b	k_{obs} (min^{-1})^c
0.014	1.09	0	0.94 ± 0.05
0.10	0.98	10	1.22 ± 0.02
0.20	0.97	19.7	1.27 ± 0.05
0.40	0.88	29.7	1.70 ± 0.03

[a] pH 7.0 and 25 °C. [b] pH 7.4 and 25 °C. [c] Mean value from three runs ±SD.

of 13 and 14. The data in Figure 1 between pH 12 and pH 13 could not by themselves support the conclusion that the rate is pH-independent in this range, and rate constants at higher pH values could not be obtained while maintaining the ionic strength at 0.1. The insensitivity of the hydrolysis rate between pH 13 and pH 14 was, therefore, verified in separate experiments in which the ionic strength was maintained at 1.0. With K^+ as the counterion at 25 °C, the hydrolytic rate constants were found to be 6.8×10^{-3} min^{-1} at pH 13.0, 8.0×10^{-3} min^{-1} at pH 13.5, and 10.3×10^{-3} min^{-1} at pH 14.0. With Li^+ as the counterion at 25 °C, the rate constants were found to be 0.25×10^{-3} min^{-1} at pH 13.0 and 0.28×10^{-3} min^{-1} at pH 13.5. Thus, the pH-rate profile in Figure 1 levels out at pH values above 13. The rates with 1 M Li^+ and 1 M K^+ differ because Li^+ is inhibitory at high concentrations.[6]

The rate constants we determined are 9–25 times larger than those reported by Loewus and Eckstein between pH 8 and pH 10.[6] The difference is probably due to the presence of excess lithium ions in the earlier experiments. Loewus and Eckstein reported a 4.6-fold increase in rate when the concentration of Li^+ was decreased from 315 to 36 mM;[6] the inhibitory effect of Li^+ at high concentrations has been confirmed in this laboratory (data not shown).

Effects of Medium Polarity on the Hydrolysis of MTP. The effects of tetramethylammonium chloride and dioxane on the hydrolysis of MTP are given in Table III. Tetramethylammonium chloride at concentrations up to 0.4 M has a very slight inhibitory effect on the rate at pH 7. Thus, complexation by tetramethylammonium ions, which almost certainly occurs under our conditions,[23] and the ionic strength have little effect. An alternative interpretation is that the effects of complexation and solvent polarity are compensatory in the case of the tetramethylammonium ion. Overall, the data in Table III do not indicate a substantive effect of ionic strength on the rate of MTP hydrolysis.

The hydrolytic rate constants for MTP in 25 mM MOPS buffer at pH 7.4 at various concentrations of dioxane are also given in Table III. Dioxane significantly enhances the rate at which MTP undergoes hydrolysis at pH 7.4.

Table IV. Rate Constants for the Hydrolysis of Me_4MTP and Related Compounds

compd	$k_{obs} \times 10^5$ (s^{-1})	T (°C)	ref
Me_4MTP	2020	25	this work
Me_4PP_i	42	25	24
μ-thio[*bis*(2,2-dimethyltrimethylene)]-thiopyrophosphate^a	103^b	80	25
μ-oxo[*bis*(2,2-dimethyltrimethylene)]-thiopyrophosphate^a	0.52^b	80	25

[a] *Bis*(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanyl) sulfide and its isomer with the sulfur nonbridging. [b] 0.2 mole fraction dioxane

Table V. Activation Parameters for the Hydrolysis of MTP and Related Compounds^a

compd	$\Delta H^\ddagger$ ($kcal \cdot mol^{-1}$)	$\Delta S^\ddagger$ ($cal \cdot deg^{-1} \cdot mol^{-1}$)	ref
$HMTP^{3-}$ (pH 7.0)	20	+0.2	this work
MTP^{4-} (pH 10.0)	21	−9.6	6
$H_2PP_i^{2-}$	28	−1.6	17
PP_i (pH 4.0)	27	−5	18
PP_i (pH 7.0)	29	−1	
PP_i (pH 10)	39	+18	
n-$BuSPO_3H^-$	23	+3.1	21
Me_4MTP	13	−22.7	this work
Et_4PP_i	10	−47.3	17

[a] Activation parameters are reported to two significant figures, with an error of ±5%.

