On the Mechanism of Buffer-Catalyzed Hydrolysis of RNA Models

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An alternative mechanism is proposed for the simultaneous hydrolysis and isomerization of 3′,5″-uridyluridine and related dialkyl phosphates in aqueous morpholine buffers, which was studied by Breslow and Xu. This alternative mechanism leads to kinetic equations that reproduce the experimental data.

Introduction

Breslow and Xu[1] have studied the chemistry of 3′,5″-uridyluridine (UpU, **1**) in aqueous morpholine buffers at 80 °C. They found that hydrolysis, to form uridine 2′,3′-cyclic phosphate (**2**) plus uridine, is accompanied by isomerization, to form 2′,5″-uridyluridine (**3**). By HPLC and the initial-rate method they could measure the rate constants k_c and k_i for cleavage and isomerization, respectively, at three different buffer ratios.

One of the unusual features of these kinetics is that the rate of isomerization decreases with increasing buffer concentration. In an earlier paper[2] this was called "negative catalysis", involving negative rate constants, which elicited vehement objection.[3] The fallacy is in assuming that the uncatalyzed reaction is an independent one that proceeds normally in the presence of buffer.[4] However, much of this is a semantic dispute.

For other reasons we felt that the mechanism warrants reinterpretation, especially since the original interpretation has been affirmed in reviews.[5] Accounts of obstacles to publishing criticisms of these papers have also been presented.[6] It is important to understand the mechansim of this reaction, since it serves as a nonenzymatic model for the hydrolysis of RNA, although the enzyme ribonuclease differs by using one imidazole as a catalytic base and an imidazolium ion as a catalytic acid.[7] We now propose an alternative mechanism for the simultaneous nonenzymatic hydrolysis and isomerization of dialkyl phosphates that are models for RNA.

Discussion

Previous Interpretation. Below 1 M buffer the rate constants of Breslow and Xu[1] could be fit to eqs 1 and 2, where the buffer concentration C and buffer ratio r are specified in eqs 3 and 4 and where the six fitting parameters $a-f$ are defined in eqs 5−10. Above 1 M buffer there were small deviations that required an additional seventh parameter, but we shall ignore these conditions as being too far from ideality.[8]

$$k_c = \frac{r(1-r)dC^2 + eC + f}{bC + c} \tag{1}$$

$$k_i = \frac{rC + a}{bC + c} \tag{2}$$

$$C = [\mathrm{B}] + [\mathrm{BH}^+] \tag{3}$$

$$r = \frac{[\mathrm{BH}^+]}{[\mathrm{B}] + [\mathrm{BH}^+]} \tag{4}$$

$$a = \frac{k''_w}{k_1} \tag{5}$$

$$b = \frac{rk_{-1} + (1-r)k_2}{k_1k_3} \tag{6}$$

$$c = \frac{k_3 + k_w}{k_1k_3} \tag{7}$$

$$d = \frac{k_2}{k_3} \tag{8}$$

$$e = \frac{(1-r)k' + rk''}{k_1k_3} \tag{9}$$

$$f = \frac{k'_w}{k_1k_3} \tag{10}$$

These equations correspond to the mechanism in Scheme 1. The key feature is a phosphorane intermediate (**4**), which partitions between cleavage and isomerization. The observed decrease in k_i with increasing C is then due simply to the partitioning of that intermediate, whose cleavage is catalyzed by buffer. Indeed, the form of eq 2, with C in the denominator, can adequately account for this decrease.

According to this mechanism, the processes of formation, reversion, and cleavage of intermediate **4** are respectively general-acid-, general-acid-, and general-

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[®] Abstract published in *Advance ACS Abstracts,* February 15, 1995.

(1) Breslow, R.; Xu, R. *J. Am. Chem. Soc.* **1993**, *115*, 10705.

(2) Breslow, R.; Huang, D.-L. *J. Am. Chem. Soc.* **1990**, *112*, 9621.

(3) Menger, F. M. *J. Org. Chem.* **1991**, *56*, 6251.

