served for $Pt^{IV}(TTP)Cl_2$ to the (π,d) CT state. The short lifetime is consistent with the fact that the compound does not emit,[2c] since the $^3T(\pi,\pi^*)$ appears to relax to the CT in $\leq$10 ps.

Similar spectral and kinetic results were obtained for both $Pt^{II}(TPP)$ and $Pt^{IV}(TTP)Cl_2$ following excitation with 532-nm flashes. The lack of excitation-wavelength dependence to the photophysics supports the view that the spectral and kinetic behavior we have reported are due to the lowest excited states of these two compounds.

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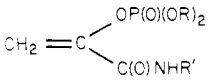
Registry No. $Pt^{II}(TPP)$, 14187-14-5; $Pt^{IV}(TPP)Cl_2$, 74194-58-4.

Phosphoenolpyruvamides. Amide–Phosphate Interactions in Analogues of Phosphoenolpyruvate

Ronald Kluger,* Jane Frances Chow, and James J. Croke

Contribution from the Lash Miller Laboratories, Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1. *Received November 8, 1983*

Abstract: Ethyl esters of nitrogen-substituted carboxamides of phosphoenolpyruvate (**1** and **2**) were obtained from the reactions

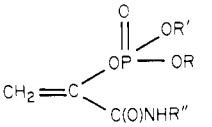


1, R = Et, R′ = *n*-Pr
2, R = Et; R′ = Ph

of triethyl phosphite with the corresponding nitrogen-substituted 3-bromopyruvamide. The hydrolysis of the ethyl ester portions of **1** and **2** occurs with an observed first-order rate constant that is 4 orders of magnitude larger than is estimated for triethyl phosphate under comparable conditions, indicating that participation by the neighboring carboxamide group is occurring. However, the enol phosphate ester substituent is cleaved much more slowly than are vinyl phosphate esters. The results are consistent with a mechanism in which the amide adds to the adjacent phosphate to form a reactive cyclic intermediate. The data support the proposal that amides can become phosphorylated during processes that involve interactions of peptides and nucleotides or during phosphate-transfer processes. The hydrolysis products of **1** and **2** may also be useful analogues of phosphoenolpyruvate in studies of enzyme mechanisms and in the design of inhibitors.

The covalent interaction of amide and phosphate functional groups has potential importance in many biochemical processes. For example, enzymic phosphate transfer may involve intermediate formation of a phosphorylated amide.[1,2] In the association complex of a nucleic acid or a phosphate ester with a protein,[3] the possibility that peptide functions can add to phosphodiester bonds is made favorable by the decreased entropic barrier provided by the macromolecular association. The chemical mechanism of such an interaction can be studied by combining the groups of interest into a single molecule.[4-7]

We have shown previously that esters of phenylphosphonic acid with an amide in the ortho position of the benzene ring undergo hydrolysis by a mechanism involving addition of the amide to the phosphonate center.[4,5] Although phosphonates share many reaction patterns with phosphates,[8,9] there are very significant differences in their interactions with enzymes.[10,11] Therefore, we sought a system that would permit the phosphorus reaction site to be a phosphate group. We also required that the amide and phosphate be in reactive proximity. Esters of derivatives of the carboxamide of phosphoenolpyruvate meet these requirements (structures **1** and **2**).



1, R, R′ = Et; R″ = *n*-Pr
2, R′, R = Et; R″ = Ph
3, R = H; R′ = Et; R″ = *n*-Pr
4, R, R′ = H; R″ = *n*-Pr

It has been shown that esters of phosphoenolpyruvate are highly reactive in hydrolysis reactions due to the participation of the carboxyl group at the phosphate ester center.[12] Amide analogues of these materials should allow facile interaction between the functional groups as well as being potentially useful for enzyme studies.[13] In this paper we report the synthesis of substituted