Hydrolysis of Me_4MTP. The hydrolysis of Me_4MTP at pH 6 produces dimethyl phosphorothionate and dimethyl phosphate, as indicated by the ^{31}P NMR chemical shifts and coupling constants of the hydrolysis products (data not shown). These products exhibit a chromophore (probably dimethyl phosphorothionate) with a λ_{max} at 207 nm and a molar extinction coefficient of about 6000. The background absorbance is minimized by monitoring the progress of the hydrolysis at 218 nm.

Me_4MTP undergoes hydrolysis at pH 7.0 in 10 mM MOPS (I = 0.1 with KCl) in a first-order process, the rate of which is independent of buffer concentration below 0.1 M. The rate at pH 7.0 is nearly identical to that at pH 6.0 in 20 mM MES. The rate constant is compared in Table IV with those for the hydrolysis of tetramethyl pyrophosphate (Me_4PP_i) and other related compounds reported in the literature. The rate for Me_4MTP is about 48 times that for Me_4PP_i.

Activation Parameters for Hydrolysis of MTP and Me_4MTP. The activation parameters for the hydrolysis of MTP and Me_4MTP at pH 7.0 (25 mM MOPS (I = 0.1 M with KCl) are given in Table V, along with values for related compounds. The value of $\Delta S^\ddagger$ for MTP^{3-} is in the range of values reported for PP_i^{3-}, alkyl phosphate, and alkyl phosphorothiolate monoanions. The values of $\Delta H^\ddagger$ for MTP^{3-} are much smaller than those for PP_i and alkyl phosphates, and similar to that for n-butyl phosphorothiolate. The P–S bond is weaker than the P–O bond,[26] and the bond strengh presumably is expressed in the enthalpy of activation rather than the entropy of activation. The lower values of $\Delta H^\ddagger$ for P–S cleavage compared with P–O cleavage indicates that bond cleavage is important in the transition state. The entropy of activation is also typical of a dissociative transition state, in which the bond to the leaving group is largely cleaved and there is little bonding to an entering nucleophile.

For the hydrolysis of Me_4MTP at pH 7.0, $\Delta H^\ddagger$ is smaller than that for MTP, and $\Delta S^\ddagger$ is large and negative. These values are typical of associative reaction mechanisms, in which bond cleavage to the leaving group is not so advanced in the transition state and bond formation to the entering nucleophile is well advanced (Benkovic and Schray, 1973).

Discussion

Acid Dissociation Constants for MTP. The pK_a value of 8.49 for MTP, which was determined kinetically from the pH-rate profile for hydrolysis, is assigned to the dissociation of a proton from the trianion (K_4) on the basis of its similarity to the fourth pK_a of PP$_i$. The effect of ionic strength on pK_3 and especially pK_4 of PP$_i$ strongly depends on the nature of the salt chosen to hold ionic strength,[15] and the correlation between decreasing pK_a and decreasing unhydrated size of the cation results from increasing complexation.[27,28] The value of pK_4 for PP$_i$ of 8.40[16] offers the most pertinent comparison with MTP, because both were determined in the presence of 0.1 M potassium.

The values of pK_3 for MTP and PP$_i$ in Table I were determined at different temperatures, 5 and 25 °C, respectively; however, the difference probably cannot be attributed to temperature dependence because the values for PP$_i$ change very little with temperature.[14] Moreover, the difference in the values of pK_2 for *n*-butyl phosphate and *S*-*n*-butyl phosphorothiolate is similar to that between pK_3 for MTP and PP$_i$. These differences are probably due to electronic factors in the structures. One might expect the oxy compounds to be the stronger acids on the basis of electronegativity considerations; however, the reverse is the case. Sulfur does not engage in π-back-bonding to phosphorus to the degree that oxygen does;[29] this effect has been suggested to explain the acid strengthening effect of sulfur in phosphorothioate anions.[30]

Effects of Bridging S on the Hydrolysis of Phosphoanhydrides. The rate constants for all ionic species of MTP are many orders of magnitude larger than those for the comparable species of PP$_i$. The calculated rate constant for the hydrolysis of PP$_i$ at pH 7 and 25 °C is 1.4×10^{-9} s^{-1} (Table II) while that for MTP is 2.37×10^{-2} s^{-1} at 25.0 °C and pH 7. Thus, MTP is 17-million-fold more reactive than PP$_i$ at pH 7. If the rate constant for MTP at pH 7 is compared with those reported for the trianion of PP$_i$ between 60 and 70 °C (Table II), the ratios fall between 1 and 10 million. The dianion of MTP is 58 million times more reactive than that of PP$_i$ (Table II). By analogy to PP$_i$, we prefer a mechanism of hydrolysis in which the minor tautomer of the dianion of MTP, that with both protons on one phosphoryl group, is the most reactive species.