(4) If $k_{obs} = (a[\mathrm{buffer}] + b)/(c[\mathrm{buffer}] + d)$, it is not justified to derive a rate constant "corrected for the buffer independent rate" by subtracting b/d, the rate constant at zero buffer.

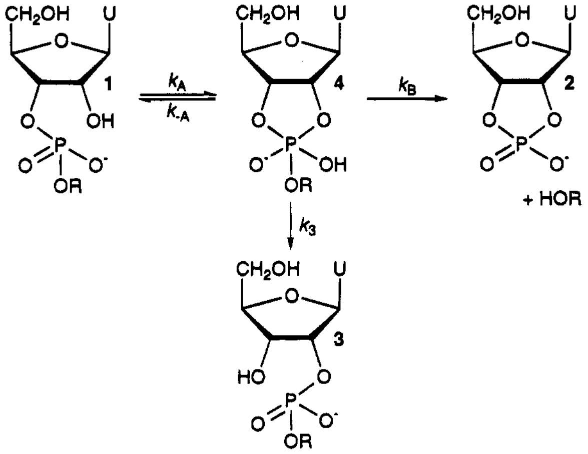
(5) Anslyn, E.; Breslow, R.; Huang, D.-L. *Tetrahedron* **1991**, *47*, 2365. Breslow, R. *Acc. Chem. Res.* **1991**, *24*, 317.

(6) Menger, F. M.; Haim, A. *Nature* **1992**, *359*, 666. Zurer, P. *Chem. Eng. News* **1992**, *70* (Oct 26), 6.

(7) Blackburn, R.; Moore, S. In *The Enzymes*; Academic: New York, 1982; Vol. 15, p 317.

(8) Above 1 M buffer there could be a generalized solvent effect due to the morpholine.

Scheme 1. Breslow's Mechanism for Buffer-Catalyzed UpU Hydrolysis



base-catalyzed, with rate constants as in eqs 11–13, but

$$k_A = k_1[BH^+] + k_{1w} + k_H[H^+] \quad (11)$$

$$k_{-A} = k_{-1}[BH^+] + k_{-1w} + k_{-H}[H^+] \quad (12)$$

$$k_B = k_2[B] + k_{2w} + k_{OH}[OH^-] \quad (13)$$

conversion of **4** to the isomeric UpU **3** is simply water-catalyzed, with rate contant k_3. The relations between the rate constants of Scheme 1 or eqs 11–13 and the parameters of eqs 5–10 are given in eqs 14–18. However, it must be kept in mind that there are only six independent parameters that were fit, so that not all ten rate constants of Scheme 1 can be evaluated. Actually none of these rate constants were evaluated, so there is no test of whether they have the proper dependence on $[H^+]$ or $[OH^-]$. Below it is shown that they do not.

