Phosphoryl Transfer between Pyridines[1]

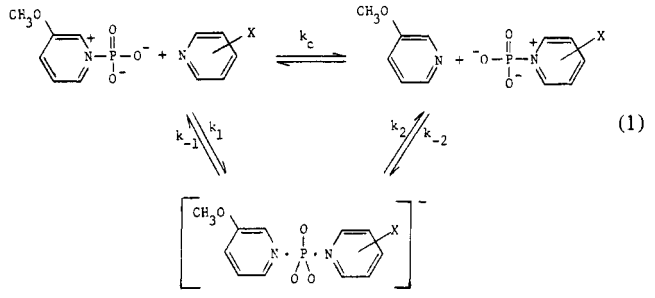
Mark T. Skoog and William P. Jencks*

Graduate Department of Biochemistry, Brandeis University Waltham, Massachusetts 02254†

Received December 13, 1982

We describe here a linear Brønsted-type relation for phosphoryl transfer from 3-methoxypyridine to pyridines of both larger and smaller basicity that provides evidence for a preassociation mechanism with similar or identical bonding to the nucleophile and to the leaving group in the transition state (Figure 1). The simplest interpretation is that the reaction proceeds through a concerted preassociation mechanism with no metaphosphate anion intermediate; however, a stepwise preassociation mechanism with weak bonding to the entering and leaving pyridines in the transition states of both steps is not excluded.

Most hydrolysis and transfer reactions of the monoanions and dianions of monosubstituted phosphates in hydroxylic solvents proceed through transition states that closely resemble the monomeric metaphosphate anion, and it has been suggested, but not proved, that these reactions proceed through a metaphosphate anion intermediate.[2,3] If there is an intermediate in these reactions, its lifetime is too short to permit diffusion in mixed nucleophilic solvents, so these reactions are required to proceed through a preassociation mechanism.[3d,e,4] The question remains whether the preassociation mechanism is stepwise with a metaphosphate anion intermediate (k_1 and k_2, eq 1) or concerted with no intermediate (k_c, eq 1).



(1)

The essential difference between these two mechanisms is that there is a single, central transition state for the concerted mechanism with identical entering and leaving groups, whereas the stepwise mechanism involves two different transition states and must undergo a change in the rate-limiting step with changing basicity of the nucleophile (Figure 2). If phosphoryl transfer from 3-methoxypyridine to substituted pyridines occurs through a stepwise mechanism with a metaphosphate anion intermediate (k_1 and k_2, eq 1), there must be a change in the rate-limiting step as the reactivity of the nucleophile is varied. When the substituted pyridine is less nucleophilic than 3-methoxypyridine, reaction of the substituted pyridine with PO_3^- would be slower than the reaction of 3-methoxypyridine with PO_3^- and the step described by k_2 and transition state b would be rate limiting (solid line,

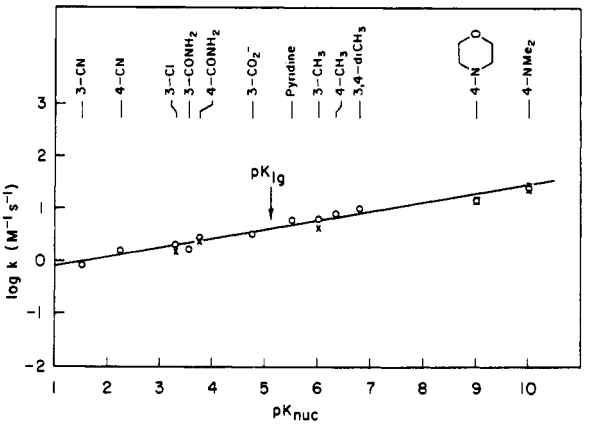


Figure 1. Brønsted plot for phosphoryl transfer from 3-methoxypyridine to a series of substituted pyridines at 25 °C, ionic strength 1.0 M (KCl). × represents rate constants calculated without correcting for self-association of the nucleophile.[3b,7]

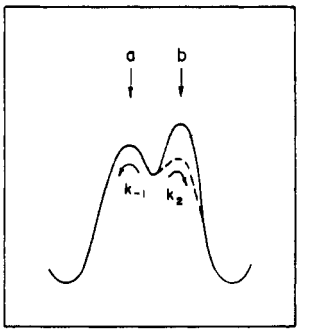


Figure 2. Reaction coordinate diagram illustrating a change in the rate-limiting step in the stepwise pathway of eq 1 as the substituted pyridine changes from less nucleophilic (solid line) to more nucleophilic (dashed line) than 3-methoxypyridine.