The tetraanion of MTP reacts at an appreciable rate, whereas the hydrolysis of PP$_i$ tetraanion is difficult to observe. McGilvery and Crowther reported rate constants at 65.5 °C for the trianion and tetraanion of PP$_i$, which they reported to differ by 110-fold[20] (Table II). The corresponding rate ratio for MTP^{3-}/MTP^{4-} at 25 °C is 430. If these ratios hold for PP$_i$ at 25 °C, the rate constant for PP$_i^{4-}$ is between 0.33×10^{-11} and 1.3×10^{-11} s^{-1}. On this basis, MTP^{4-} reacts between 6×10^6 and 2.4×10^7 times faster than PP$_i^{4-}$.

The increased reactivity of MTP relative to PP$_i$ is greater than might be expected on the basis of the chemistry of alkyl phosphates and alkyl phosphorothiolates (Table II). *S*-*n*-butyl phosphorothiolate is about 1-million-fold more reactive than methyl phosphate.[21] With the *p*-nitrophenyl moiety in place of an alkyl group, the substitution of sulfur for oxygen in the bridging position leads to rate increases from 300 for the monoanion to 340 000 for the dianion.[31,22] It is interesting to compare the hydrolysis of MTP with that of *p*-nitrophenyl phosphorothiolate. The dianion of the latter undergoes hydrolysis at 25 °C with a rate constant of 1.24×10^{-3} s^{-1} [22] compared with 2.32×10^{-2} s^{-1} for the trianion of MTP (Table II). Yet the pK_a of 4-nitrothiophenol is 4.43 compared with 5.4 for $H_2OPSO_2^-$.[30] Thus, the MTP trianion reacts 18 times faster than *p*-nitrophenyl phosphorothiolate despite having a leaving group with a pK_a value one unit higher for the conjugate acid.

The entropy of activation for MTP hydrolysis at pH 7 is similar to the values for PP$_i$ at pH 7, whereas the enthalpy of activation is much smaller. The comparison between MTP and PP$_i$ is similar to that between the monoanions of *n*-butyl phosphorothiolate and methyl phosphate (Table V). The greater reactivity of butyl phosphorothiolate is due almost entirely to its lower activation enthalpy; the entropies of activation are similar.

The Hydrolytic Mechanism for MTP Anions. All evidence indicates that bond cleavage between P and the leaving group is important in the transition states for hydrolysis of dianionic and trianionic MTP and PP$_i$. (1) The rate of MTP hydrolysis is more than 10^7 times faster than the hydrolysis of PP$_i$, and the value of $\Delta H^{\ddagger}$ for MTP is much smaller than that for PP$_i$. These facts are most easily explained on the basis that the P–S bond is much weaker (about 50 kcal/mol[26]) than the P–O bond, and this weakness is partially expressed in the rate to the extent that P–S bond cleavage is important in the transition state. The full difference in bond energy is not expressed because the mechanism is polar, whereas the bond energies refer to homolytic cleavages. (2) The entropies of activation for the di- and trianions of MTP and PP$_i$ are near zero, which is generally regarded as being consistent with a unimolecular decomposition. In a solvolysis reaction this can also be consistent with a preassociation bimolecular process with a loose transition state.[32] (3) The enthalpies of activation for the hydrolysis of MTP and *S*-alkyl phosphorothiolates are smaller than those for PP$_i$ and the corresponding alkyl phosphates. This is consistent with the weakness of the P–S bond compared with the P–O bond and the importance of the cleavage of this bond in the transition state. (4) There is no evidence of proton transfer in the transition state for MTP hydrolysis because of the absence of buffer catalysis and the absence of a kinetic isotope effect in D_2O.

On the evidence of the present findings, and by analogy with other phosphoryl group transfers, the mechanism of MTP hydrolysis probably proceeds through one of the mechanisms in Scheme I. In both mechanisms the transition state is dissociative; that is, there is little bonding to the reacting P by the leaving group and attacking water. In mechanism 1 a molecule of water associated with solvated MTP in a preassociation complex partially bonds to the metaphosphate-like transition state at the same time that the P–S bond is breaking. However, there is little bonding between P and either the leaving group or the attacking water in the transition state. In mechanism 2 the preassociated water does not interact covalently with the incipient metaphosphate in the transition state, but it immediately captures the discrete metaphosphate within the solvation sphere. The dianion probably undergoes hydrolysis through an analogous mechanism, most likely with both protons associated with the leaving group. The tetraanion probably also undergoes hydrolysis by a dissociative mechanism that may involve a slightly smaller degree of bond cleavage to the leaving group than the reactions of the di- and trianions. It is possible that MTP undergoes hydrolysis by mechanism 2 and PP$_i$ by mechanism 1, but our data do not bear on this question.