$$k_w = k_{-1w} + k_{2w} + k_{OH}[OH^-] + k_{-H}[H^+] \quad (14)$$

$$k'_w = (k_{1w} + k_H[H^+])(k_{2w} + k_{OH}[OH^-]) \quad (15)$$

$$k' = k_{1w}k_2 + k_1k_{OH}[OH^-][BH^+]/[B] \quad (16)$$

$$k'' = k_1k_{2w} + k_Hk_2[H^+][B]/[BH^+] \quad (17)$$

$$k''_w = k_{1w} + k_H[H^+] \quad (18)$$

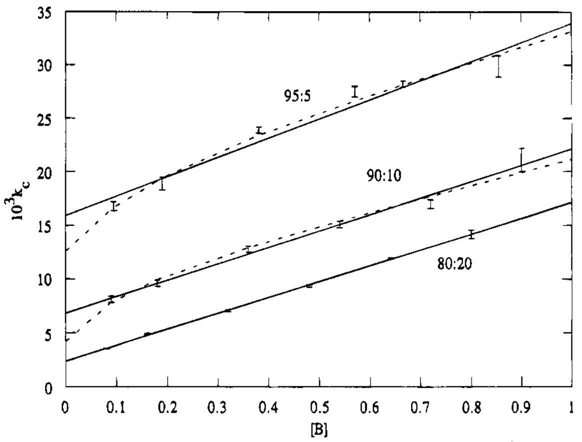


Figure 1. Rate constants for cleavage of UpU at 80 °C in aqueous buffers of designated [morpholine]:[morpholinium]. Data, with error bars, are from Breslow and Xu.[1] Dashed lines are fits to eq 1 using the parameters of eqs 5–10 as listed in their Table IV. Solid lines are from the fits to the linearized eq 24.

Questions. A "curious" [9] feature of this mechanism is that the first step is catalyzed by acid and the second, leading to cleavage, is catalyzed by base, even though cleavages of two nearly identical alkoxy groups ought to proceed by the same catalytic pathway.[10] This duality of catalysis is what leads to the term in eq 1 that is first order in [B] and first order in $[BH^+]$, or second order overall in *C*. The experimental evidence for this term was an apparent maximum in plots of k_c vs the state of protonation of an imidazole buffer.[9,11] However, the initial data came from studies where the ionic strength was not controlled for reactions of a polyelectrolyte.[9] Even if the ionic strength is maintained constant, 1 M ImH^+Cl^- is so far beyond the Debye–Hückel limiting region that specific-ion effects intrude, and 1 M imidazole could produce a medium effect.[12] Besides, all rates in true buffers are nearly the same, within experimental error, and a maximum appears only because rates are lower in solutions with zero or infinite buffer ratios.[11] Since these are not truly buffered, pH varies widely. When the experiments were carried out under controlled conditions but with morpholine buffers,[1] the maxima were not confirmed. Since the maxima were the key evidence for Scheme 1, this mechanism warrants reinvestigation and reinterpretation.

The cumbersome form of the above equations makes it difficult to analyze the data. Moreover, different equations, including terms introduced in an ad hoc manner, were presented in an earlier version,[2] rates were labeled as rate constants,[11] there were transcription errors, and rates at zero buffer were subtracted to obtain "corrected rates", so that an attempt to verify the fit of the data to the equations was a formidable task.[13]

Of course the data are likely to fit the equations, since there are so many parameters. Of the six parameters that were fit (Table IV of Breslow and Xu),[1] two have errors (standard deviations) comparable to the values themselves, and in their Table V four of seven have such errors. Therefore those parameters are meaningless, i.e., not significantly different from zero. There can be no justification for including meaningless parameters in the fitting. This conclusion suggests that it ought to be possible to obtain a simpler expression.

Alternative Mechanism. Before proposing a mechanism, it is wise to look at the data. For reasons that will become apparent, it is more convenient to focus on k_c/k_i, the ratio of the two rate constants, along with k_c. These two quantities, in each of the three buffers, from Tables I–III of Breslow and Xu,[1] are plotted in Figures 1 and 2. The error bars shown are theirs, as obtained from duplicate kinetic runs. Unlike previous figures,[1,2,9,11]

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[9] Breslow, R.; Labelle, M. *J. Am. Chem. Soc.* **1986**, *108*, 2655.

[10] Were the leaving groups truly identical, this would be a violation of the principle of microscopic reversibility, in that the forward reaction, to form the intermediate, would be acid-catalyzed while the reverse would be base-catalyzed.

[11] Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 4473.

[12] The apparent maximum might be due to retardation by nonpolar imidazole (at high concentrations) or to a positive salt effect of ImH^+ (which is lost at high proportions of buffer base), superimposed on base catalysis. Either of these is reasonable for the reaction of an anionic substrate with hydroxide.

[13] Haim, A. *J. Am. Chem. Soc.* **1992**, *114*, 8384.

