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Reactions of Pyridines and Primary Amines with N-Phosphorylated Pyridines[1]

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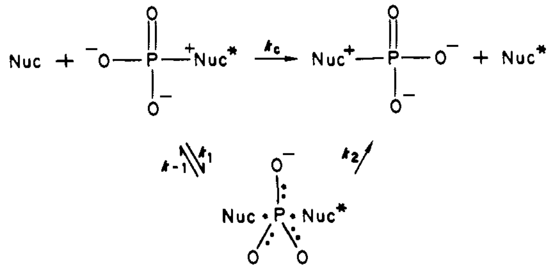
Abstract: Rate constants for the reactions of pyridines and primary amines of pK_a = 1–11 follow Brønsted-type correlations with $\beta_{nuc} = 0.17$ and 0.19 for reaction with phosphorylated 3-methoxypyridine and $\beta_{nuc} = 0.22$ and 0.28 for reaction with phosphorylated 4-morpholinopyridine, respectively. The rate constants show significant deviations from the behavior expected for a model with a metaphosphate intermediate and a change in rate-limiting step; they are consistent with a concerted reaction mechanism with a single, "open" transition state that changes in structure with changing basicity of the leaving and attacking amines. These changes may be described by the cross-coefficient $p_{xy} = \partial\beta_{nuc}/\partial pK_{lg} = \partial\beta_{lg}/\partial pK_{nuc} = 0.014$ and the direct interaction coefficients $p_x = p_y = \partial\beta_{nuc}/-\partial pK_{nuc} = \partial-\beta_{lg}/\partial pK_{lg} \leqslant 0.006$. The smaller value of p_x is consistent with the behavior expected from a linear perturbation of a reaction coordinate–energy contour diagram. It is difficult to reconcile the observed differences in activation barriers for different nucleophiles, of up to 7 kcal mol^{-1}, with a reaction mechanism that proceeds through an intermediate complex that has too short a lifetime to undergo diffusional separation. Equilibrium constants for phosphoryl transfer between pyridines depend on pyridine basicity with a value of $\beta_{eq} = 1.05 \pm 0.05$ for a correlation of log K and pK_a.

Reactions of the monoanions and dianions of monosubstituted phosphate derivatives generally proceed through transition states that closely resemble the monomeric metaphosphate monoanion, with a large amount of bond breaking to the leaving group and little bond formation to the entering nucleophile.[2-9] The metaphosphate monoanion has been observed in the gas phase[10] and reactions in the gas phase or in nonhydroxylic solvents have been shown to proceed through HPO_3 or PO_3^- intermediates.[3b,c] It has been suggested, but not proved, that reactions in hydroxylic solvents also proceed through such intermediates.[2,4,7] However, attempts to trap a free intermediate in such solvents have failed and several lines of evidence show that any metaphosphate intermediate that might be formed has an insufficient lifetime to diffuse through the solution, so that the reaction must proceed through a preassociation mechanism in which the phosphoryl acceptor is present at the time that the bond to the leaving group is cleaved.[7,11-14]

The question remains whether the preassociation mechanism proceeds through an intermediate with a significant lifetime or through the single transition state of a concerted reaction. It is difficult to design experiments that will answer this question. One approach is based on the essential difference between the two mechanisms: i.e., the stepwise mechanism must proceed through an intermediate and through two transition states that differ sufficiently from the intermediate in structure and energy to allow the existence of an energy well between them (Figure 1A), whereas the concerted mechanism proceeds through a single transition state that is symmetrical for a symmetrical reaction (Figure 1B). The first step of the stepwise mechanism is bond breaking to form the intermediate, k_1 in eq 1, and the second step is nucleophilic attack on the intermediate, k_2. The stepwise mechanism must undergo a change in rate-limiting step with changing reactivity of the nucleophile or leaving group. With a weak nucleophile, attack on the intermediate is rate limiting (k_2, eq 1; $k_{-1} > k_2$), whereas bond breaking becomes rate limiting with a strong nucleophile as shown in Figure 1C (k_1, eq 1; $k_2 > k_{-1}$). This change in rate-limiting step should appear as a change in the slope of a structure–reactivity correlation as the structure of the nucleophile or leaving group is changed.[15] The concerted mechanism cannot show a change in rate-limiting step, but it can undergo a change in the structure of the transition state that will cause curvature in a structure–reactivity correlation (Figure 1D). There may even be a bulge along the side of the reaction coordinate for the concerted mechanism if the symmetrical reaction has a relatively flat surface around the region corresponding to the structure of PO_3^-, as shown by the dotted lines in Figure 1, parts B and D.



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Communications from this laboratory and from Bourne and Williams have reported rate constants for phosphoryl transfer between pyridines and related compounds that are consistent with a linear structure–reactivity correlation of log k against the pK_a of the attacking pyridine for pyridines of larger and smaller basicity than the leaving group, as expected for a concerted reaction mechanism.[16,17] This result excludes a mechanism in which a metaphosphate intermediate is formed by bond breaking in one step, followed by bond making in the second step, and shows that the transition state always involves nucleophilic attack of the entering pyridine.

However, it is impossible to exclude a small amount of curvature in a structure–reactivity correlation, and the data are also con-

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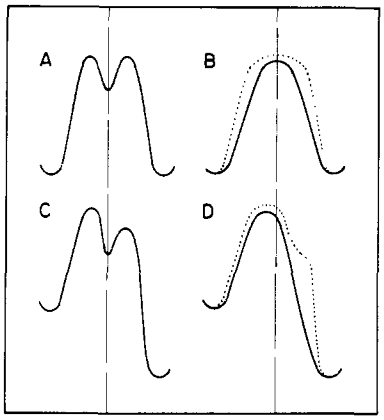

Figure 1. Reaction coordinate diagrams for stepwise phosphoryl transfer through a metaphosphate monoanion intermediate, A and C, and for concerted reactions with no intermediate, B and D. The lower diagrams show the change to completely rate-limiting bond cleavage for the stepwise mechanism, C, or a change to an earlier transition state for the concerted mechanism, D, when the attacking nucleophile is made more basic.

sistent with a stepwise mechanism in which there is nucleophilic assistance to rate-limiting expulsion of the leaving group in the first step,[18] with slopes in the structure–reactivity correlation of $\beta_{\text{nuc}} = 0.1$ and 0.2 for the two steps of the reaction, for example. Curvature is also consistent with a concerted reaction mechanism when there is a change in transition-state structure, as noted above. There is precedent for such changes in transition-state structure in the smaller dependence of reaction rate on the basicity of attacking pyridines for 2,4-dinitrophenyl phosphate compared with *p*-nitrophenyl phosphate[8] and the relatively large dependence on leaving group basicity for the reactions of weakly basic nucleophiles with phosphorylated 4-(dimethylamino)pyridine and 4-methylpyridine.[9] These changes represent interactions between the entering and leaving groups and may be described by the cross-interaction coefficient p_{xy} (eq 2).[19]

$$p_{xy} = \frac{\partial \beta_{\text{nuc}}}{\partial pK_{\text{lg}}} = \frac{\partial \beta_{\text{lg}}}{\partial pK_{\text{nuc}}} \tag{2}$$

These questions are relevant to the difficult problem of the mechanism by which enzymes catalyze phosphoryl transfer.[20] It would be of interest to distinguish between mechanisms that proceed through an in-line displacement or through a metaphosphate intermediate with inversion, but if the metaphosphate ion has no lifetime in the presence of a nucleophile, there is no distinction between these mechanisms. The problem then reduces to the quantitative question of how much bonding or metaphosphate character there is in the transition state of a concerted reaction.

The experiments described here were carried out in order to extend the range of reactivity that was examined in the earlier work[16,17] in the hope of providing a better understanding of the mechanism of phosphoryl transfer between pyridines and other amines in aqueous solution.

Experimental Section

Materials. Substituted pyridines, other amines, and phosphorous oxychloride were purified by distillation or recrystallization. 4-Morpholinopyridine[9] and the hydrochloride salt of 3-methoxypyridine[21] were synthesized and purified by reported procedures. Dioxane was dried with sodium metal and distilled prior to use. All salts and buffers were of the finest commercial grades and were used without further purification. Glass-distilled water was used throughout.

Procedures. Phosphorylation of 3-methoxypyridine was effected by the forceful injection with a pipettor (Pipetman) of 0.14 mL of a solution containing 0.3 M 3-methoxypyridine in 0.9 M KOH into a small tube containing ~4 μL, 1 equiv, of $POCl_3$. All solutions were at 4 °C. This mixture was agitated for <5 s with a vortex mixer. An aliquot (5–100 μL) of this synthesis mixture was applied immediately to the side of a quartz cell in the spectrophotometer cell holder. The reaction was initiated by forceful injection against the same side of the quartz cell from a 5-mL pipettor (Pipetman) equipped with a tip of wide bore that has a small exit (Rainin) of a solution containing the desired concentration of nucleophile, 0.05 M carbonate buffer and 1.0 M KCl at pH 10.3 and 25 °C. Typically, collection of data began within 15 s of addition of 3-methoxypyridine to $POCl_3$ and 1–3 s after injection of the nucleophile into the quartz cell. When the nucleophile was ethylamine, pyrrolidine, piperidine, or quinuclidine this solution was buffered by the amine at a pH near the pK_a of the conjugate acid of the amine. When the nucleophile was 0.05 M KOH the reaction solution was unbuffered. Disappearance of the phosphorylated 3-methoxypyridine was monitored by following the decrease in absorbance at wavelengths in the range 290–307 nM. Good first-order kinetics were observed for >3 half-lives in each case. Second-order rate constants were obtained from the slopes of plots of values of k_{obsd} against the concentration of nucleophile in the range 0–0.5 M; the observed increase in k_{obsd} was always >100%. No catalysis by buffers was observed.