Other phosphoryl group transfer reactions proceed through dissociative transition states analogous to those in Scheme I. Solvolyses of alkyl and aryl phosphate monoanions and analogous dianions with very good leaving groups exhibit entropies of ac-

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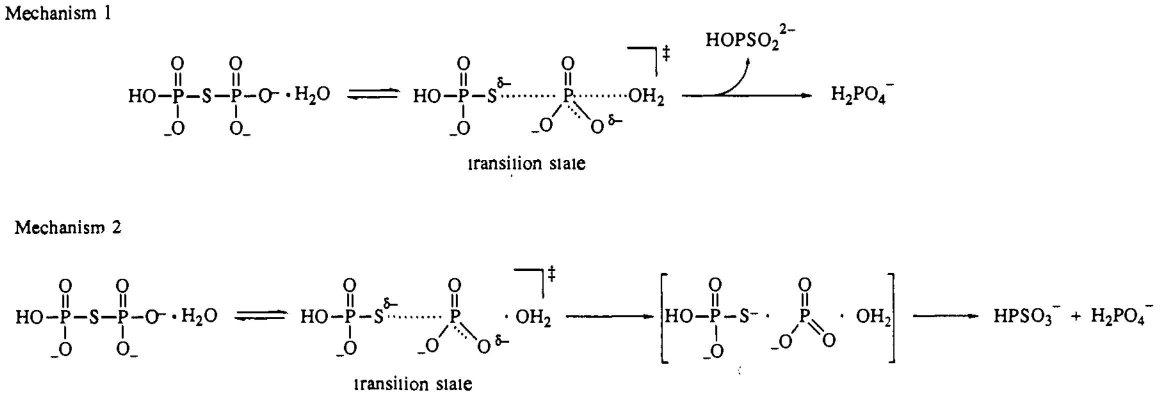
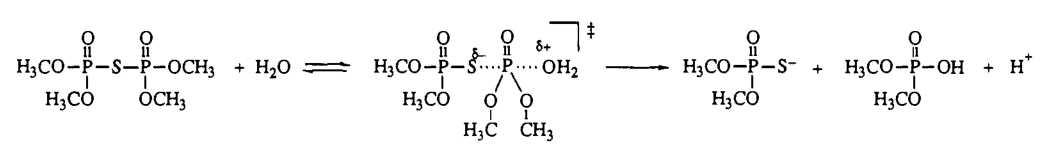
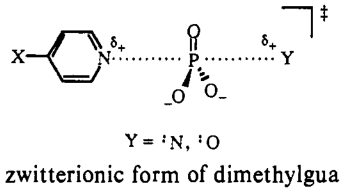
Scheme I



Scheme II



tivation near zero and little or no kinetic isotope effects in D_2O, as does MTP. Electronic effects in structure-reactivity correlations show that the rates are relatively insensitive to electronic effects in the leaving groups, which suggests that there is little bonding between P and the leaving group in the transition state.[4] The methanolysis of aryl [(R)-^{16}O,^{17}O,^{18}O]phosphates proceeds with inversion of configuration at P;[33] therefore, either there must be a degree of bonding between P and the attacking methanol in the transition state, analogous to mechanism 1 of Scheme I, or a discrete [PO_3^-] must be so short-lived that it cannot escape the solvation sphere, analogous to mechanism 2 of Scheme I. Alcoholysis of P-chiral alkyl phosphates and phosphoanydrides in poorly solvating organic media can proceed with racemization at P.[34,35] These findings suggest but do not prove that discrete and diffusible [PO_3^-] might be an intermediate under special conditions. However, the transfer of the phosphoryl group from substituted pyridinium phosphates to nitrogen and oxygen nucleophiles in aqueous solutions proceeds through dissociative transition states, in which significant bonding exists between P and both the leaving group and the attacking nucleophile, and there is no discrete intermediate [PO_3^-].[3,32,36,37] The transition state for the latter reactions is as illustrated below.