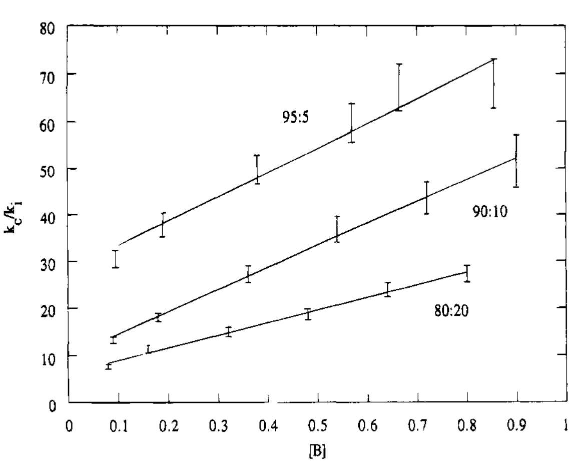


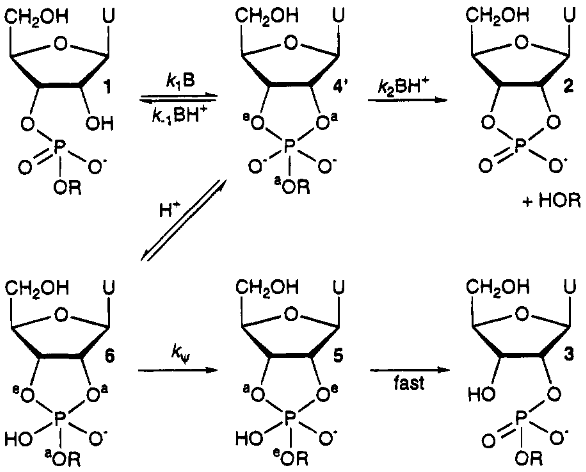
Figure 2. Ratio of rate constants for cleavage and isomerization of UpU at 80 °C in aqueous buffers of designated [morpholine]:[morpholinium]. Data, with error bars, are from Breslow and Xu.[1] Solid lines are fits to eq 21.

which were plotted against total buffer concentration, these are plotted against the concentration of the buffer base.

For each buffer the cleavage rate constant or the ratio can be fit to a linear equation. As can be seen from the solid lines in Figures 1 and 2, the fits are quite good (although Figure 1 actually represents a fit to an almost linear equation, as described below). The lowest correlation coefficient is 0.977. For comparison, the fits of k_c to the nonlinear eq 1,[1] using the parameters in their Table IV, are shown as dashed.[14] In the 95:5 buffer the data suggest curvature, but these data have the largest errors, and they do not deviate markedly from the linear fit. The data for the 90:10 buffer certainly seem linear. This linearity was acknowledged[1] but viewed as arising from two terms, arbitrarily separated, whose curvatures compensate.[15] The linearity of k_c/k_i as seen in Figure 2 has been noted by Haim[13] and also observed in imidazole buffers.[1]

In Figure 1, not only do the rates increase with buffer concentration but also the intercepts increase with increasing pH. This looks like a textbook example of general-base catalysis. It certainly does not provide support for a mechanism whose first step is acid-catalyzed, as claimed by Breslow and Xu.[1]

Scheme 2. Proposed Mechanism for Buffer-Catalyzed UpU Hydrolysis



The recognition of general-base catalysis suggests an alternative mechanism that is consistent with the data of Breslow and Xu.[1] This is presented in Scheme 2, where B can be either OH^- or morpholine. A key feature is that the first step is catalyzed by base, with pseudo-first-order rate constant $k_{1B}[B] + k_{1OH}[OH^-]$ (B = morpholine). Then since the reactant is a monoanion, the immediate product of that first step must be the dianionic form of the phosphorane intermediate (**4'**). This differs from Scheme 1, where intermediate **4** is a monoanion. It further follows by microscopic reversibility that only the dianion can revert to reactant and that that reversion must be general-acid-catalyzed, with rate constant $k_{-1B}[BH^+] + k_{-1OH}$. Cleavage of **4'** to product is also assumed to require the dianion and to be general-acid-catalyzed, with rate constant $k_{2B}[BH^+] + k_{2OH}$. Isomerization to **3** cannot proceed, since the 3' oxygen in **4'** is equatorial (e) and a pseudorotation to **5**, with rate constant k_ψ, is necessary to make this oxygen apical (a). It is further assumed that only the monoanion **6** undergoes pseudorotation, since pseudorotation of the dianion **4'** would require placing an nonapicophilic oxyanion in the apical position.[16] Since $k_c > k_i$, **5** undergoes cleavage more rapidly than it pseudorotates again, so that the pseudorotation is the rate-limiting step for isomerization. This might not be the case at lower pH in imidazole buffers,[2,11] where pseudorotation becomes reversible. The mechanism can be adapted for this complication, as well as for pseudorotation of the neutral phosphorane.