(1) Buchanan, J. *Adv. Enzymol.* **1973**, *39*, 91.
(2) Griffith, O. W.; Meister, A. *J. Biol. Chem.* **1981**, *256*, 9981.
(3) Schnarr, M.; Durand, M.; Maurizot, J. *Biochemistry* **1983**, *22*, 3563.
(4) Kluger, R.; Chan, J. L.-W. *J. Am. Chem. Soc.* **1973**, *95*, 2362.
(5) Kluger, R.; Chan, J. L.-W. *J. Am. Chem. Soc.* **1976**, *98*, 4913.
(6) Kluger, R.; Davis, P. P. *J. Am. Chem. Soc.* **1979**, *101*, 5995.
(7) Kluger, R.; Lam, J. C.-H. *Can. J. Chem.* **1977**, *55*, 640.
(8) Hudson, R. F.; Keay, L. *J. Chem. Soc.* **1956**, 2463.
(9) Cadogan, J. I. G.; Eastlick, D.; Hampson, F.; Mackie, R. K. *J. Chem. Soc. B* **1969**, *144*.
(10) Lazarus, R. A.; Benkovic, P. A.; Benkovic, S. J. *Arch. Biochem. Biophys.* **1979**, *197*, 218.
(11) Yount, R. *Adv. Enzymol.* **1975**, *43*, 3.
(12) Clark, V. M.; Kirby, A. J. *J. Am. Chem. Soc.* **1963**, *85*, 3705.

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phosphoenolpyruvamides and the elucidation of their reactivity patterns.

Experimental Section

Spectra. Proton NMR spectra were measured on a Varian T-60 instrument using chloroform-*d* ($CDCl_3$) or deuterated dimethyl sulfoxide with tetramethylsilane as an internal standard. Spectra for kinetics were done in solutions of deuterium oxide and acetone-d_6 (1:1 by volume) with deuterium chloride. Phosphorus NMR spectra were carried out on a Bruker WP-80 instrument. Infrared spectra were measured on a Perkin-Elmer 337 Grating Infrared Spectrophotometer. Melting points are uncorrected. Deuterated solvents were purchased from Merck, Sharp & Dohme or from the Aldrich Chemical Co. Mass spectra were run on an AEI MS-30 high-resolution instrument by Carmencita Sordan.

Synthesis. Diacetyltartaric Anhydride.[14] Tartaric acid (100 g, 0.66 mol) was dissolved in a mixture of acetic anhydride (220 mL, 2.33 mol) and concentrated sulfuric acid (3 mL). This addition resulted in an exothermic reaction. When the initial reaction had subsided, the mixture was heated under reflux for 10 min to bring the reaction to completion. Crystals appeared as the solution was cooled to room temperature. Filtration of the mixture followed by washing with benzene gave the product as a white crystalline solid: (108 g, 75%) mp 135 °C; proton NMR ($CDCl_3$), 2.21 (6 H, s, CH_3CH_3), 5.70 (2 H, s, CHCH).

Pyridine Salt of Hydroxymaleic Anhydride.[15] Diacetyltartaric anhydride (30 g, 0.1 mol) was dissolved in 60 mL of dry acetone. The resulting solution was cooled in an ice bath to 10 °C, and 22.5 mL of dry pyridine was added. After 5 min the color of the mixture turned light green. Then 18 mL of glacial acetic acid was added. The precipitate, which formed as the flask was kept in a freezer (−10 °C) for 12 h, was collected by suction filtration and washed with anhydrous ether (3 × 10 mL) and dry, cold acetone (2 × 25 mL). The desired product is a pale brown solid: (10 g, 40%) mp 108–110 °C; proton NMR (deuterated dimethyl sulfoxide), 4.90 (H, s, OH), 7.32–8.78 (7 H, m, C_5H_5, NH, CH).

Pyruvanilide.[14] Freshly distilled aniline (6 mL, 0.06 mol) was added to the pyridine salt of hydroxymaleic anhydride (6 g, 0.03 mol). A vigorous reaction took place with the evolution of carbon dioxide. When the bubbling had ceased, hydrochloric acid (15%, 15 mL) was added to the stirred mixture leading to precipitation of the product as a yellow solid. The solid was collected by suction filtration and recrystallized twice from water. The compound is a yellow solid: (0.87 g, 33%) mp 104 °C; proton NMR ($CDCl_3$) 2.54 (3 H, s, CH_3), 7.20–7.77 (5 H, m, C_6H_5), 8.45 (H, s br, NH).