Approximate rate constants for the reactions of phosphorylated 3-methoxypyridine with 4-morpholinopyridine and 4-(dimethylamino)pyridine were determined by spectrophotometric analysis of the products, because the large absorbance of reactants and products interferes with direct measurements of these reactions. The phosphorylation of 3-methoxypyridine was effected as described above. A 50-μL aliquot of the product was mixed as quickly as possible into a tube containing 7.5 mL of 0.05 M carbonate buffer and 1.0 M KCl at pH 10.3 and 4 °C. This solution was then split into aliquots, which were frozen in dry ice/acetone. An aliquot of the frozen solution containing phosphorylated 3-methoxypyridine and $PO_2Cl_2^-$ was thawed and kept immersed in an ice-water bath. Aliquots of this solution were added to tubes containing 4-morpholinopyridine or 4-(dimethylamino)pyridine, 0.05 M carbonate buffer, and 1.0 M KCl at pH 10.3 and 25 °C. The total change of absorbance at 295 nm from reaction of phosphorylated 3-methoxypyridine and $PO_2Cl_2^-$, ΔA_{tot}, was determined by comparing the absorbance of reaction mixtures to which aliquots had been added directly or after standing at 25 °C for 20 min to allow hydrolysis of the phosphorylating agents before addition. The contribution to the absorbance change from phosphorylation by $PO_2Cl_2^-$, ΔA_2, was estimated by comparing the absorbance of reaction mixtures prepared from aliquots that had stood at 25 °C for 100 s, to allow hydrolysis of >90% of the phosphorylated 3-methoxypyridine and <30% of the $PO_2Cl_2^-$, and aliquots that had stood at 25 °C for 20 min to give complete hydrolysis. This procedure was carried out in triplicate for each of nine concentrations of 4-morpholinopyridine and 4-(dimethylamino)pyridine. The results were plotted against the concentration of the nucleophilic reagent, as illustrated in Figure 2 for the reaction with 4-(dimethylamino)pyridine. The data for reaction with $PO_2Cl_2^-$ were fit to a curve that was calculated from the observed values of ΔA_2 (triangles, Figure 2) and the measured rate constants for hydrolysis and reaction with 4-(dimethylamino)pyridine of $PO_2Cl_2^-$ (see below). The absorbance changes and the rate constant for the reaction with phosphorylated 3-methoxypyridine were estimated from $\Delta A_{\text{tot}} - \Delta A_2$ and the measured rate constant for hydrolysis of phosphorylated 3-methoxypyridine. The second-order rate constant does not depend on the amount of the reaction with $PO_2Cl_2^-$, within the error of the estimation, because it is obtained from the rate constant for hydrolysis and the ratio of the products from hydrolysis and reaction with 4-(dimethylamino)pyridine of phosphorylated 3-methoxypyridine, rather than from the absolute yields of products from the two phosphorylating agents. The upper line in Figure 2 was calculated from the rate constants and absorbance changes for the reactions of the two phosphorylating agents. The reaction with 4-morpholinopyridine was examined by the same procedure; however, the fraction of the total reaction from reaction with phosphorylated 3-methoxypyridine was 36% instead of 51%.

A solution of $PO_2Cl_2^-$ was generated prior to each kinetic run by adding ~4 μL of $POCl_3$ to 0.15 mL of 1.0 M NaOH (>3 equiv) at 4 °C, and the mixture was agitated with a vortex mixer. The formation of $PO_2Cl_2^-$ from $POCl_3$ in water occurs with a half-time of <0.1 s.[22] An aliquot (20–80 μL) of this synthesis mixture was mixed into 2 mL of 0.05 M carbonate buffer and an equal-sized aliquot was applied with a pipettor (Pipetman) to the side of a 3-mL quartz cell in the spectrophotometer cell holder. The reaction was initiated by forceful injection, as described above, of a solution containing the desired concentration of

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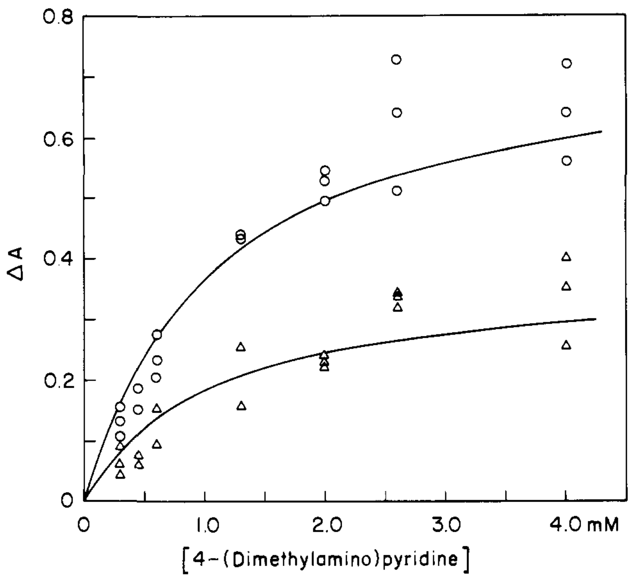

Figure 2. Estimation of the rate constant for reaction of phosphorylated 3-methoxypyridine with 4-(dimethylamino)pyridine by analysis of products. Circles: total change in absorbance at 295 nm, ΔA_{tot}, from the reactions of phosphorylated 3-methoxypyridine and $PO_2Cl_2^-$. Triangles: change in absorbance, ΔA_2, from the reaction of $PO_2Cl_2^-$. The lines were calculated from rate constants for hydrolysis and nucleophilic reaction as described in the text.

4-morpholinopyridine or 4-(dimethylamino)pyridine, 0.05 M carbonate buffer and 1.0 M KCl at pH 10.3 and 25 °C. The appearance of phosphorylated aminopyridine was monitored by following the increase in absorbance at wavelengths in the range 295–305 nm. Collection of data started <5 s after addition of nucleophile to the quartz cell. Good first-order kinetics were observed for >1 half-life with the nucleophile in 10-fold excess over $PO_2Cl_2^-$ and for >3 half-lives when the nucleophile was in 20-fold excess over $PO_2Cl_2^-$. Second-order rate constants were obtained from the slopes of plots of values of k_{obsd} against the concentration of nucleophile in the range 0–0.018 M; the observed increase in k_{obsd} was always >4-fold.

Phosphorylation of 4-morpholinopyridine was effected by mixing $POCl_3$ with a mixture of 0.28 M 4-morpholinopyridine and 2.52 M KOH. Three aliquots each of 1 equiv of $POCl_3$ were added with vigorous mixing, and the synthesis mixture was cooled in an ice-water bath between additions. The resulting solution containing phosphorylated 4-morpholinopyridine was stored frozen with negligible loss of the absorbance of phosphorylated 4-morpholinopyridine at 280 nm over a period of weeks. An aliquot of aqueous phosphorylated 4-morpholinopyridine sufficient to yield a concentration in the range of 0.02–0.07 mM was added to a 3-mL quartz cell in the spectrophotometer cell holder. The reaction was initiated by addition of 1–3 mL of a solution containing the desired concentration of nucleophile, 0.05 M carbonate buffer, and 1.0 M KCl at pH 10.3 and 25 °C. When the nucleophile was ethylamine, pyrrolidine, piperidine, or quinuclidine, this solution was buffered by the amine at a pH near the pK_a of the conjugate acid of the amine. Disappearance of the phosphorylated 4-morpholinopyridine was monitored by following the decrease in absorbance in the range 295–312 nm. The quartz cells were sealed tightly with stoppers and Parafilm. Good first-order kinetics was observed for >3 half-lives in each case. Second-order rate constants were obtained from the slopes of plots of k_{obsd} against the concentration of nucleophile in the range 0–1.0 M; the observed increase in k_{obsd} was always more than 3-fold.

The rate of the reaction of 4-(dimethylamino)pyridine with phosphorylated 4-morpholinopyridine could not be measured directly because of the similar absorbance of the reactants and products. Approximate rate constants were determined by a discontinuous assay. The reaction mixture consisted of the desired concentration of 4-(dimethylamino)pyridine in 10-fold excess over phosphorylated 4-morpholinopyridine, 0.05 M carbonate buffer, and 1.0 M KCl at pH 10.3 and 25 °C. Aliquots were removed from the reaction mixture and diluted into 0.05 M carbonate buffer and 1.0 M KCl at pH 10.0 20–30 times during the first 2–3 half-lives of the reaction, and five aliquots were removed and diluted after ≥10 half-lives. The absorbance in the range 296–300 nm of the diluted aliquots was read in a double-beam spectrophotometer with one of the end-point samples in the reference cell, and these absorbance readings were used to construct first-order plots of log $(A_t - A_\infty)$ against time. Satisfactory first-order kinetics was observed for at least 1 half-life in each case. The reaction was estimated to have proceeded >99% toward completion because 4-(dimethylamino)pyridine was present in ≥10-fold excess over 4-morpholinopyridine and the equilibrium constant for phosphorylation of the more basic pyridine is ~10 ($\beta_{eq} = 1$; see Discussion).