To derive the rate expression associated with this mechanism, it is necessary to know the state of protonation of the intermediate. Its pK_1 has been estimated as 6.5,[17] but this is uncertain, of unknown temperature dependence, and uncomfortably close to the pH of the buffer solutions (pK_a 7.4 extrapolated[18] to 80 °C). For simplicity we assume that under the experimental conditions the dominant form of the intermediate is the monoanion **6**. The steady-state treatment then leads to eq 19 for the rate constant for cleavage and eq 20 for the

$$k_c = \{k_{1OH}[OH^-] + k_{1B}[B]\}[k_{2OH}(K_2/K_w)[OH^-] + k_{2B}(K_2/K_a)[B]]/[(k_{-1OH} + k_{2OH})(K_2/K_w)[OH^-] + (k_{-1B} + k_{2B})(K_2/K_a)[B] + k_\psi] \quad (19)$$

$$k_i = \{k_{1OH}[OH^-] + k_{1B}[B]\}[k_\psi/[(k_{-1OH} + k_{2OH})(K_2/K_w)[OH^-] + (k_{-1B} + k_{2B})(K_2/K_a)[B] + k_\psi]] \quad (20)$$

rate constant for isomerization, where K_2 is the second acid-dissociation constant of the phosphorane intermediate, K_a is the acid-dissociation constant of the buffer, and $K_w = [H^+][OH^-]$. Although rate constants k_{-1} and k_2 correspond to acid-catalyzed reactions in Scheme 2, they involve the conjugate base **4'** of the dominant form **6** and

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(14) The curve for the 95:5 buffer differs from that shown in Breslow and Xu's Figure 4B, but the y intercept must be $10^3 f/c$, or 12.6, not the 6 shown in their figure.

(15) With their parameters for the 90:10 buffer in Table IV, eq 1 becomes $k_c = (3.06C^2 + 3.6C + 0.18)/(300C + 43)$. This numerator can be factored as $(300C + 15.7)(0.0102C + 0.0115)$. Since $c = 43 \pm 36$ in the denominator, 15.7 is within experimental error of 43. Therefore $300C + 15.7$ cancels between numerator and denominator and k_c simplifies to the linear form, $0.0102C + 0.0115$.

(16) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.

(17) Guthrie, J. P. *J. Am. Chem. Soc.* **1977**, *99*, 3991.

(18) Hetzer, H. B.; Bates, R. G.; Robinson, R. A. *J. Phys. Chem.* **1966**, *70*, 2869.

therefore enter as terms that are first order in base. If there is appreciable neutral phosphorane intermediate, with the same k_ψ for its pseudorotation (for simplicity), K_2 must be replaced throughout by $K_2K_1/([H^+] + K_1)$.

These are multiparameter nonlinear expressions that can be fit to the data. They are actually less complicated than they appear, since the rate and equilibrium constants that are required to express both the state of protonation and the partitioning of the intermediate in Scheme 2 could have been combined into a smaller number of fitting parameters. However, the data for k_c in Figure 1 appear to be simply linear in [B]. Consequently there are too many parameters to be evaluated, and the errors associated with them will be large.