3-Bromopyruvanilide. Pyruvanilide (500 mg, 0.003 mol) was added to 15 mL of carbon tetrachloride. After 15 min the solid dissolved. The temperature of the reaction vessel was brought to 50 °C. A mixture of bromine (0.08 mL, 0.003 mol) and glacial acetic acid (1.04 mL) was added dropwise to the stirred solution. After 6 h, the product precipitated. The solid was collected by suction filtration, redissolved in chloroform, and recrystallized by the addition of hexane to the chloroform solution. The product is a pale white solid: mp 139 °C (60%); proton NMR ($CDCl_3$) 4.52 (2 H, s, CH_2), 7.10–7.78 (5 H, m, C_6H_5), 8.55 (H, s br, NH).

Diethyl *N*-Phenylphosphoenolpyruvamide. A mixture of triethyl phosphite (0.85 mL, 0.004 mol) and dry ethanol (1.18 mL) was added to 3-bromopyruvanilide (1 g, 0.004 mol). An exothermic reaction took place. The solution was stirred overnight. Removal of the solvent under reduced pressure left a viscous oil. The oil was chromatographed on a column of silica gel (Mesh 200, 40 × 1.5 cm) using hexane/acetone (2:1) as eluant to give the product as a viscous oil. Proton NMR ($CDCl_3$) 1.20 (6 H, t, J = 6 Hz, CH_3), 4.30 (4 H, q, J = 7 Hz, CH_2), 5.50 (H, t, J = 2 Hz, CH), 6.09 (H, t, J = 2 Hz, CH), 7.00–7.80 (5 H, m, C_6H_5); exact mass of parent peak in high-resolution MS, m/e calcd for $C_{13}H_{18}NO_5P$ 299.0922, found: 299.0922.

***N*-Propylactamide.** Ethyl lactate (114 g, 1 mol, prepared by Fischer esterification of lactic acid) was added to *n*-propylamine (108 g, 2 mol) and the solution stirred at room temperature in a stoppered flask for 3 days. Ethanol and excess amine were removed under reduced pressure, leaving a yellow liquid: IR (Neat) 1710 cm^{-1} (C=O), 1550 cm^{-1} (C—N); proton NMR ($CDCl_3$) 0.85 (3 H, t, J = 8 Hz), 1.38 (2 H, d, J = 8 Hz), 1.17–1.84 (2 H, m), 3.35 (2 H, t br, J = 6 Hz, CH_2), 4.17 (1 H, q, J = 7 Hz, CH(OH)), 5.18 (H, s br, OH), 7.17 (H, s br, NH).

***N*-Propylpyruvamide.** *N*-Propyllactamide (2 g, 0.015 mol) was dissolved in acetone (10 mL). A solution of 8.4 mL of Jones reagent (70 g CrO_3/61 mL of concentrated H_2SO_4/300 mL of H_2O) was added dropwise to this solution with the temperature of the reaction maintained below 20 °C. The resulting mixture was stirred at room temperature for 3 h. Sodium bisulfite (0.5 g) was added to remove excess Jones reagent. The solution was extracted with ether, and the combined extracts were washed with sodium bicarbonate solution. The ether solution was then washed with saturated sodium chloride solution and dried over magnesium sulfate. Removal of solvent under reduced pressure gave the product as a yellow viscous liquid (90%): IR (neat) 1725, 1680, 1530 cm^{-1}; proton NMR ($CDCl_3$) 0.92 (3 H, t, J = 7 Hz, CH_3), 1.28–1.94 (2 H, m, CH_2), 2.44 (3 H, s, CH_3), 3.26 (2 H, q of d, J = 7 Hz, CH_2), 7.49 (H, s br, NH).