The neutral zwitterionic form of dimethylguanidinium phosphate is also highly reactive in solvolysis relative to most phosphoryl compounds and undergoes hydrolysis with a rate constant of 0.0858 min^{-1} at 37 °C.[38] Solvolysis of this compound in 1:1 mixtures of methanol/water or ethanol/water proceeds with equal partitioning of the phosphoryl group between water and alcohol. This is consistent with a discrete metaphosphate being transiently formed and randomly captured by water or alcohol. Haake and Allen propose that dimethylguanidinium phosphate may undergo hydrolysis through a metaphosphate intermediate (mechanism 2, Scheme I), whereas less reactive phosphates react with nucleophilic participation (mechanism 1, Scheme I). In comparison, MTP trianion undergoes hydrolysis at 25 °C with a rate constant of 1.39 min^{-1}, or about 100 times the rate for dimethylguanidinium phosphate when the temperature difference is taken into account. Thus, if dimethylguanidinium phosphate is near the borderline between mechanisms 1 and 2 in Scheme I, MTP should be well on the side of mechanism 2.

Effects of Solvent Polarity on the Hydrolysis of MTP. The modest inhibitory effect of ionic strength on the hydrolysis of MTP, and the rate enhancement brought about by increasing dioxane in H_2O/dioxane mixtures, may be interpreted as supporting a dispersal of charge in the transition state. The transition state of a unimolecular decomposition of a charged species is larger than the ground state; therefore, the charge will be dispersed in the transition state.[39] A transition state with dispersed charge will be stabilized by less polar solvents and destabilized by more polar solvents.

The solvent effects are complex, however, and medium polarity may influence observed rates for other reasons. Thus, it is possible that the effects of ionic strength and complexation by tetramethylammonium ion in the experiments of Table III are algebraically additive and lead to the observed slight inhibition. Moreover, one must consider other mechanisms by which dioxane may increase the observed rate. The pK_a values of MTP should be increased in solvents of decreasing polarity, so that an increased fraction exists in the dianionic form in dioxane/water mixtures at a "measured pH" relative to pure water, with no change in the rate constants for the species themselves. The second pK_a of potassium phosphate is increased by about 0.6 unit in the presence of 25% (v/v) dioxane.[7] If we assume that the magnitude of the perturbation is the same on the third dissociation constant for MTP, and that the rate constant for dianion hydrolysis is 25 min^{-1} at 25 °C, we can calculate a rate constant of 1.8 min^{-1} at pH 7. This is very near the value in Table III for 29.7% dioxane at pH 7.4. The perturbation of the pK_a of ADPβS by organic solvents is also the dominant factor enhancing the reactivity of its tributylammonium salt in organic solvents.[40] It appears that medium effects are not very helpful for characterizing the transition state in the case of MTP.

In the hydrolysis of *S-n*-butyl phosphorothiolate, the addition of 10% (v/v) dioxane has little effect on the rate of hydrolysis of the monoanion, but it increases the rate at pH 8 by 1.7-fold.[16] At pH 8 the rate is due mainly to hydrolysis of the monoanion. This solvent effect is significantly greater than that for MTP in Table III, and perturbation in the pK_a probably can account for

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part but not all of the rate enhancement. The rate of hydrolysis of the monoanion of *S*-(4-nitrophenyl) phosphorothiolate is retarded by organic solvents, whereas the rate for the dianion is increased. The latter observation is consistent with solvent stabilization of a transition state with dispersed charge and not with perturbation of the pK_a. This is because the rate for the dianion is faster than that for the monoanion in the case of *S*-(4-nitrophenyl) phosphorothiolate;[22] therefore, a perturbation of pK_a would lead to a decreased rate rather than the observed increase.

Hydrolysis of Me$_4$MTP. The rate enhancement induced by bridging S in Me$_4$MTP, relative to bridging O in Me$_4$PP$_i$, is profoundly smaller than the enhancements in the phosphoanhydride and phosphoryl ester anions (Table IV). The hydrolytic reactivity of Me$_4$MTP is only 48-fold greater than that of Me$_4$PP$_i$. Moreover, the activation parameters for the hydrolysis of the tetramethyl esters are very different from those for MTP and PP$_i$, respectively. The entropies of activation for both Me$_4$MTP and Me$_4$PP$_i$ are large and negative, and the enthalpies of activation are much smaller than those for MTP and PP$_i$. The values of $\Delta S^\ddagger$ for Me$_4$MTP and Et$_4$PP$_i$ are consistent with bimolecular processes, and the values of $\Delta H^\ddagger$ are consistent with there being much less bond cleavage to the leaving groups in the transition state than in the reactions of MTP and PP$_i$. The nucleophile in the hydrolysis of Me$_4$MTP appears to be water and not hydroxide because the rate is insensitive to pH between pH 6 and pH 7.