One simplification of eqs 19 and 20 results from considering the ratio of the two rate constants, as in eq 21. According to this equation, a plot of this ratio ought

$$\frac{k_c}{k_i} = \frac{k_{2OH}(K_2/K_w)[OH^-] + k_{2B}(K_2/K_a)[B]}{k_\psi} \qquad (21)$$

to be linear in the concentration of the base component of the buffer, with constant slope, and the intercept ought to be proportional to the hydroxide concentration, or inversely proportional to $[H^+]$. This decrease of isomerization with increasing base or buffer arises because cleavage is a general-acid-catalyzed reaction of the dianion **4′** and thus apparently general-base-catalyzed, whereas isomerization requires the monoanion **6**. Such a plot is shown in Figure 2, for three different buffer ratios. The slopes are not truly constant, but the reduction in the 80/20 buffer may be due to the presence of an appreciable proportion of the neutral form of the intermediate, which can be shown to decrease k_c/k_i. From the two slopes in the more alkaline solutions the average value of $k_{2B}K_2/K_ak_\psi$ is 50 ± 4 M^{-1}. From the three intercepts and a buffer pK_a of 7.4, the average value of $k_{2OH}K_2/k_\psi$ is $(5.6 \pm 1.0) \times 10^{-8}$. The small standard deviations associated with these estimates are evidence that eq 21 describes k_c/k_i well. However, Breslow's mechanism also leads to an expression for k_c/k_i that is linear in buffer concentration.[1,13] Thus both mechanisms account for the observation that k_i decreases with increasing buffer concentration.

Another simplification of eq 19 comes from considering the partitioning of intermediate **4′**. Let P_2 be the probability that **4′** proceeds to cleavage product, rather than revert to reactant, as in eq 22. For simplicity we assume that P_2 is the same regardless of catalyzing acid. Then eq 19 becomes eq 23, which is still nonlinear in [B] but where the right-hand fraction can be evaluated independently.

$$P_2 = \frac{k_{2OH}}{k_{-1OH} + k_{2OH}} = \frac{k_{2B}}{k_{-1B} + k_{2B}} \qquad (22)$$

$$k_c = \{k_{1OH}P_2[OH^-] + k_{1B}P_2[B]\}[k_{2OH}K_2[OH^-]/K_wk_\psi + k_{2B}K_2[B]/K_ak_\psi]/[k_{2OH}K_2[OH^-]/K_wk_\psi + k_{2B}K_2[B]/K_ak_\psi + P_2] \qquad (23)$$

A value for P_2 can be estimated from hydrolysis of the model compound, methyl ethylene phosphate. At pH 5–6, pseudorotation occurs fast enough to transform the initially formed intermediate into one analogous to **6**, with alkoxy apical. Under these conditions the proportion of exocyclic cleavage is 25%.[19] (The deviation from the statistical 50% is a small effect that arises because a 2-alkoxyethoxide is a better leaving group than methoxide.)

With $P_2 = 0.25$ and with the above $k_{2B}K_2/K_ak_\psi$ and $k_{2OH}K_2/k_\psi$ from the slopes and intercepts of Figure 1, the right-hand fraction in eq 23 can be evaluated for each buffer. It is almost exactly unity for all. This then is a correction that can be applied to the observed k_c to obtain a corrected rate constant $k_{c,corr}$, defined in eq 24. The correction is exceedingly small, and it is only slightly sensitive to the value of P_2. Comparison with eq 23 then leads to the expression on the right-hand side of eq 24.

$$k_{c,corr} \equiv \frac{k_{2OH}K_2[OH^-]/K_wk_\psi + k_{2B}K_2[B]/K_ak_\psi + P_2}{k_{2OH}K_2[OH^-]/K_wk_\psi + k_{2B}K_2[B]/K_ak_\psi}k_c = k_{1OH}P_2[OH^-] + k_{1B}P_2[B] \qquad (24)$$