***N*-Propyl-3-bromopyruvamide.** *N*-Propylpyruvamide (1 g, 0.007 mol) in a 100-mL round-bottom flask was heated to 50 °C in an oil bath. The bath was removed and bromine (1.24 g, 0.007 mol) in carbon tetrachloride (1.5 mL) was added dropwise. After the addition was complete, the solution was left stirring at room temperature for 10 min. The solution became cloudly and viscous. Cyclohexene was added to remove unreacted bromine. Washing with carbon tetrachloride and recrystallizing from hexane afforded the required product as a pale white solid (50%): mp 54–56 °C; proton NMR ($CDCl_3$) 1.00 (3 H, t, J = 7 Hz, CH_3), 1.20–1.90 (2 H, m, CH_2), 3.18–3.57 (2 H, q of d, J = 7 Hz, CH_2), 4.50 (2 H, s, CH_2), 6.90 (H, s br, NH).

Diethyl *N*-Propylphosphoenolpyruvamide. Treatment of *N*-propyl-3-bromopyruvamide (0.53 g, 0.002 mol) in absolute ethanol (2 mL) with triethyl phosphite (0.44 mL, 0.002 mol) resulted in an exothermic reaction. After stirring overnight, volatiles were removed under reduced pressure, leaving a viscous oil. The oil was chromatographed on a column of silica gel (mesh 200, 40 × 1.5 cm) using ether/hexane (2:1) as a solvent. The product is a yellow/green oil. Proton NMR ($CDCl_3$) 1.00 (3 H, t, J = 7 Hz, CH_3), 1.40 (6 H, t, J = 6 Hz, CH_3), 1.60 (2 H, m, CH_2), 3.18–3.57 (2 H, q of d, J = 7 Hz, CH_2), 4.20 (4 H, q, J = 7 Hz, CH_2), 5.4 (H, t, J = 1 Hz, CH), 6.00 (H, t, J = 1 Hz, CH), 6.80 (H, s br, NH); Exact mass of parent peak in high-resolution MS, calcd for $C_{10}H_{20}NO_5P$ 265.1079, found 265.1081.

Sodium Ethyl *N*-Propylphosphoenolpyruvamide. Sodium iodide (0.14 g) in 2 mL of butanone was added to diethyl *N*-propylphosphoenolpyruvamide (0.22 g). The solution was heated at reflux for 3 h. This was kept at 4 °C overnight. Trituration of the gummy precipitate with ether afforded the product as a hygroscopic brown solid: mp 80 °C; proton NMR ($CDCl_3$) 0.90 (3 H, t, J = 7 Hz, CH_3), 1.20 (3 H, t, J = 6 Hz, CH_3), 1.55 (2 H, m, CH_2), 3.20 (2H, t, J = 7 Hz, CH_2), 3.90 (2 H, q of d, J = 7 Hz, CH_2), 5.25 (H, t, J = 1 Hz, CH), 5.60 (H, t, J = 1 Hz, CH).

Dimethyl 1-Methylvinyl Phosphate. Bromoacetone (11.6 mL) and trimethyl phosphite (14 mL) were reacted in 70 mL of dry methanol. Distillation of the reaction solution produced the material as has been reported by Clark.[16] Proton NMR ($CDCl_3$) 3.80 (6 H, d, J = 12 Hz, OCH_3), 1.95 (3 H, s, CH_3) 4.54 (H, t, J = 1 Hz, CH), 4.74 (H, J = 1 Hz, =CH).

Kinetic Measurements. Hydrolysis of the esters of the enolpyruvamides was followed by proton NMR spectroscopy. All reactions were conducted by using tightly capped 5-mm NMR tubes. Under all conditions, it was observed that only the ethyl groups departed (and not the enol ester group). Samples were kept in a silicone oil bath maintained at 75 ± 2 °C with a Yellow Springs controller. The reaction was followed by comparing the integrated intensity of the signal of the methylene protons signal of ethanol with the integrated methylene proton absorbance of the ethyl groups of the substrate. The extent of reaction was obtained from the integral of the ethanol methylene peak over the sum of the integrals of the ester and ethanol methylene peaks. For the hydrolysis of diethyl *N*-propylphosphoenolpyruvamide, the integrated methyl signal of the propyl group was used as an internal standard for comparison against the integrated methylene signal of ethanol. The slope