The effect of pH on the rate of hydrolysis of phosphorylated pyridine and phosphorylated 3,4-dimethylpyridine was studied by methods similar to those used to study the reactions of phosphorylated 3-methoxypyridine. The phosphorylation of pyridine and 3,4-dimethylpyridine was effected by the forceful injection with a pipettor of 0.5 mL of a solution containing the pyridine at a concentration of 0.15 M in 2.25 M KOH into a small tube containing ~30 μL, 6 equiv, of $POCl_3$. All solutions were at 4 °C. This mixture was agitated for <5 s with a vortex mixer. This synthesis mixture was applied with a pipettor to the side of a 3-mL quartz cell in the spectrophotometer cell holder, and the reaction was initiated by forceful injection with a 5-mL pipettor of 3 mL of a solution containing 0.05 M of the desired buffer and 1.0 M KCl at the desired pH. The buffers and the pH at which each was used are carbonate, 10.5 and 9.5, borate, 8.5, phosphate, 7.5 and 6.5, acetic acid, 5.5 and 4.5, and chloroacetic acid, 3.5 and 2.5. At pH 1.5 and under conditions of greater acidity, HCl was used to obtain the desired activity of hydrogen ions. When the concentration of HCl was greater than 1.0 M, KCl was omitted from the reaction solution. Disappearance of phosphorylated pyridine was monitored by following the decrease in absorbance at wavelengths in the range 260–270 nm (260–275 nm for phosphorylated 3,4-dimethylpyridine). Good first-order kinetics were observed for >3 half-lives in each case. No catalysis by buffers was observed.

Initial rates for the reaction of 4-(dimethylamino)pyridine with *p*-nitrophenyl phosphate dianion at pH 10.3, 25 °C, and ionic strength 1.0 M (KCl) were determined by monitoring the increase in absorbance at 400 nm due to the appearance of *p*-nitrophenolate anion. End points for these reactions were obtained from aliquots of the reaction mixtures that had been adjusted to pH 5 and kept at 100 °C in sealed tubes for >3 h. First-order rate constants were calculated from the linear plots of absorbance against time by converting to concentration units and dividing by the initial concentration of phosphate ester using the determined end points and $\epsilon = 18\,320$ for *p*-nitrophenolate ion at 400 nm.[23] The second-order rate constant for this reaction is 9.2×10^{-6} M^{-1} s^{-1}. The self-association equilibrium constant for 4-(dimethylamino)pyridine derived from these data by the method of Kirby and Jencks[6] is 9 M^{-1} (Table I).

Results

Rate constants for the reactions of phosphorylated 3-methoxypyridine and phosphorylated 4-morpholinopyridine with substituted pyridines and with other amines in aqueous solution at pH 10.3, 25 °C, and ionic strength 1.0 M (KCl) are shown in Tables I and II. Under these conditions the rate constant for hydrolysis of phosphorylated 3-methoxypyridine is 0.026 s^{-1}, and that for phosphorylated 4-morpholinopyridine is 3.4×10^{-6} s^{-1}. The rate constant of 0.027 s^{-1} for hydrolysis of phosphorylated 3-methoxypyridine in the presence of 0.05 M dioxane suggests that the increased rate of disappearance of this substrate in the presence of low concentrations of weakly basic pyridines is due to a nucleophilic reaction rather than a solvent effect. A very similar rate constant of 0.025 s^{-1} was observed for the hydrolysis of phosphorylated 3-methoxypyridine in the presence of 0.05 M KOH. A rate constant of 0.7×10^{-4} M^{-1} s^{-1} was determined for the reaction of 2-methylpyridine with phosphorylated 4-morpholinopyridine. The small value of this rate constant compared with those for other methylpyridines (Table I) is consistent with steric hindrance by the *o*-methyl group to nucleophilic attack.

At high amine concentrations the rates of reactions of phosphorylated pyridines with pyridine and some substituted pyridines do not increase linearly with increasing concentration of the nucleophile (Figure 3). Downward curvature has been observed in plots of k_{obsd} against the concentration of pyridine for some other reactions and can be accounted for as an activity coefficient effect or an association of the pyridine nucleophile.[6,24] Strong self-association of 4-(dimethylamino)pyridine has been observed

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Table I. Rate Constants for the Reactions of Phosphorylated 3-Methoxypyridine and Phosphorylated 4-Morpholinopyridine with Substituted Pyridines at Ionic Strength 1.0 M (KCl), 25 °C

			phosphorylated 3-methoxypyridine		phosphorylated 4-morpholinopyridine	
substituted pyridine	pK_a[a]	K_{as},[b] M^{-1}	k_2(uncor),[c] M^{-1} s^{-1}	k_2,[d] M^{-1} s^{-1}	10^4k_2(uncor),[c] M^{-1} s^{-1}	10^4k_2,[d] M^{-1} s^{-1}
3-CN	1.52			0.84		1.93
4-CN	2.25			1.55		
3-Cl	3.30[e]	2.0	1.38	1.88	3.4	4.7
3-$CONH_2$	3.55[e]	0.35[f]	1.55	1.65	4.0	5.8
4-$CONH_2$	3.76	2.0	2.37	2.92	5.5	9.0
3-COOH	4.75			3.23		10.3
3-OCH_3	5.12					14.6
pyridine	5.51[g]	0.50[f]	5.2	6.0	15.1	18.4
3-CH_3	6.02	3.0	4.2	6.2	17	30
4-CH_3	6.33[g]	3.0[f]	5.8	8.1	19	36
3,4-$(CH_3)_2$	6.79[g]	10[e]	5.7	9.7	30	45
4-N O	9.01			14 ± 2		
4-$N(CH_3)_2$	10.06[h]	9[i]		23 ± 6[j]	58 ± 20	112 ± 40

[a] Determined by the method of Jencks and Salveson (Jencks, W. P.; Salveson, K. *J. Am. Chem. Soc.* **1971**, *93*, 4433) in 1 M KCl at 25 °C, except as noted. [b] Apparent association constants for the pyridine were determined by the method of Kirby and Jencks[6] except as noted. [c] Uncorrected rate constant based on the slope of a straight line drawn through the curved plots of k_{obsd} vs. concentration of pyridine nucleophile (see text). [d] Rate constants for those pyridines that undergo association were corrected as described previously.[6] [e] Reference 21c. [f] Reference 6. [g] Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031. [h] Palling, D., unpublished experiments. [i] Determined[f] from the reaction of 4-(dimethylamino)pyridine with *p*-nitrophenyl phosphate at pH 10.3 and 25 °C in the presence of 1.0 M KCl (see text). [j] The effect of self-association of the nucleophile is negligible at the low concentrations of nucleophile used in these experiments.

Table II. Rate Constants for the Reactions of Phosphorylated 3-Methoxypyridine and Phosphorylated 4-Morpholinopyridine with Other Amines at Ionic Strength 1.0 M (KCl), 25 °C

amine	pK_a[a]	phosphorylated 3-methoxypyridine k_2, M^{-1} s^{-1}	phosphorylated 4-morpholinopyridine 10^4k_2, M^{-1} s^{-1}
semicarbazide	3.86	0.15	0.52
methoxyamine	4.72[b]	0.35	1.33
trifluoroethylamine	5.86	0.35	1.21
ethylenediamine monocation	7.42	4.3[c]	18.5[c]
glycinamide	8.32[b]	1.8	10.8
ethylenediamine	10.18	2.9[c,d]	29[c,d]
ethylamine	10.97	2.8	42
triethylenediamine	9.20	9.7[d]	60[d]
piperidine	11.42	3.8	63
quinuclidine	11.45[e]	3.6	59
pyrrolidine	11.51[f]	6.2	138

[a] In 1 M KCl at 25 °C (Jencks, W. P.; Gilchrist, M. J. *J. Am. Chem. Soc.* **1968**, *90*, 2622). [b] Palling, D., unpublished experiments. [c] Calculated from rate constants determined by three ratios of ethylenediamine monocation to ethylenediamine free base, extrapolated to 100% ethylenediamine monocation (see text). [d] Statistically corrected for the presence of two amine groups. [e] Gresser, M. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 6963. [f] Ewing, S. P.; Lockshon, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 3072.

spectrophotometrically.[24d] The data obtained in this work are satisfactorily described by self-association of the pyridine nucleophile. The solid lines in Figure 3 have been calculated as described previously[6] using the association constants reported in Table I. An apparent association constant of 9 M^{-1} for 4-(dimethylamino)pyridine was determined by the same method[6] from rate constants for the reaction of this pyridine with *p*-nitrophenyl phosphate at pH 10.3, 25 °C, and ionic strength 1.0 M (KCl).

Second-order rate constants for the reaction of phosphorylated 3-methoxypyridine with ethylenediamine were determined at pH 9.34, 10.68, and 11.5, at which 13%, 76%, and 100%, respectively, of the ethylenediamine is unprotonated and the remainder is monoprotonated. The reaction of phosphorylated 4-morpholinopyridine with ethylenediamine was examined at pH 9.56, 10.83, and 11.50, at which the ethylenediamine is 20%, 84%, and 100% unprotonated, respectively. The rate constants for the reactions of monoprotonated ethylenediamine were determined from extrapolation to 0% unprotonated ethylenediamine of plots of the second-order rate constants against the percent unprotonated ethylenediamine. The rate constants for the reactions of unprotonated ethylenediamine were determined directly from the experiments in the presence of 100% unprotonated ethylenediamine.