Figure 2). When the substituted pyridine is more nucleophilic than 3-methoxypyridine, the step described by k_1 and transition state a would be rate limiting (dotted line, Figure 2). These two transition states (a and b, Figure 2) must differ in the amount of bond formation and cleavage to the entering and leaving pyridines. Thus, a stepwise reaction mechanism is expected to show a nonlinear Brønsted-type correlation against the pK_a of the attacking pyridine, with slopes corresponding to the β_{nuc} values for steps one and two and a break at the pK_a of the leaving pyridine.[5] A fully concerted reaction with a single, symmetrical transition state will give a linear Brønsted plot or a gently curving plot if there is some change in transition state structure.

The Brønsted-type plot for phosphoryl transfer between 3-methoxypyridine and a series of substituted pyridines is consistent with a linear correlation and a slope of $\beta_{nuc} = 0.17$ (Figure 1). This means that for both weakly and strongly basic pyridines there is a small amount, ~15%, of positive charge development and bond formation in the "open" transition state of these reactions, similar to that observed for other reactions of monosubstituted phosphates.[3] Since phosphoryl transfer between pyridines is a symmetrical reaction, there must also be ~15% positive charge and bonding for the leaving pyridine. There is no evidence for a change in the rate-limiting step.

The results are consistent with a concerted reaction mechanism in which a single, symmetrical transition state involves weak bonding to both the entering and leaving groups and there is no intermediate. They exclude a preassociation stepwise mechanism in which bond formation or cleavage involves only one pyridine molecule in each step. Such a mechanism would give $\beta_{nuc} = 0$ for basic pyridines. The maximum curvature of the Brønsted line that is consistent with the estimated error of the data corresponds to $\delta\beta/-\delta pK_{nuc} = p_x = 0.015$. Consequently, the results are also

† Contribution No. 1444.

(1) Supported by grants from the National Science Foundation (PCM 81-17816) and the National Institutes of Health (GM20888). M.T.S. was supported by a postdoctoral fellowship from the National Institutes of Health (GM08317).

(2) (a) Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313. (b) Satterthwait, A. C.; Westheimer, F. H. *J. Am. Chem. Soc.* **1981**, *103*, 1177. (c) Rebek, J., Jr.; Gaviña, F.; Navarro, C. *Ibid.* **1978**, *100*, 8113. (d) Ramirez, F. Marecek, J. F. *Ibid.* **1979**, *101*, 1460.

(3) (a) Benkovic, S. J.; Schray, K. J. In "Transition States of Biochemical Processes"; Gandour, R. D. Schowen, R. L., Eds.; Plenum Press, New York, 1978; Chapter 13, p 493. (b) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209. (c) Kirby, A. J.; Varvoglis, A. G. *J. Chem. Soc. B* **1968**, 135. (d) Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1963**, *85*, 1181. (e) Kirby, A. J.; Varvoglis, A. G. *Ibid.* **1967**, *89*, 415. (f) Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 53. (g) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 1438.

(4) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345.

(5) Knier, B. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 6789.

0002-7863/83/1505-3356$01.50/0

consistent with a change in the structure of the transition state for a concerted reaction with changing basicity of the nucleophile or a stepwise preassociation mechanism in which there is weak nucleophilic interaction with the attacking pyridine in *both* steps, with β_{nuc} = 0.1 and 0.2, for example.