Table I. Observed Rate Constants for the Hydrolysis of Diethyl Esters of *N*-*n*-Propylphosphoenolpyruvamide (**1**) and *N*-Phenylphosphoenolpyruvamide (**2**)[a]

[DCl], M	10^6k_{obsd}, s^{-1}		[DCl], M	10^6k_{obsd}, s^{-1}	
	1	**2**		**1**	**2**
0.05	4.90	3.35	0.90	41.1	19.2
0.10	7.54	6.88	1.00	43.0	22.7
0.30	16.00	7.55	1.14	48.1	26.3
0.42	19.3	8.75	1.50	90.6	59.2
0.66	23.9	15.0			

[a] At 50 °C, in deuterium oxide, acetone-d_6 (50:50 by vol) containing deuterium chloride.

(13) Stubbe, J. A.; Kenyon, G. L. *Biochemistry* **1972**, *11*, 338.
(14) Wohl, A.; Oesterlin, C. *Chem. Ber.* **1901**, *36*, 1144.
(15) van Dorp, D. A.; Arens, J. F. *Recl. Trav. Chim. Pays*-Bas **1948**, 67.
(16) Clark, V. M.; Kirby, A. J. *Tetrahedron* **1965**, *21*, 1969.

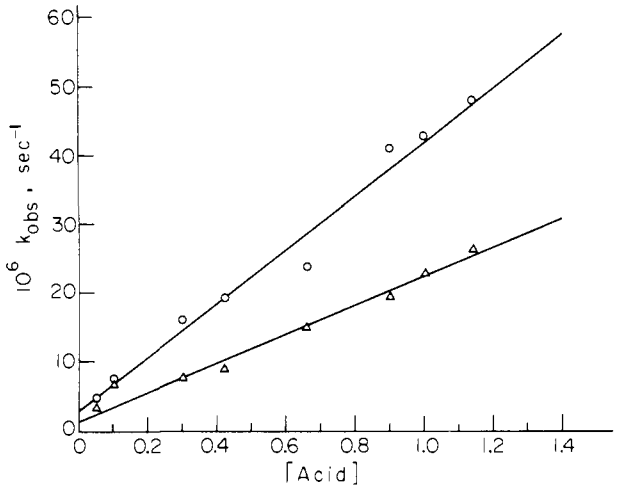


Figure 1. Observed rate constant for hydrolysis of **1** (○) and **2** (△) as described in the text.

of a plot of the extent of reaction against time was used to obtain observed rate constants. At least 10 points over four half-times were obtained for each sample. Since the half-life for the reaction can be measured most accurately from the integration procedure, the rate constants were also estimated by dividing the natural log of 2 by the half-time. The values of rate constants reported in Table I and Figure 1 are the average of the rate constants determined by the two procedures and of duplicate runs. Since the NMR method with the Varian T-60 spectrometer is subject to considerable error, rate constants are estimated to be have confidence limits of ±15%, based on the reproducibility of each measurement.

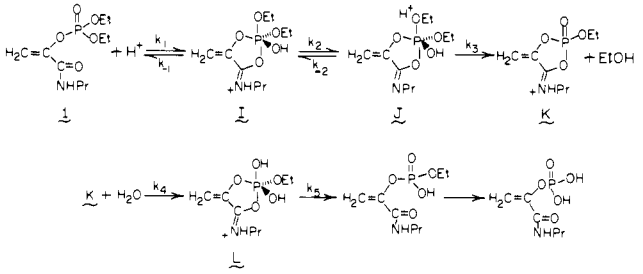
Results

Ethyl esters of nitrogen-substituted phosphoenolpyruvamides (**1–4**) result from the reaction of *N*-ethyl-3-bromopyruvamides (prepared by bromination of the corresponding pyruvamides) with triethyl phosphites, in analogy to reactions of other α-bromo ketones.[16–19] Owen has reported that N-alkylated pyruvamides can be prepared by oxidation of the corresponding lactamides with permanganate,[20] but the experimental details have not been published (although Professor Owen did generously provide us with additional information from a thesis). We were not successful in our attempts to use permanganate to accomplish the oxidation efficiently, but we found that oxidation of *N*-propyllactamide with chromic acid is effective. We also were able to prepare *N*-phenylpyruvamide by published procedures that are unique for this derivative.[14] Since those procedures may not be widely available, we have reported details in the Experimental Section.