Approximate rate constants for the reactions of phosphorylated 3-methoxypyridine with 4-morpholinopyridine and 4-(dimethylamino)pyridine were determined from the rate constant for hydrolysis and spectrophotometric analysis of the ratio of products, as described in the Experimental Section and illustrated in Figure 2. Rate constants for the reaction of phosphorylated 4-morpholinopyridine with 4-(dimethylamino)pyridine under pseudo-first-order conditions were determined as described in the Experimental Section. These rate constants have large error limits because of the small difference in the absorbance of the reactants and products, as shown in Figure 4. A second-order rate constant of 112 ± 40 M^{-1} s^{-1} was obtained from the limiting slopes shown in Figure 4, after correction for nonlinearity using K_{as} = 9 M^{-1} for 4-(dimethylamino)pyridine.

Rate constants for the hydrolysis of phosphorylated pyridine and phosphorylated 3,4-dimethylpyridine were determined as a function of pH (Table III, Figure 5). Under conditions more acid than pH 1 the data were fit to an acidity function, H_-, based on the protonation equilibria of indicators derived from phosphoric acid in aqueous hydrochloric acid.[25] The data are adequately described by the rate law of eq 3, where Py represents the pyridine

$$k_{obsd} = k_+[PyPO_3H_2]^+ + k_o[PyPO_3H] + k_-[PyPO_3]^- \quad (3)$$

moiety. The species $(PyPO_3H_2)^+$ has been assigned a pK_a of 0.1 and $PyPO_3H$ a pK_a of 3.0, for both substrates. The lines in Figure 5 were calculated by assuming these values of pK_a and rate

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(25) Phillips, J. N. *Aust. J. Chem.* **1961**, *14*, 183-189.

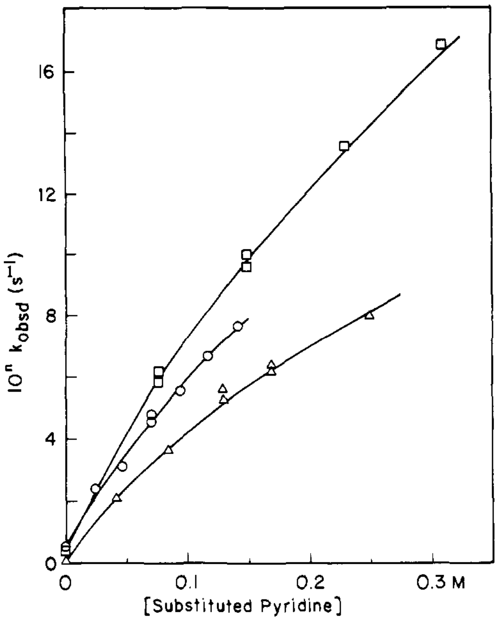

Figure 3. Plots of k_{obsd} against the concentration of substituted pyridines at pH 10.3 and 25 °C in the presence of 1.0 M KCl for the reactions of phosphorylated 4-morpholinopyridine with 3-chloropyridine (○), $k(1.5 \times 10^5)$, isonicotinamide (□), $k10^5$, and 3-methylpyridine (△), $k(2 \times 10^4)$. Where only one data point is shown at a concentration of pyridine, duplicate points were found to overlap. The solid curves were calculated with $K_{as} = 2.0$ M^{-1} and $k_1 = 4.7 \times 10^{-4}$ for 3-chloropyridine, $K_{as} = 2.0$ M^{-1} and $k_1 = 9.0 \times 10^{-4}$ for isonicotinamide, and $K_{as} = 3.0$ M^{-1} and $k_1 = 2.05 \times 10^{-3}$ for 3-methylpyridine.

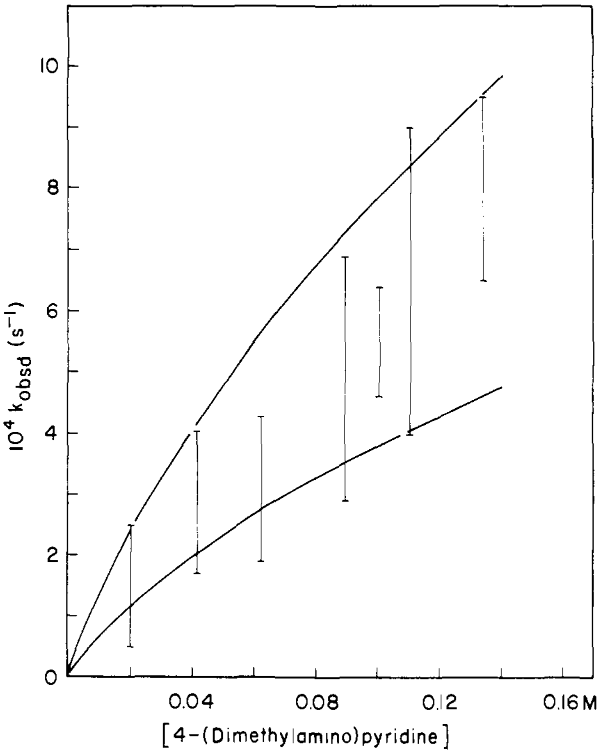

Figure 4. Dependence of the pseudo-first-order rate constants for the reaction of 4-(dimethylamino)pyridine with phosphorylated 4-morpholinopyridine on the concentration of 4-(dimethylamino)pyridine. The lines were calculated[6] from $K_{as} = 9.0$ M^{-1} and limiting values for the second-order rate constant of 73 and 152 M^{-1} s^{-1}.

constants for the hydrolysis of phosphorylated pyridine of $k_+ = 2.9$ s^{-1}, $k_o = 0.025$ s^{-1}, and $k_- = 0.0127$ s^{-1} and for phosphorylated 3,4-dimethylpyridine of $k_+ = 0.29$ s^{-1}, $k_o = 0.0025$ s^{-1}, and $k_- = 6.83 \times 10^{-4}$ s^{-1}. Attempts to fit the data with only one ionization in the region examined gave calculated curves that deviate by ≥60% from at least two of the observed rate constants.

Table III. Rate Constants for the Hydrolysis of Phosphorylated Pyridine and Phosphorylated 3,4-Dimethylpyridine as a Function of pH and H_-[a]

	[HCl], M	phosphorylated pyridine, $10^2 k_{hydrol}$, s^{-1}	phosphorylated 3,4-dimethyl-pyridine $10^2 k_{hydrol}$, s^{-1}
H_-[a]			
	7.86		19.5
	6.74		18.7
	5.61		20.5
	4.49		14.3
	3.37		16.6
	2.25		11.4
	1.12		6.95
	1.00	60.7	7.35
	0.45	40.0	3.60
pH			
		14.1	1.26
		3.70	0.322
		1.40	0.116
		1.26	0.070
		1.25	
		1.36	
		1.32	0.066
		1.33	
		1.14	
		1.23	0.069

[a] At ionic strength 1.0 (KCl) for [H$^+$] < 1 M, 25 °C. The values of H_- are from ref 25.

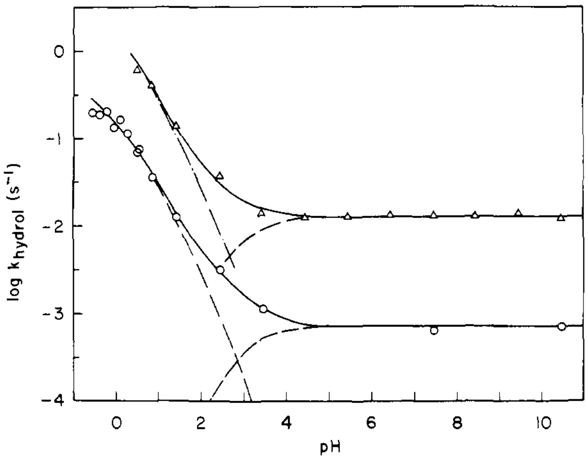

Figure 5. Dependence of rate constants for the hydrolysis of phosphorylated pyridine (△) and phosphorylated 3,4-dimethylpyridine (○) on pH and H_-. The solid lines were drawn to fit the rate law of eq 3 and the rate constants given in the text. The contributions to the observed rate constant k_- and k_+, for reaction of the monoanion and monocation, are shown by dashed lines.

Rate constants of 2.6 M^{-1} s^{-1} and 4.9 M^{-1} s^{-1} were obtained for the reactions of $PO_2Cl_2^-$ with 4-morpholinopyridine and 4-(dimethylamino)pyridine, respectively, at pH 10.3 in 0.05 M carbonate buffer and 1.0 M KCl at 25 °C. Extrapolation to zero pyridine concentration gave $k = 5 \times 10^{-3}$ s^{-1} for the hydrolysis of $PO_2Cl_2^-$, in agreement with reported values of 3.21×10^{-3} s^{-1} in the absence of added salt and 6.0×10^{-3} s^{-1} in the presence of 1.5 M KCl at pH 7.[22]

Discussion

Brønsted-type plots for the reactions of N-phosphorylated 3-methoxypyridine and 4-morpholinopyridine with pyridines of pK_a = 1.5–10 and with primary amines of pK_a = 3.9–11 are shown in Figure 6. Pyridines are about 10-fold more reactive than primary amines of the same pK_a as nucleophiles and 3-methoxypyridine (pK_a = 5.1) is 10^3–10^4 times more reactive than 4-morpholinopyridine (pK_a = 9.0) as a leaving group. Rate constants for the reactions of basic secondary amines and quinuclidine are close to those for basic primary amines; however,