3-Methoxypyridine was synthesized by the method of Prins.[6] Phosphorylation of 3-methoxypyridine was effected by the forceful injection of a solution containing 0.3 M 3-methoxypyridine in 0.90 M KOH into a small tube containing 1 equiv of $POCl_3$. All solutions were at 4 °C. An aliquot of this synthesis mixture was applied immediately to the side of a quartz cell in the spectrophotometer cell holder, and the reaction was initiated by forceful injection of a solution containing the desired concentration of nucleophile, 0.050 M carbonate buffer, and 1.0 M KCl at pH 10.3 and 25 °C. 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 more than 3 half-lives in each case. Second-order rate constants were obtained from the slopes of plots of values of k_{obsd} vs. the concentration of nucleophile in the range 0–0.2 M; the observed increase in k_{obsd} was always >100%. The rates of reaction with 4-morpholinopyridine and 4-(dimethylamino)-pyridine were determined by spectrophotometric analysis of the ratio of products. No catalysis by buffers was observed.

Acknowledgment. We appreciate correspondence with Dr. A. Williams.

Registry No. 3-Cyanopyridine, 100-54-9; 4-cyanopyridine, 100-48-1; 3-chloropyridine, 626-60-8; 3-pyridinecarboxamide, 98-92-0; 4-pyridinecarboxamide, 1453-82-3; 3-pyridinecarboxylate, 3308-39-2; pyridine, 110-86-1; 3-methylpyridine, 108-99-6; 4-methylpyridine, 108-89-4; 3,4-dimethylpyridine, 583-58-4; 4-morpholinopyridine, 2767-91-1; 4-(dimethylamino)pyridine, 1122-58-3; 3-methoxypyridine, 7295-76-3.

(6) Prins, D. A. *Recl. Trav. Chim.* Pays-Bas **1957**, *76*, 58.
(7) (a) Andon, R. J. L.; Cox, J. D.; Herington, E. F. G. *J. Chem. Soc.* **1954**, 3188. (b) Ibl, N.; Dändliker, G.; Trümpler, G. *Helv. Chim. Acta* **1954**, *37*, 1661.

The Question of Concerted or Stepwise Mechanisms in Phosphoryl Group ($-PO_3^{2-}$) Transfer to Pyridines from Isoquinoline-*N*-phosphonate

Nicholas Bourne and Andrew Williams*

University Chemical Laboratories, Canterbury, England

Received December 13, 1982

The existence of the metaphosphate monomer as an intermediate has been the subject of considerable scrutiny in the hydrolysis and transfer reactions of monophosphate esters and amides.[1-3] Recent studies on the methanolysis of phenyl phosphates bearing an asymmetric phosphorus atom[3] have indicated inversion of configuration at phosphorus, consistent with either a concerted mechanism or a preassociation stepwise mechanism where metaphosphate monomer reacts in the encounter complex before it can escape into bulk solution. It is possible that inversion observed in enzyme-catalyzed phosphonyl group transfer reactions[4] could be due to a mechanism similar to the latter with the metaphosphate group reacting at the active site before it can "tumble" and hence cause racemization.

We decided to study phosphonyl group transfer from a pyridine-*N*-phosphonate donor to a series of unhindered pyridine acceptors because the reaction must be symmetrical when acceptor and donor basicities are identical. The preassociation mechanism (eq 1) predicts a curved Brønsted plot and has a theoretical rate

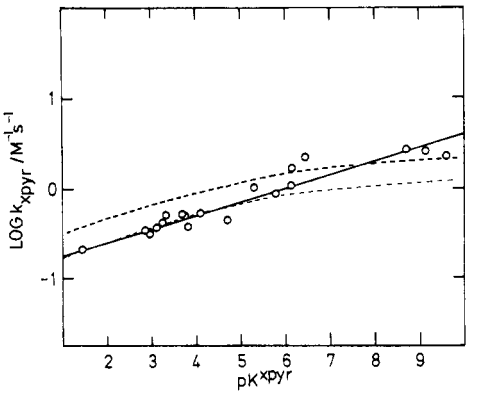


Figure 1. Reactivity of pyridines against isoquinoline-*N*-phosphonate as a function of the pK of the pyridine; 25 °C, ionic strength 0.2 M. Identity of the pyridines in increasing order of pK: 3-CN, 3-Br, 3-Cl, 3-MeOCO, 3-Ac, 3-CHO, 4-Br, 4-Cl, 3-CH_2CN, 4-CHO, H, 3-Me, 4-Me, 3,5-Me_2, 3,4-Me_2, 4-morpholino, 4-NH_2, 4-Me_2N. The line is calculated from the equation log k_{xpyr} = (0.15 ± 0.01)pK^{xpyr} − (0.87 ± 0.07) (r = 0.946), which is the best linear fit to the data points. The dashed curves are theoretical (see equation in text) for β_N = 0.2 drawn to fit either high or low pK points.