The phosphate esters derived from phosphoenolpyruvamides undergo relatively rapid hydrolysis of their ester groups. Table I summarizes the rate constants we observed for the hydrolysis of the ethyl groups of diethyl *N*-propyl- and diethyl *N*-phenylphosphoenolpyruvamide (**1** and **2**) and the data are plotted in Figure 1. All reactions were measured by using solutions of deuterium chloride in 50:50 (v/v) acetone-d_6 and deuterium oxide[5] with substrate concentrations of 0.2 M. The hydrolysis of the monoethyl ester monosodium salt of *N*-propylphosphoenolpyruvamide (**2**) was examined at a single acidity (1 M deuterium chloride, in duplicate). The observed rate constant is 7.7×10^{-5} s^{-1}, which is almost twice that observed for the corresponding diester under the same conditions.

The observed rate at which the ethyl ester groups are hydrolyzed is several orders of magnitude faster than that of the corresponding esters that do not possess the enolpyruvamide function at phosphorus. The hydrolysis of triethyl phosphate in 0.3 M perchloric

Scheme I



acid (60% aqueous dioxane) occurs with a rate constant of 4.2×10^{-7} s^{-1} at 80 °C,[21] while the hydrolysis of the ethyl ester **1** has a rate constant of 1.6×10^{-5} at 50 °C. This corresponds to a rate factor of about 10^4 at a common temperature. Since we cannot observe a bimolecular reaction between an amide and phosphate, we cannot estimate an "effective concentration"[22] for the internal amide but the value must be very high.

The enol ester group of the phosphoenolpyruvamide derivatives is cleaved much more slowly than the enol group of unconjugated vinyl phosphates. The product of treatment of **1** and **2** with aqueous acid is the corresponding material with hydroxyl groups in place of the ethoxyl groups of the starting ester. This is not what is expected from direct attack of water on the phosphate since the enol is a stronger acid than an alcohol and therefore it should be bonded more weakly to phosphorus and depart more readily. This is strong evidence that direct attack at phosphorus by water is not occurring. Furthermore, the hydrolysis of unconjugated vinyl phosphates occurs much more readily (via C–O cleavage) than does the hydrolysis of the enol ester of the phosphoenolpyruvamide derivatives.[23] For comparison, we prepared the enol phosphate diethyl ester derived from acetone, dimethyl 1-methylvinyl phosphate. We observed that in acid solution it produces dimethyl phosphate and acetone but no methanol. For

$$(CH_3O)_2P(=O)OC(CH_3)=CH_2 \xrightarrow{H^+} (CH_3O)_2PO_2H + CH_3COCH_3$$

this reason, although this molecule is a better model electronically than triethyl phosphate for a rate comparison with **1** and **2**, a comparison with that aspect of reactivity is not available.

The reaction pattern of the phosphoenolpyruvamides is similar to that of phosphoenolpyruvate esters, in which the enol also remains intact while the alkyl esters are cleaved.[12,24]

Discussion

The reactivity patterns of the phosphoenolpyruvamide esters indicate that the adjacent amide group is participating in the reaction at phosphorus. The high degree of reactivity and the preference for the departure of the ethyl group over the enol are consistent with formation of a cyclic derivative by nucleophilic addition of the amide to the phosphate.[4] A mechanism that is consistent with the experimental results is presented in Scheme I. The reaction has been formulated as proceeding by participation of the oxygen of the amide although it is possible but less likely that the nitrogen is the participant. We feel oxygen participation is more likely since, in the case of urea participation at phosphorus, it has been shown that the oxygen atom adds preferentially.[6] It has also been shown that amides react with protons at oxygen since the O-protonated species is lower in energy than the N-protonated species.[25]

The initial intermediate, I, should be resistant to pseudorotation for the following reasons, based on arguments that have been presented by Westheimer.[26] The five-membered ring should span