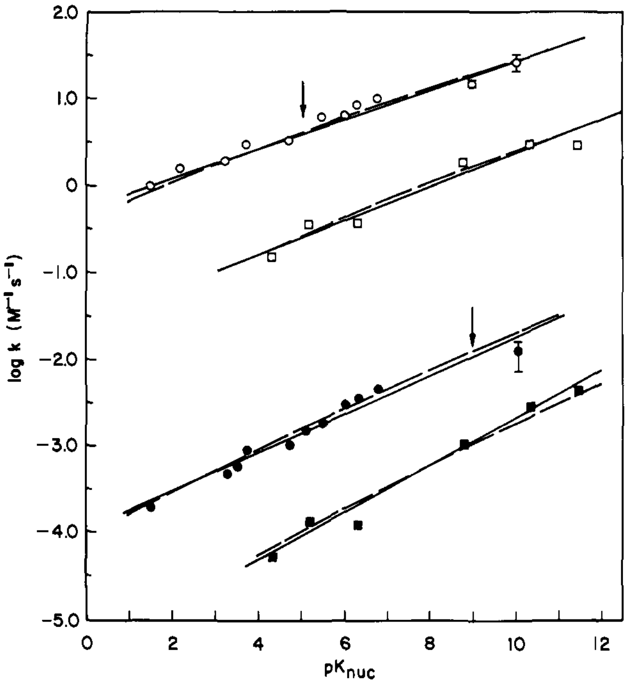

Figure 6. Brønsted-type plots for the reactions of phosphorylated 3-methoxypyridine (○, □) and phosphorylated 4-morpholinopyridine (●, ■) with pyridines (●, ○) and primary amines (■, □). The solid lines are the least-squares straight line to fit the experimental data. The dashed curves are calculated assuming a reaction that occurs through a single transition state of variable structure as described in the text. The pK_a values for primary amines are statistically corrected.

triethylenediamine reacts at a rate similar to that of pyridines of comparable pK_a (Tables I and II). The monocation of ethylenediamine exhibits a positive deviation compared with other primary amines (Table II) that may be attributed to an electrostatic effect.[6] The weakly basic "α-effect" compounds, semicarbazide and methoxyamine, show no enhanced reactivity compared with other primary amines, in agreement with previous results; hydrazine and hydroxylamine (which reacts at both nitrogen and oxygen[5]) have been reported to show small rate enhancements.[9]

The data are consistent with linear correlations of log k against pK_a of the attacking amines, as shown by the solid lines in Figure 6. The slopes of the lines, β_{nuc}, are larger for the less reactive substrate with the poorer leaving group: for pyridines the values of β_{nuc} are 0.17 ± 0.01 and 0.22 ± 0.01 for the reactions with phosphorylated 3-methoxypyridine and 4-morpholinopyridine, respectively, and for primary amines the corresponding values are $\beta_{nuc} = 0.19 \pm 0.02$ and 0.28 ± 0.02; the limits refer to the standard error. The difference in slope for the two leaving groups is statistically significant, with $p = 0.004$ for the pyridines and $p = 0.019$ for the primary amines. The data are also consistent with a small amount of curvature in the correlations, as shown by the dashed lines in Figure 6.

These small slopes show that the transition state resembles the attacking amine more closely than a protonated amine and suggest that there is little bond formation between the amine and phosphorus in the transition state. Since the reaction of pyridines is symmetrical, this conclusion applies to both the attacking and leaving pyridine. The reactions behave as if the "effective charge" that is seen by polar substituents on both the attacking and leaving pyridine in the transition state is approximately 20% of a full positive charge.

The results for phosphorylated 3-methoxypyridine and isoquinoline are inconsistent with a stepwise reaction mechanism in which only bond breaking occurs in the first step, to form a metaphosphate intermediate, followed by nucleophilic attack to form the product in a second step, as reported previously.[16,17] Such a mechanism corresponds to a value of $\beta_{nuc} = 0$ for the initial bond-breaking step. However, it is impossible to exclude a small amount of curvature that corresponds to a stepwise mechanism in which there is nucleophilic assistance to bond breaking in the first step followed by a change in rate-limiting step to nucleophilic attack on an unstable intermediate that is still stabilized by interaction with the leaving group. Values of $\beta_{nuc} = 0.1$ and 0.25 for these two steps would not be inconsistent with the data for phosphorylated 3-methoxypyridine, for example.

This possibility could be tested if the range of reactivity could be extended over a much larger range than was examined in the original work. This is not possible for a single series of attacking pyridines because of the limited range of basicity that can be obtained in such a series, but the range of reactivity can be extended by utilizing primary amines, which have a lower nucleophilic reactivity than pyridines, and by examining the reactions of a phosphorylated pyridine with a poor leaving group, 4-morpholinopyridine, so that nucleophilic attack (k_2, eq 1) would make a larger contribution to the observed rate constant in the stepwise mechanism.

The stepwise mechanism is expected to show the following behavior:

(1) The dependence of the rate on nucleophilic reactivity is larger when nucleophilic attack is rate limiting (k_2) than when bond breaking is rate limiting (k_1). For the reactions of pyridines with phosphorylated 3-morpholinopyridine there is a change in rate-limiting step when the basicities of the entering and leaving groups are equal, shown by the arrow in Figure 6, but with values of $\beta_{nuc} = 0.1$ and 0.25 for the two steps, both steps contribute significantly to the observed rate constant for all of the pyridines. Primary amines are less nucleophilic (smaller k_2) than pyridines of the same basicity, so that nucleophilic attack contributes more to the observed rate constant. However, in the reactions with phosphorylated 4-morpholinopyridine nucleophilic attack is predominantly rate limiting for all amines except 4-(dimethylamino)pyridine. Since the dependence of the rate on nucleophilicity is larger when nucleophilic attack is rate limiting, the difference between the rate constants for the reactions of pyridines and of the less nucleophilic primary amines should be larger for the reactions with phosphorylated 4-morpholinopyridine than with phosphorylated 3-methoxypyridine. For the same reason the ratio of the rate constants for the two different leaving groups should be larger for primary amines than for pyridines.

(2) The difference between the values of β_{nuc} for pyridines and primary amines should be larger for reactions of phosphorylated 3-methoxypyridine than for phosphorylated 4-morpholinopyridine. Nucleophilic attack is predominantly rate limiting for both reactions of the latter compound so that the values of β_{nuc} should be similar, whereas with phosphorylated 3-methoxypyridine both steps are rate limiting for pyridines but nucleophilic attack is predominantly rate limiting for the less nucleophilic primary amines. For the same reason primary amines, which would always react with predominant rate-limiting attack, should show a smaller change in β_{nuc} for the reactions with the two different leaving groups than do pyridines, for which bond breaking is largely rate limiting with the better leaving group.

(3) The curvature in the Brønsted plots should be larger for the reactions of pyridines with phosphorylated 3-methoxypyridine, which should give a change in rate-limiting step in the center of the line, than for the other reactions.

None of these expectations is realized in the data. There is no significant difference in the relative reactivities of pyridines and primary amines in the two reactions, the increase in β_{nuc} for primary amines compared with pyridines is, if anything, larger for the 4-morpholinopyridine than for the 3-methoxypyridine leaving group, and the change in β_{nuc} with the two different leaving groups is as large or larger for primary amines than for pyridines. For nucleophiles of pK_a = 5, pyridines are 16-fold more reactive than primary amines with both leaving groups. Furthermore, there is no significant difference in the small curvature that may exist in the correlation lines for reactions of pyridines and amines with the two different leaving groups; if there is a larger curvature it occurs with the 4-morpholinopyridine leaving group.

The relationship of the different rate constants is shown most clearly by a plot of the ratio of the rate constants for the reactions

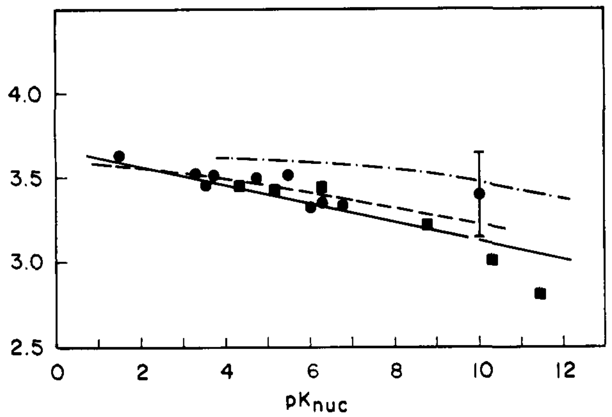

Figure 7. The logarithm of the ratios of the rate constants for reactions of phosphorylated 3-methoxypyridine to those of phosphorylated 4-morpholinopyridine plotted against the pK_{nuc} for primary amine (■) and pyridine (●), nucleophiles. The solid line was calculated from eq 8, for a reaction with a single transition state of variable structure. The dashed lines were calculated from eq 4–6, for a mechanism involving a change in rate-limiting step. The pK_a values for primary amines are statistically corrected.

of the two phosphorylated pyridines against the pK_a of the attacking amine, as shown in Figure 7. It is apparent that there is no significant difference in the rate constant ratios for the two leaving groups, with the possible exception of a slightly smaller ratio for the most basic primary amines. The negative slope is statistically significant ($p < 0.001$) and reflects the larger values of β_{nuc} with the poorer leaving group.[26] The negative deviations for basic primary amines in Figure 7 reflect the relatively large rate constants for their reactions with phosphorylated 4-morpholinopyridine that are responsible for the relatively large value of β_{nuc} for this reaction.