$$^{+}\text{isq}\text{–}PO_3^{2-} + \text{xpyr} \underset{}{\overset{k_1}{\rightleftharpoons}} {}^{+}\text{isq}\text{–}PO_3^{2-}\cdot\text{xpyr} \overset{k_2}{\rightleftharpoons} \text{isq}\cdot\text{xpyr}\cdot PO_3^{-} \overset{k_3}{\rightleftharpoons} \text{isq}\cdot{}^{+}\text{xpyr}\text{–}PO_3^{2-} \overset{k_4}{\rightleftharpoons} \text{isq} + {}^{+}\text{xpyr}\text{–}PO_3^{2-} \quad (1)$$

law $k/k_0 = 1/(1 + 10^{\Delta pK\beta_N})$ where $\Delta pK = pK^{isq} - pK^{xpyr}$, k_0 is the value of k when k_2 is rate limiting, and β_N is the Brønsted exponent when attack of acceptor pyridine is rate limiting (k_3). The mechanism assumes that k_2 and k_3 are independent of the "spectator" pyridines xpyr and isq in the respective steps.

The Brønsted relationship for the theoretical equation gives a family of normalized theoretical curves for log k/k_0 dependent only on β_N and ΔpK and these are illustrated in the following paper.[5] The concerted mechanism would give a linear Brønsted plot consistent with a transition state that does not change its structure over the range in question for a series of structurally related nucleophiles.[6]

The pyridinolysis of isoquinoline-*N*-phosphonate[7] was conveniently measured spectrophotometrically at 350 nm[8] by using buffers composed of hindered amines at low concentrations at pHs between 8 and 12. Isoquinoline-*N*-phosphonate was prepared in solution by mixing 0.5 mL of a stock of 0.5 mL of isoquinoline in 40% acetonitrile/water (v/v, 10 mL) with 10 mg of ammonium phosphoramidate; the stock was used directly in kinetics without attempting to isolate a solid product and was discarded after 2 h. The reaction with pyridines obeys good first-order kinetics and the rate law is $k_{obsd} = k_{intercept} + k_{xpyr}[xpyr]$ and is independent of the acid form of the pyridine. The value of $k_{intercept}$ is composed of buffer and water reaction; amines possess a significant reactivity toward pyridine-*N*-phosphonates.[9] The second-order rate constant (k_{xpyr}) obeys an excellent linear Brønsted-type relationship (Figure 1).

The dependence of the rate constant for phosphonyl group transfer on the concentration of the acceptor nucleophile excludes

(1) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345.
(2) Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313.
(3) Buchwald, S.; Knowles, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 1438.
(4) Frey, P. A. *Tetrahedron* **1982**, *38*, 1541.
(5) Hopkins, A.; Bourne, N.; Williams, A., following paper in this issue.
(6) Bordwell, F. G.; Hughes, D. L. *J. Org. Chem.* **1982**, *47*, 3224.
(7) The rate constant for the aqueous decomposition of the stock solution (k_{H_2O}) as measured by the decay at 350 nm agreed well with the value predicted from the Brønsted relationship for the hydrolysis of substituted pyridine-*N*-phosphonates;[9] we are thus confident that we are measuring the degradation of isoquinoline-*N*-phosphonate. It is not possible to characterize reactive pyridine-*N*-phosphonates in the normal way.
(8) The pyridine concentrations were kept below 0.05 M to prevent complications due to self-association. Experiments at different pHs with basic pyridines confirmed that only the base form was reacting.
(9) Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc., Perkin Trans. 2* **1970**, 53.

0002-7863/83/1505-3357$01.50/0