(17) Perkow, W.; Ullerich, K.; Meyer, F. *Nature (London)* **1952**, *39*, 353.
(18) Lichtenthaler, F. W. *Chem. Rev.* **1961**, *61*, 607.
(19) Jacobson, H. I.; Griffith, M. J.; Preis, S.; Jensen, E. W. *J. Am. Chem. Soc.* **1957**, *79*, 2608.
(20) Young, P. R.; Howell, L. G.; Owen, T. C. *J. Am. Chem. Soc.* **1975**, *97*, 6544.
(21) Thain, E. M. *J. Chem. Soc.* **1957**, 4694.
(22) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 283.
(23) Tldwell, T. T.; Lyznicki, E. P. *J. Am. Chem. Soc.* **1973**, *95*, 4935.
(24) Schray, K. J.; Benkovic, S. J. *J. Am. Chem. Soc.* **1971**, *93*, 2522.
(25) McClelland, R. A.; Reynolds, W. F. *J. Chem. Soc., Chem. Commun.* **1974**, 824.
(26) Westheimer, F. H. *Acc. Chem. Res.* **1968**, 1, 70.

equatorial and axial positions. Therefore, any pseudorotation would interchange the axial and equatorial substituents. Since the imino nitrogen center will be protonated in acidic solutions, there is a high electronic barrier to placing the group (the oxygen atom bears part of the positive charge) in an equatorial position. As a result, either of the ethoxy groups, but not the enol group, can occupy the other axial position from which departure occurs.

Since we observe that the reaction is acid catalyzed, it is necessary to propose a role for a proton in arriving at the rate-determining transition state. Because the barrier to ethanol expulsion should be larger than for amide expulsion from the addition intermediate, the rate-determining step is probably the acid-catalyzed decomposition of pentavalent intermediate J, k_3. Furthermore, the tautomeric equilibrium (k_2/k_{-2}) will favor I over J since the imino site is much more basic than the ether oxygen. It is reasonable to expect that k_{-1} will be larger than k_2 because of the thermodynamic barrier associated with k_2. Since k_{-2} is associated with a thermodynamically favorable proton shift,[27] it should be on the order of 10^7 s^{-1}. The expulsion step, k_3, should be slower and thus rate determining in the conversion of **1** to intermediate K.

The cyclic phosphate product, K, should be highly reactive, as are other five-membered ring phosphates.[26] Strain and electronegativity effects require that it cleave exclusively after addition of water to expel the amide, yielding the monoester **3**, which can proceed by an analogous route to the diacid **4**. We observe that the rate constant for hydrolysis of the monoester is larger than the rate of hydrolysis of the diester. As a result, we conclude that the k_3 step is rate determining overall.

The rate law associated with Scheme I is given in eq 1. Since

$$v = k_3[\mathrm{J}] = k_{\mathrm{obsd}}[1] \quad (1)$$

I and J are present in very low concentration as steady-state intermediates, we can use eq 2. Since we assume an equilibrium

$$k_1[\mathbf{1}][\mathrm{H}^+] - k_{-1}[\mathrm{I}] - k_3[\mathrm{J}] = 0 \quad (2)$$

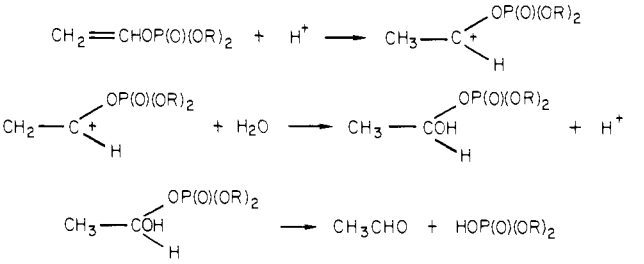
between I and J, we let K_2 replace the rate constants for the interconversion. The observed first-order rate constant for the hydrolysis of **1** is then given by eq 3.