The difference of 48-fold between Me$_4$MTP and Me$_4$PP$_i$ is 4-fold less than that between bis(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanyl) sulfide and its isomer, in which the sulfur atom is nonbridging (Table IV). These latter compounds are MTP and μ-oxo-thiopyrophosphate esterified with 2 equiv of 2,2-dimethyl-1,3-propanediol. One would expect that the ester of μ-oxo-thiopyrophosphate would be hydrolyzed at a rate similar to that of the same ester of pyrophosphate, on the basis of the similar reactivities of tetraethyl pyrophosphate and tetraethyl μ-oxo-thiopyrophosphate, in which the sulfur does not bridge.[25]

The tetraalkyl esters of MTP and PP$_i$ appear to react with water by an associative mechanism, in which there is substantial bonding between the reacting P and both the leaving group and attacking water in the transition state (Scheme II). An associative transition state is one in which the sum of bond orders between P and the attacking and leaving groups is the same as or greater than in the ground state.[41] Bonding between phosphorus and the leaving group is much stronger than in a dissociative transition state. This mechanism is consistent with the activation parameters, and it explains why Me$_4$MTP reacts only 48-fold faster than Me$_4$PP$_i$ despite the weakness of the P–S bond. In contrast to the reaction of MTP compared with PP$_i$, the P–S bond weakness is only slightly expressed in the rate because cleavage of the bond to the leaving group is not well advanced in the transition state for the hydrolysis of the tetramethyl esters.

The activation entropy is much less negative for Me$_4$MTP than for Et$_4$PP$_i$, although still negative enough to be typical of associative character for the transition state.[42] Moreover, the enthalpy of activation for Et$_4$PP$_i$ is smaller than that for Me$_4$MTP. These differences suggest that the transition state for the reaction of Et$_4$PP$_i$ may be more associative than that for Me$_4$MTP.

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Registry No. MTP, 136862-87-8; Me$_4$-MTP, 71861-22-8.

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Surface-Mediated Cycloaddition: 1,4-Addition of Atomically Adsorbed Oxygen to 1,3-Butadiene on Ag(110)

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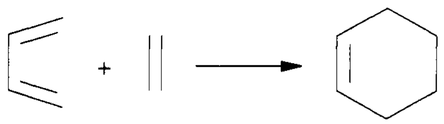
Abstract: The reaction of 1,3-butadiene with atomically adsorbed oxygen on Ag(110) has been studied with use of temperature-programmed reaction mass spectrometry. Oxygen adds to C$_1$ and C$_4$ of 1,3-butadiene via a reaction that is formally a chelotropic 1,4-cycloaddition. 2,5-Dihydrofuran and furan, the cycloaddition products, evolve into the gas phase at 465 K. Other reaction products are water, carbon dioxide, 2(5*H*)-furanone, maleic anhydride, 4-vinylcyclohexene, and styrene. The reaction of oxygen and 1,3-butadiene on Ag(110) resembles a number of other reactions in which simultaneous bond making and breaking occur on the pathway leading from reactants to products. Among these reactions are the epoxidation of norbornene and styrene on silver, the Diels–Alder dimerization of 1,3-butadiene on clean Ag(110), and the trimerization of acetylene to benzene on Pd(111) and Cu(110). We propose (i) that these reactions are all formally related, (ii) that they may also be mechanistically related, and (iii) that they be classified as surface-mediated cycloaddition processes.

The word cycloaddition refers to an extremely broad family of chemical reactions in which a cyclic molecule is formed via a reaction that involves the concurrent making and breaking of bonds in the reacting molecule(s). Cycloaddition reactions can be concerted (that is, bond making and breaking occur simultaneously) or nonconcerted (bond making and breaking occur in a stepwise fashion). Perhaps the best known cycloaddition process is the Diels–Alder reaction, exemplified by the addition of ethylene to 1,3-butadiene to form cyclohexene (Scheme I). Cycloaddition is an extremely powerful synthetic tool because (i) bonds are made and broken at the same time, (ii) an extremely broad range of molecular types undergo cycloaddition, and (iii) cycloaddition processes can often be executed with an impressive degree of

Scheme I



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