According to eq 24 this corrected $k_{c,corr}$ is simply linear in the concentration of the base component of the buffer, with constant slope, and with an intercept proportional to the hydroxide concentration, or inversely proportional to $[H^+]$. Such a plot is shown in Figure 1 for three different buffer ratios. The plot is of k_c itself, but the solid lines are taken from the least-squares fits to the linearized eq 24. The indetectability of curvature in those lines is further evidence that the correction in converting the observed k_c to $k_{c,corr}$ is small. The slopes are indeed reasonably constant, corresponding to an average value for $k_{1B}P_2$ of $(1.6 \pm 0.2) \times 10^{-2}$ M^{-1} h^{-1}. The intercepts are indeed proportional to $[OH^-]$, and with K_w at 80 °C[20] the constant of proportionality corresponds to a value for $k_{1OH}P_2$ of $(1.2 \pm 0.2) \times 10^2$ M^{-1} h^{-1}. Again the small standard deviations are evidence that eq 24 describes k_c well. This is good support for the mechanism of Scheme 2 and suggests that it is not necessary to invoke the more complicated mechanism of Scheme 1, with its attendant eqs 1–18. Indeed, the constancy of the slopes provides no evidence for a maximum versus buffer ratio, which was the evidence for the term in k_c that is second order in C and for a mechanism in which the first step is catalyzed by acid and the second is catalyzed by base.[9,11]

Thus one advantage of our mechanism is that it leads to eqs 21 and 24 that reproduce the experimental linearities with buffer concentration as well as the pH dependence of the intercepts, at least over the narrow range studied. A further advantage is that it restores the "symmetry" of the reaction, in that cleavages of three nearly identical alkoxy groups are all general-acid-catalyzed, rather than one catalyzed by buffer acid, one by buffer base, and one by neither. This mechanism may be wrong or oversimplified, especially since further pathways may operate in buffers of different pK_a. Anslyn and Breslow[11] had proposed such a mechanism but rejected it on the basis of a maximum in plots of k_c vs buffer ratio, but this maximum has not been confirmed.

Does the mechanism of Breslow and Xu[1] fit the data? It would seem that it has enough adjustable parameters that it cannot fail to do so. However, the behavior of the y intercepts in Figure 1 is not consistent with Scheme 1.

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(19) Kluger, R.; Covitz, F.; Dennis, E.; Williams, L. D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1969**, *91*, 6066.

(20) Shugar, G. J.; Dean, J. A. *The Chemist's Ready Reference Handbook*; McGraw-Hill: New York, 1990; Chapter 17, p 2.

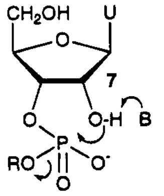
Experimentally, those intercepts, obtained from the linear fits to eq 24 (solid lines), are proportional to $[OH^-]$. Alternatively, the intercepts obtained from the fit to eq 1 (dashed lines) increase with $[OH^-]$, although proportionality is less certain, since the error in the parameter c of their Table IV is larger than its value. (This increase is also seen with the parameters in their Table V, from a seven-parameter fit.) Yet according to eqs 2, 7, 10, 14, and 15, this intercept is $(k_{1w} + k_H[H^+])(k_{2w} + k_{OH}[OH^-])/(k_3 + k_{-1w} + k_{2w} + k_{OH}[OH^-] + k_{-H}[H^+])$. For this to be proportional to $[OH^-]$, the inequalities $k_{1w} \gg k_H[H^+]$, $k_{2w} \ll k_{OH}[OH^-]$, and $k_3 + k_{-1w} + k_{2w} + k_{-H}[H^+] \gg k_{OH}[OH^-]$ must all hold. The principle of microscopic reversibility requires that $k_{-1w}/k_{-H}[H^+] = k_{1w}/k_H[H^+]$, and the dominance of cleavage over isomerization means that $k_3 < k_{2w} + k_{OH}[OH^-]$. The only way to satisfy these requirements is if $k_{-1w} \gg k_{2w}$, which is unprecedented in phosphate chemistry and unreasonable for two cleavage reactions that are so similar.