The stepwise mechanism may be treated quantitatively according to eq 4–6, in which $k_b = k_1k_2/k_{-1}$ (rate-limiting nucleophilic attack, eq 1), $C_1 = 0.14$ and $C_b = -0.63$ for phosphorylated 3-methoxypyridine, and $C_1 = -2.93$ and $C_b = -4.28$ for phosphorylated 4-morpholinopyridine. The values of C_1 and C_b were obtained from log k_{obsd} for a pyridine with the same pK_a as the leaving group, using the Brønsted plots of Figure 6. For this value of log k both steps of the reaction are equally rate limiting, and the rate constants are related by $k_2 = k_{-1}$, $k_1 = k_b$, and $k_{obsd} = k_1/2 = k_b/2$. The data for the pyridines can be fit satisfactorily according to eq 4–6 using the values of $\beta_{nuc} = 0.1$ for rate-limiting pyridine leaving (eq 5) and $\beta_{nuc} = 0.25$ for rate-limiting nucleophilic attack (eq 6). The calculated rate constant ratios are shown by the lower dashed line in Figure 7. The nucleophilic reactivity of primary amines toward phosphorylated 4-morpholinopyridine, for which nucleophilic attack is largely rate limiting, corresponds to the reactivity of pyridines that are less basic by 5.5 pK units. Using this difference to include primary amines in the correlation gives the upper dot–dashed line for the rate constant ratios in Figure 7. The calculated lines provide a satisfactory fit to the data for the reactions with pyridines but deviate significantly for the reactions with primary amines. The positive deviation corresponds to the failure of expectation 1 above. We conclude that the data do not support the stepwise model.

$$k_{obsd} = \frac{k_1k_b}{k_1 + k_b} \tag{4}$$

$$\log k_1 = 0.10\text{p}K_{nuc} + C_1 \tag{5}$$

$$\log k_b = 0.25\text{p}K_{nuc} + C_b \tag{6}$$

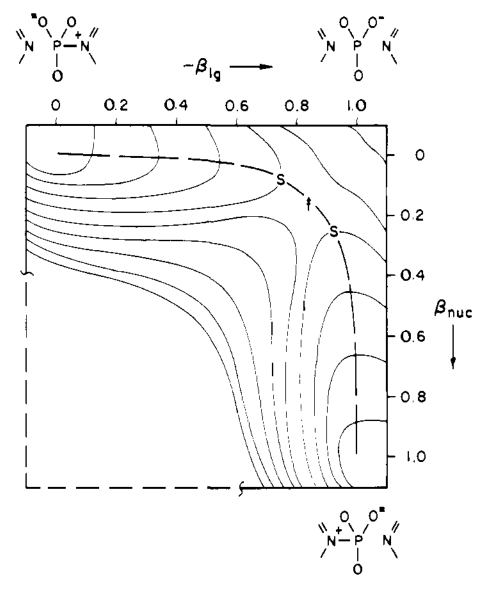

Figure 8. Reaction coordinate–energy contour diagram for phosphoryl transfer between pyridines, with $\beta_{nuc} = 0.17$. The position of the transition state for a concerted mechanism is indicated by ‡, and positions of the two transition states that surround a potential well for a stepwise mechanism are indicated by S. The values of $-\beta_{lg}$ and β_{nuc} are normalized, using $\beta_{eq} = 1.05$.

There is some difficulty in visualizing how an intermediate can be formed along a reaction coordinate when it is surrounded by two transition states of very similar structure. Figure 8 shows a hypothetical energy contour diagram for the concerted substitution reaction of phosphorylated 3-methoxypyridine, in which the axes are defined by the observed structure–reactivity parameters β_{nuc} and β_{lg}. The values of β_{nuc} and β_{lg} in the figure are normalized to a range of 1.0; this is close to the maximum value of $\beta = 1.05$ for phosphoryl transfer at equilibrium that is described below. The position of the transition state for the concerted reaction is shown, with $\beta_{nuc} = 0.17$. The positions of the two transition states for a stepwise reaction, with values of $\beta_{nuc} = 0.1$ and 0.25 for the two steps, are indicated by the letter S. The stepwise mechanism requires that instead of the single ridge at the transition state of the concerted reaction, the upper right corner of the diagram must contain two ridges for the two transition states with a well for the intermediate between them. There is little room on the energy surface for an intermediate that has a significant barrier for its collapse; it appears that any such intermediate would require that there be a very sharp curvature of the energy surface in order to produce a potential well. If the P–N bond orders to the two pyridines are 0.1 and 0.25 in the transition states and the same total bonding is maintained to stabilize the intermediate, the bond orders in the intermediate are 0.175 and the change in bond order between the transition states and the intermediate is only 0.075. This would seem to allow little change in structure between the intermediate and the transition states. A literal application of the BEBO (bond energy–bond order) approximation to this system gives an energy difference of only 0.1 kcal between the transition states and the intermediate for these changes in bond order.[27] We do not suggest that this calculation should be taken seriously, because of uncertainties in the relationship of these bond orders to values of β_{nuc} and to possible changes in the energy of PO_3^-, but it does illustrate the difficulty of accounting for the stability of an intermediate when the changes in bond order be-

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[26] The ratios of the rate constants for reactions of amines with phosphorylated 4-methylpyridine and 4-(dimethylamino)pyridine, reported by Jameson and Lawlor,[9] show similar behavior with small negative deviations for methylamine and triethylenediamine but no such deviations for piperidine, morpholine, and ammonia.

[27] The energy of the intermediate, E_f^1, was calculated from a value of $E_f^{\ddagger} = 16$ kcal mol^{-1} and the equation $E_f = V_1 - V_1n_1^{p_1} - V_2n_2^{p_2}$ in which V is bond energy, n is bond order, and p is a constant equal to approximately 0.9 (Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891–899. Johnson, H. S. *Adv. Chem. Phys.* **1960**, *3*, 131–170.). For a symmetrical reaction, in which $V_1 = V_2$, and for $n_1 = 0.1$, $n_2 = 0.25$ in the transition state and $n_1 = n_2 = 0.175$ in the intermediate, the ratio $E_f^{\ddagger}/E_f^1$ is 1.006 and $E_f^1 = 15.9$ kcal mol^{-1}, giving an activation barrier of 0.1 kcal mol^{-1} for collapse of the intermediate. The result is the same for values of $p = 0.87$ and 0.93.

tween the intermediate and transition states are small.

It is even more difficult to understand how there can be barriers for collapse of an intermediate in the presence of both weak and strong nucleophiles when the intermediate reacts with the weak nucleophile faster than it diffuses away. Diffusional separation would give racemization and a product ratio in mixed solvents that is independent of the source of the intermediate, which are not observed in reactions of monosubstituted phosphate derivatives.[7,11-13] This means that the reaction proceeds through a preassociation mechanism in which all of the reacting species are present during all of the chemical steps and collapse of any intermediate to products, k_2 in eq 7, is faster than diffusional separation, k_d.

$$\mathrm{Nuc} + {}^{2-}\mathrm{O_3P{-}X^+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{k_d\downarrow}{\mathrm{Nuc{\cdot}PO_3^-{\cdot}X}} \xrightarrow{k_2} \mathrm{Nuc^+{-}PO_3^{2-}} \quad (7)$$

The second-order rate constant for the reaction of phosphorylated 4-morpholinopyridine with water is 10^5 times smaller than that with 4-(dimethylamino)pyridine as the nucleophile, which corresponds to a difference of 7 kcal $\mathrm{mol^{-1}}$ in the observed activation barriers. There must also be a large difference of up to 7 kcal $\mathrm{mol^{-1}}$ between the barriers for reaction of an intermediate with water and with 4-(dimethylamino)pyridine during the hydrolysis of phosphorylated 4-(dimethylamino)pyridine, yet both barriers must be smaller than the barrier for diffusion of water away from the intermediate. A rate constant for diffusional separation of $10^{10}\ \mathrm{s^{-1}}$ corresponds to $\Delta G^{\ddagger} = 3.8$ kcal $\mathrm{mol^{-1}}$, and if there is less than 10% diffusion away before reaction with either nucleophile, both rate constants for collapse of the intermediate must be $>10^{11}\ \mathrm{s^{-1}}$, corresponding to barriers of <2.4 kcal $\mathrm{mol^{-1}}$. However, if the barrier for the stronger nucleophile is 7 kcal $\mathrm{mol^{-1}}$ lower than 2.4 kcal $\mathrm{mol^{-1}}$ it does not exist, there is no intermediate, and the reaction must occur in one step. Stated another way, a rate constant for collapse of $10^5 \times 10^{11} \simeq 10^{16}\ \mathrm{s^{-1}}$ is larger than a vibration frequency so that the "intermediate" has a lifetime less than a vibration frequency and does not exist. Even if the difference in the two barriers is only half as large as the observed difference in $\Delta G^{\ddagger}$, the difference of 3.5 kcal $\mathrm{mol^{-1}}$ and the rate constant of $10^{13.5}\ \mathrm{s^{-1}}$ are not consistent with the existence of an intermediate.[28]

We conclude that an intermediate with a significant lifetime is unlikely to exist along the path for the reaction of amines or water with phosphorylated pyridines. This does not, of course, mean that there cannot be an intermediate in other reactions of monosubstituted phosphate derivatives with weak nucleophiles.