$$k_{\mathrm{obsd}} = (k_1K_2k_3[\mathrm{H}^+])/(k_{-1} + k_3K_2) \quad (3)$$

The observed rates of hydrolysis of **1** (derived from propylamine) and **2** (derived from aniline) differ by about a factor of 2. The value of K_2 should be smaller for **1** than for **2** by a factor corresponding to the difference in the basicity of the amine substituents, about 10^5. The value of k_3 should be the same for both cases since the transition state should resemble the intermediate which is high in energy relative to the product. To simplify our further analysis, we assume $k_{-1} \gg k_3K_2$ as a consequence of the high energy involved in shifting a proton from the imino nitrogen to the ether oxygen. Without considering the conversion of the substrate to I, we would then have predicted that the formation of product from **2** would be favored by a factor of 10^5. The observed result of nearly equal rates requires that the formation of I from **1** is favored by about a factor of 10^5 over the formation of the analogue of I from **2**, to overcome the imbalance in the second step. The favoring of **1** in the first step is anticipated from the fact that the amine moiety acquires considerable positive character in going from the starting amide to the iminium ion in I. We can also cite this similarity in rates of reaction of **1** and **2** to rule out the first step as being rate limiting. In that case we would expect a much larger rate factor in favor of **1** over **2**.

(27) Bernasconi, C. F.; Hibdon, S. A.; McMurry, S. E. *J. Am. Chem. Soc.* **1982**, *104*, 3459.

Scheme II



In contrast, vinyl phosphates whose enol moiety is not conjugated normally undergo hydrolysis in acid solution by a mechanism involving protonation of the carbon–carbon double bond at the carbon atom β to the ether oxygen.[23] This produces an oxocarbonium ion to which water will add rapidly, as shown in Scheme II.

In the case of phosphoenolpyruvamides, the double bond is conjugated with the amide function. Protonation is retarded by the electronic effect of the amide as a substituent as well as by the resonance interaction of the two groups. This provides resistance to hydrolysis by the C–O cleavage mechanism of Scheme II. In the case of the hydrolysis of phosphate esters of phosphoenolpyruvate, Schray and Benkovic also found a relatively high barrier to reaction by this mechanism.[24]

The ease with which an amide will add to an adjacent phosphate supports proposals that have been made which suggest that amides can become phosphorylated during the course of an enzymic reaction.[1] Griffith and Meister have shown recently that oxoprolinase catalyzes a reaction that can be interpreted as involving this process.[2] It has been our contention that amide bonds of proteins are potentially involved as intermediate carriers of phosphate groups and may add to associated nucleotides,[4,5] and Knowles has considered that stereochemical scrambling phenomena may be associated with these intermediates.[28]

There are difficulties involved in establishing the existence of a phosphorylated amide in an enzymic process due to the inherently high reactivity of such a species. In order to develop means of trapping these species or developing inhibitors on the basis of the mechanisms of their reaction, further chemical studies will be necessary. We are currently examining the properties of phosphorylated amides in protein environments, including investigating the use of phosphoenolpyruvamides as mechanism-based inhibitors related to phosphoenolpyruvate.

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Registry No. **1**, 90367-73-0; **2**, 90367-74-1; I, 90367-75-2; L, 90367-76-3; $PhNH_2$, 62-53-3; $CH_3C(O)C(O)NHPh$, 46114-86-7; $BrCH_2C(O)C(O)NHPh$, 90367-77-4; $(EtO)_3P$, 122-52-1; $CH_3CH(OH)C(O)NHPr$, 74421-70-8; $CH_3CH(OH)C(O)OEt$, 97-64-3; $CH_3CH(OH)CO_2H$, 50-21-5; $PrNH_2$, 107-10-8; $CH_3C(O)C(O)NHPr$, 34907-01-2; $BrCH_2C(O)C(O)NHPr$, 90367-78-5; NaI, 7681-82-5; $BrCH_2C(O)CH_3$, 598-31-2; $(MeO)_3P$, 121-45-9; diacetyltartaric anhydride, 6283-74-5; hydroxymaleic anhydride, 52060-79-4; pyridine, 110-86-1; sodium ethyl *N*-propylphosphoenolpyruvamide, 90367-79-6; dimethyl 1-methylvinyl phosphate, 4185-82-4.

(28) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877.