Indeed, Breslow and Xu[1] recognized that the rates extrapolated to zero buffer are lower in the less basic buffer, and they suggested an additional new mechanism (their Figure 7). However, they made no attempt to attach rate constants to the various steps or to fit their data to this mechanism. The need for an additional mechanism is an acknowledgment that the original one (Scheme 1) is inadequate to account for the data. It does not support the assertion that "the quantitative curve fitting possible in the present study ... confirms the correctness of the general mechanism previously proposed". Indeed, it is strange that a reaction that is said to be general-acid-catalyzed is faster at higher pH.

Stepwise or Concerted? Must there be an intermediate in the cleavage reaction, or can it occur by an independent concerted displacement? Isomerization certainly proceeds via an intermediate that can pseudorotate, since it is impossible for both the displacing oxygen and the displaced oxygen to be apical. A common, stepwise mechanism for isomerization and cleavage then seems simpler than separate reactions for two very similar nucleophilic substitutions. Nevertheless, secondary kinetic isotope effects[21] (although with a *p*-nitrophenoxide leaving group, which may not be comparable) are consistent with a concerted displacement, and molecular-orbital calculations at the 3-21+G* level indicate that a dianionic phosphorane (in the gas phase) cannot be an intermediate, although a monoanionic phosphorane can.[22]

Despite Haim's objection[13] we agree with Breslow[9] that his data are evidence for an intermediate in the reaction. The key result[1] is that the rate of isomerization decreases with increasing buffer concentration (although the decrease is not large and ought to be confirmed[23]). If isomerization, which must proceed via an intermediate, were a reaction independent of the cleavage, its rate might be increased by added buffer, but it could not be decreased. Therefore cleavage must proceed via that same intermediate.

There are further difficulties with a concerted cleavage[21] whose rate is observed[1] to increase with buffer base. Such a mechanism requires general base to remove the proton from an attacking alcohol while another alcohol is cleaved (**7**). It must be cleaved as alkoxide, without accepting a proton, or else the rate would also be proportional to the concentration of buffer acid, or second order in buffer. (Water as general acid would be consistent with the kinetics but ineffective, since proton transfer from water to alkoxide provides no stabilization of the transition state.) The reverse reaction would then be attack of alkoxide while the other alkoxide is cleaved with general-acid assistance. Therefore a concerted mechanism, with general-base assistance in one direction and alkoxide attack in the other, does not satisfy the principle of microscopic reversibility. This problem does not arise if the reaction is stepwise, via a phosphorane intermediate.



An unusual feature of the isomerization reaction is that it is not subject to buffer catalysis because its rate-limiting step is pseudorotation of the intermediate, as recognized by Breslow. Since buffer catalysis can divert the intermediate toward cleavage, k_i decreases with increasing buffer concentration, but this also follows from our mechanism (eq 20). However, according to our mechanism the pH dependence of k_c/k_i arises because dianion **4′** of the phosphorane intermediate undergoes cleavage whereas only monoanion **6** undergoes pseudorotation, leading to isomerization.

Summary and Conclusions

Breslow's mechanism, despite a profusion of adjustable parameters, does not fit the data. However, our mechanism (Scheme 2) does lead to eqs 21 and 24 that reproduce the experimental linearity of k_c/k_i and k_c with buffer concentration as well as the pH dependence of the intercepts. A further advantage is that it restores the "symmetry" of the reaction, in that cleavages of three nearly identical alkoxy groups are all general-acid-catalyzed, rather than one catalyzed by buffer acid, one by buffer base, and one by neither.

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Note added in proof: A medium effect due to imidazole[12] has been observed: Kirby, A. J.; Marriott, R. E. *J. Am. Chem. Soc.* **1995**, *117*, 833.

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[21] Hengge, A. C.; Cleland, W. W. *J. Am. Chem. Soc.* **1991**, *113*, 5835.

[22] Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 5872. Lim, C.; Tole, P. *J. Am. Chem. Soc.* **1992**, *114*, 7245.

[23] Cleland, W. W., personal communication.