Changes in Transition-State Structure. The changes in the values of β_{nuc} with the different pyridine leaving groups in Figure 6 and the nonzero, negative slope of the correlation of rate constant ratios in Figure 7 indicate that the amount of bond formation between the attacking amine and phosphorus in the transition state increases when the leaving pyridine is more basic. The rate constants for the two phosphorylated pyridines give complementary changes in the dependence of log k on the basicity of the leaving group, with $\beta_{lg} = -0.98$ and -0.86 for the reactions with 3-cyanopyridine and 3,4-dimethylpyridine, respectively. These results may be described by a positive cross-coefficient $p_{xy} = \partial\beta_{nuc}/\partial pK_{lg} = \partial{-}\beta_{lg}/{-}\partial pK_{nuc}$ with a value of $p_{xy} = 0.014 \pm 0.002$. This cross-coefficient describes the interaction between the attacking nucleophile and the leaving group. The positive sign of p_{xy} corresponds to a normal "Hammond effect", with a larger sensitivity to the pK_a of the attacking or leaving group as the reaction becomes more difficult. The sign of p_{xy} is opposite to that expected for a direct electrostatic interaction between dipoles of the attacking and leaving pyridines.[19]

Evidence for a positive p_{xy} coefficient in related systems has been reported previously. The data of Jameson and Lawlor for the reactions of eight amines (excluding trimethylenediamine and methylamine) with phosphorylated 4-methylpyridine and 4-(dimethylamino)pyridine and of four phosphorylated pyridines with five different amines[9] are consistent with a value of $p_{xy} = \partial\beta_{nuc}/\partial pK_{lg} = \partial{-}\beta_{lg}/{-}\partial pK_{nuc} = 0.020$. The values of $\beta_{nuc} = 0.13$ and 0 for the reactions of pyridines with 4-nitrophenyl phosphate[6] and 2,4-dinitrophenyl phosphate[8] give a larger value of $p_{xy} = \partial\beta_{nuc}/\partial pK_{lg} = 0.043$ that suggests an even more mobile transition state than for the reactions of phosphorylated pyridines.

It is more difficult to determine whether there is a significant direct interaction coefficient $p_x = \partial\beta_{nuc}/{-}\partial pK_{nuc}$ or $p_y = \partial{-}\beta_{lg}/\partial pK_{lg}$. These coefficients describe curvature in Brønsted plots (e.g., "Hammond effects") that arise from changes in the amount of bond formation or cleavage in the transition state with increasing basicity of the attacking or leaving amine. The reactions of pyridines with phosphorylated pyridines are symmetrical, so that $p_x = p_y$ if the equilibrium constants follow a linear structure–reactivity correlation. The data for pyridines are consistent with, but do not prove, the existence of a small amount of curvature in the Brønsted plots and a positive p_x coefficient. However, the values of β_{nuc} for the less reactive primary amines are definitely larger than those for pyridines, so that if it is assumed that the two classes of amines are comparable there is an increase in β_{nuc} with decreasing reactivity of the nucleophile. The data can be fit with a value of $p_x = 0.006 \pm 0.003$. The individual Brønsted plots for pyridine nucleophiles do not show significant curvature; however, when taken together the data are consistent with a small curvature ($p = 0.05$).

The rate constants for the reactions were calculated from eq 8, using the values $p_x = p_y = 0.006$ and $p_{xy} = 0.014$. Equation

$$-\log k = \tfrac{1}{2}p_x\mathrm{p}K_{nuc}{}^2 + \tfrac{1}{2}p_y\mathrm{p}K_{lg}{}^2 - p_{xy}\mathrm{p}K_{nuc}\mathrm{p}K_{lg} - \beta_{nuc}{}^\circ\mathrm{p}K_{nuc} - \beta_{lg}{}^\circ\mathrm{p}K_{lg} + F \quad (8)$$

8 describes the observed rate constants when the values of β_{nuc} and β_{lg} change with changing structure of the reactants and give rise to significant p_x, p_y, and p_{xy} coefficients. Equation 8 is the form of eq 9 that describes the structure–reactivity diagram of

$$-\log k = \tfrac{1}{2}p_xd^2 + \tfrac{1}{2}p_ye^2 + p_{xy}de + x_od + y_oe + F \quad (9)$$

Figure 8,[29] with $x = \beta_{nuc}$, $y = -\beta_{lg}$, $d = -\mathrm{p}K_{nuc}$, and $e = \mathrm{p}K_{lg}$. In eq 9, x and y are normalized structure–reactivity parameters, such as β, β_{nuc}, or ρ, x_0 and y_0 are the values of x and y when $d = e = 0$, d and e are parameters related to energy, such as pK_a or σ, and F is a constant. Increasing d or e increases the energy of the right side or the top of the diagram, respectively.[19] The rate constants were calculated from values of $\beta_{nuc}{}^\circ = 0.14$, $\beta_{lg}{}^\circ = -0.90$, and $F = -4.295$ for the reactions of pyridines and $\beta_{nuc}{}^\circ = 0.17$, $\beta_{lg}{}^\circ = -0.90$, and $F = -2.964$ for the reactions of primary amines. The smaller value of F for the primary amines reflects their lower nucleophilic reactivity. The larger values of β_{nuc} for primary amines than for pyridines correspond to the difference expected from the positive coefficient of $p_x = \partial\beta_{nuc}/\partial pK_{nuc} = 0.006$ if the primary amines behave like pyridines that are less basic by 6 pK units.

The calculated rate constants are shown by the dashed lines in Figure 6 and provide a satisfactory fit to the data. They also provide a satisfactory fit to the rate constant ratios in Figure 7, as shown by the solid line.

Rate constants were also calculated by using the same values of $\beta_{nuc}{}^\circ = 0.16$ and $\beta_{lg}{}^\circ = -0.90$ for both pyridines and primary amines and values of $F = -4.20$ for pyridines and $F = -3.01$ for primary amines. The results (not shown) gave a fit to the observed rate constant ratios that is indistinguishable from the solid line in Figure 7 and a slightly less satisfactory fit to the data in Figure 6 because of the larger change in β_{nuc} for primary amines than for pyridines with the two leaving groups; however, this difference is probably within the uncertainty of the β values.

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(28) If the values of β_{nuc} are 0.1 and 0.25 for rate-limiting formation and collapse of the intermediate, respectively, the difference in log k for the two steps of the reaction is $(0.25 - 0.1)/0.25 = 60\%$ of the difference in log k_{obsd} when collapse of the intermediate is rate limiting.

(29) In Figure 8 the value of β_{nuc} corresponds to the x axis and $-\beta_{lg}$ to the y axis for the equations used to calculate the properties of the saddle point.[19] The diagram was drawn in this way to conform to the conventional usage for displacement reactions.

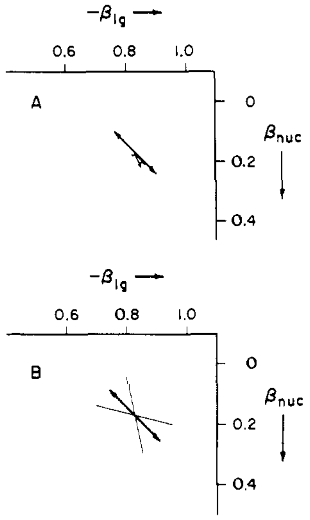
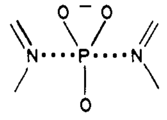


Figure 9. Reaction coordinates for the concerted mechanism shown in the diagram of Figure 8. The effects of an electron-donating substituent that stabilizes the reactants on motions of the transition state perpendicular and parallel to the reaction coordinate are shown in A. The positions of the two level lines of constant energy and of a reaction coordinate that bisects these lines, calculated as described in the text, are shown in B.

This structure–reactivity behavior is consistent with what would be expected for an open, "exploded", transition state of a concerted reaction mechanism, **1**, with a relatively small curvature of the energy surface, so that the transition state can move about easily. An electron-donating substituent in the leaving group stabilizes the reactant and lowers the energy of the left side of the energy contour diagram in Figure 8. As shown in Figure 9A, this will tend to cause movements of the transition state downhill toward the southwest corner, perpendicular to the reaction coordinate, and uphill toward the southeast corner, parallel to the reaction coordinate. The resultant of these shifts is a net movement downward on the diagram that corresponds to increased bond formation with the attacking pyridine and an increased value of β_{nuc}, as observed. The diagram also predicts that there will be relatively little change in β_{lg} for the leaving pyridine, corresponding to a "Hammond effect" or a positive coefficient $p_y = p_x$; there would be no change if the movements perpendicular and parallel to the diagonal reaction coordinate were equal. The movement perpendicular to the reaction coordinate corresponds to a decrease in metaphosphate character in the transition state, while the movement parallel to the reaction coordinate corresponds to a later transition state with increased bond breaking and bond making. Both of these changes increase the amount of bond making and β_{nuc}, so that the p_{xy} coefficient is relatively large, but they have opposite effects on the amount of bond breaking, so that the change in the amount of bond breaking and the direct Hammond effect measured by p_y are small. An analogous reinforcement and cancellation of the parallel and perpendicular effects gives large changes in β_{lg} and smaller changes in β_{nuc} when the reactivity of the nucleophile is changed.



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The amount of movement of the transition state increases when the curvature at the saddle point is smaller. If the curvature is smaller along the reaction coordinate than perpendicular to it, there will be a movement of the transition state to the right (Figure 9A). This gives a larger value of $-\beta_{lg}$ and corresponds to a positive p_y coefficient. For the reaction in the reverse direction, it gives a smaller value of β_{nuc} with increasing basicity of the attacking amine and a positive p_x coefficient. This is consistent with the data, but not proved, as indicated above. If the curvatures parallel and perpendicular to the reaction coordinate were equal, the Hammond effect and the p_x and p_y coefficients would be zero.

The energy surface in the region of the saddle point may be described by eq 10, in which a and b define the curvatures of the saddle point parallel to the x and y axes, respectively, c is a measure of the diagonal curvature that is not described by a and b, and increases in the energy of the right edge and top of the diagram are described by d and e, respectively. The movements of the transition state are determined by the curvature at the saddle point, so that the observed movements, as described by the p_{xy}, p_x, and p_y coefficients, can be used to calculate the curvatures a, b, and c.[19,29] The values of $p_{xy} = 0.014$ and $p_x = p_y = 0.006$ give curvatures of $a = 18.8$, $b = 18.8$, and $c = -87.5$. This gives the coordinates $g_1/g_2 = g_2/g_1 = 0.23$ and 4.44 for the two level lines of constant energy that pass through the saddle point.[19] The tangents of 0.23 and 4.4 are 12.7° and 77.3°, respectively, which gives an angle of 64.6° between the two level lines, as shown in Figure 9B. The reaction coordinate that bisects these level lines is 32.3° + 12.7° = 45° relative to the x and y axes. Thus, this reaction coordinate passes through a valley with steeper curvature upward of the walls perpendicular to the reaction coordinate than downward along the reaction path. The relatively small curvature along the reaction coordinate is responsible for movement of the transition state toward the direction of increased energy when the structure of the nucleophile or leaving group is changed, which corresponds to a normal "Hammond effect" and to a net movement of the transition state toward the right in Figure 9A.

$$\Delta G/(2.303RT) = ax^2 + by^2 + cxy + dx + ey + f \quad (10)$$

Equilibrium Constants. The dependence of the equilibrium constants for phosphoryl transfer between pyridines on the basicity of the pyridine, β_{eq}, may be obtained from the relationship $\beta_{eq} = \beta_{nuc} - \beta_{lg}$. *This equation must be applied to the same molecule or group of the same chemical reaction in both directions*, because different nucleophilic reagents and leaving groups have different values of β_{nuc} and β_{lg}. This requirement has not always been met in previous estimations of β_{eq}.

A value of $\beta_{eq} = 1.06$ is obtained from the value of $\beta_{nuc} = 0.17$ for reactions of pyridines of pK_a = ~5 with phosphorylated 3-methoxypyridine and the value of $\beta_{lg} = -0.89$ for reactions of a pyridine of pK_a = 5.1 with phosphorylated 3-methoxypyridine and 4-morpholinopyridine (Table I, Figure 6). A value of β_{eq} = 1.07 is obtained similarly from $\beta_{nuc} = 0.22$ for reactions of pyridines of pK_a ~5 with phosphorylated 4-morpholinopyridine and $\beta_{lg} = -0.85$ for an attacking pyridine of pK_a = 9; correction of β_{nuc} for a nucleophile of pK_a = 9, based on $p_x = \partial\beta_{nuc}/-\partial pK_{nuc} = 0.006$, gives $\beta_{eq} = 1.05$.

The observed rate constants for phosphoryl transfer in both directions between 3-methoxypyridine and 4-morpholinopyridine (Table I) give the equilibrium constant for this reaction directly, $K = 1.03 \times 10^4$. By definition the equilibrium constant for phosphoryl transfer between two molecules of 3-methoxypyridine is unity. These equilibrium constants and $\Delta pK = 3.89$ for these two pyridines give $\beta_{eq} = 1.03$. The data of Jameson and Lawlor for reactions of pyridines with phosphorylated 4-(dimethylamino)pyridine[9] give $k = 3.2 \times 10^{-4}$ M^{-1} s^{-1} for a pyridine of pK_a = 5.1. The rate constant for the reaction of 4-(dimethylamino)pyridine with phosphorylated 3-methoxypyridine (Table I) gives an equilibrium constant of 1.5×10^5 for phosphoryl transfer and a value of $\beta_{eq} = 1.05$. We conclude that the value of β_{eq} for phosphoryl transfer between pyridines is 1.05 ± 0.05.

It is reasonable to expect that the equilibrium constants for transfer of a phosphoryl group will follow a linear free energy relationship with a constant value of β_{eq}. In contrast, the rate constants for phosphoryl transfer give changing values of β_{nuc} and β_{lg} that may be attributed to changing transition-state structure, as discussed above.

This value of β_{eq} means that the influence of polar substituents on the equilibrium constant is slightly larger for addition of a phosphoryl group than for addition of a proton; the "effective charge" that interacts with the polar substituent in phosphorylated pyridines is +1.05. This indicates that the influence of the two negative charges on the phosphoryl group is slightly more than compensated for by electron withdrawal toward phosphorus, which carries a formal positive charge.

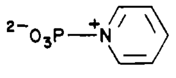
Hydrolysis. Mono- and diprotonation of the phosphoryl group of phosphorylated pyridines increases their rate of hydrolysis (Figure 5), in agreement with the behavior of other phosphorylated amines.[11,30,31] The pH-rate profiles in Figure 5 are consistent with pK_a values of 0.1 and 3.0 for the two ionizations of the diprotonated species of phosphorylated pyridine and phosphorylated 3,4-dimethylpyridine. The rate increase is in constrast with the behavior of phosphate esters and acyl phosphates, which characteristically show rapid reactions of the monoanions and dianions that are not accelerated by a proton on the phosphate group in the transition state.[2,4,8]

These results are consistent with a smaller amount of metaphosphate character in the transition states for cleavage of phosphorylated pyridines compared with oxygen esters. The rapid hydrolysis of the protonated species of phosphorylated pyridines shows that the transition state for cleavage of the unprotonated species does not have extraordinary stability because of its resemblance to metaphosphate monoanion; the protonated species will not give a transition state resembling metaphosphate monoanion, and PO_3H is presumably less stable than PO_3^-.

However, the value of $\beta_{lg} = -1.0$ for the hydrolysis of the four unprotonated phosphorylated pyridines examined here at ionic strength 1.0 shows that there is a large amount of bond cleavage in the transition states of these reactions; this value is close to a value of $\beta_{lg} = -1.13$ for a similar series at ionic strength 0.2 reported by Jameson and Lawlor.[9] These large negative values of β_{lg} for reaction with water, a weak nucleophile, may represent a continuation of the trend toward more negative values of β_{lg} with decreasing basicity of the attacking nucleophile that is described by a positive value of $p_{xy} = \partial\beta_{lg}/\partial pK_{nuc}$ for the reactions with pyridines. The value of $\beta_{lg} = -1.23$ for the cleavage of the dianions of phosphate esters and acyl phosphates is even more negative.[6,7]

The rates of hydrolysis of the mono- and diprotonated species of phosphorylated pyridines show a smaller dependence on the basicity of the leaving pyridine (Figure 5); the value of β_{lg} for these two compounds is -0.78. This is consistent with a tighter transition state with less metaphosphate character for these species. However, this value of β_{lg} is still much more negative than the value of $\beta_{lg} = -0.27$ for the hydrolysis of phosphate monoester monoanions.[7] This is consistent with the proposal that proton transfer from the phosphoryl oxygen to the leaving phenol has occurred in the transition state for the hydrolysis of phosphate monoester monoanions.[2,7]

The expulsion of pyridines from phosphorylated pyridine monoanions (**2**) is ~10^5 times faster than the expulsion of an oxygen anion of the same pK from the dianion of a phosphate ester,[6,7] in spite of the larger metaphosphate character and electrostatic advantage of the ester reactions. The pyridine reactions involve loss of the electrostatic stabilization of a zwitterion, while the ester reactions involve relief of the electrostatic repulsion in the dianion. For the bimolecular reactions with pyridine the difference in rate favors the expulsion of pyridine compared with aryl oxide[6] by a factor of ~10^6. Primary amines are even better leaving groups than pyridines of the same pK_a, by a factor of 50;[31] primary amines behave like pyridines of lower pK_a when they act as either a nucleophile or a leaving group.



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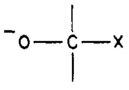

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On the other hand, amines are expelled slower than oxygen anions of the same pK_a from carbon to form a carbonyl group when the driving force arises from an oxygen anion, **3**.[32] Amines are expelled ~10^5 times faster than oxygen anions from uncharged addition compounds of a phthalimidium ion, but the expulsion of oxygen is slowed by a requirement for charge separation.[33]

The relatively slow expulsion of oxygen anions from phosphate compounds is presumably another reflection of the well-known high affinity of phosphorus for oxygen.

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Registry No. 2, 26322-03-2; $POCl_3$, 10025-87-3; $PO_2Cl_2^-$, 47986-81-2; phosphorylated 3-methoxypyridine, 92670-30-9; phosphorylated 4-morpholinopyridine, 26322-06-5; phosphorylated 3,4-dimethylpyridine, 92670-28-5; 3-cyanopyridine, 100-54-9; 4-cyanopyridine, 100-48-1; 3-chloropyridine, 626-60-8; 3-formamidopyridine, 98-92-0; 4-formamidopyridine, 1453-82-3; 3-carboxypyridine, 59-67-6; 3-methoxypyridine, 7295-76-3; pyridine, 110-86-1; 3-methylpyridine, 108-99-6; 4-methylpyridine, 108-89-4; 3,4-dimethylpyridine, 583-58-4; 4-(*N*-morpholino)pyridine, 2767-91-1; 4-(dimethylamino)pyridine, 1122-58-3; semicarbazide, 57-56-7; methoxyamine, 67-62-9; trifluoroethylamine, 29010-16-0; ethylenediamine monocation, 26265-69-0; glycinamide, 598-41-4; ethylenediamine, 107-15-3; ethylamine, 75-04-7; triethylaminediamine, 100-36-7; piperidine, 110-89-4; quinuclidine, 100-76-5; pyrrolidine, 123-75-1.

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