(3) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins", Wiley-Interscience, New York, N.Y., 1971.
(4) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. I, W. A. Benjamin, New York, N.Y., 1966, pp 1-195.
(5) M. L. Bender, *J. Am. Chem. Soc.*, **79**, 1258 (1957).
(6) M. L. Bender, Y.-L. Chow, and F. Chloupek, *J. Am. Chem. Soc.*, **80**, 5380 (1958).
(7) G. Dahlgren and N. L. Simmerman, *J. Phys. Chem.*, **69**, 3636 (1965).
(8) A. Bruylants and F. J. Kézdy, *Rec. Chem. Prog.*, **21**, 213 (1960).
(9) T. Higuchi, L. Eberson, and A. K. Herd, *J. Am. Chem. Soc.*, **88**, 3805 (1966).
(10) A. J. Kirby and P. W. Lancaster, *J. Chem. Soc., Perkin Trans. 2*, 1206 (1972).
(11) A. R. Fersht and A. J. Kirby, *Prog. Bioorg. Chem.*, **1** (1971).
(12) M. F. Aldersley, A. J. Kirby, P. W. Lancaster, R. S. McDonald, and C. R. Smith, *J. Chem. Soc., Perkin Trans. 2*, 1487 (1974).
(13) R. Kluger and J. L. W. Chan, *J. Am. Chem. Soc.*, **96**, 5637 (1974).
(14) C. H. Lam, unpublished observation.
(15) M. F. Aldersley, A. J. Kirby, and P. W. Lancaster, *J. Chem. Soc., Chem. Commun.*, 570 (1972).
(16) A. R. Fersht, *J. Am. Chem. Soc.*, **94**, 293 (1972).
(17) E. C. Lucas and M. Caplow, *J. Am. Chem. Soc.*, **94**, 960 (1972).
(18) A. C. Satterthwait and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 7018 (1974).
(19) A. Anschutz, *Chem. Ber.*, **20**, 3215 (1887).
(20) M. Siegel and D. Pressman, *J. Am. Chem. Soc.*, **76**, 2863 (1954).
(21) G. Caronna, *Gazz. Chim. Ital.*, **78**, 38 (1948); *Chem. Abstr.*, **42**, 6760e (1952).
(22) W. Herz, *J. Am. Chem. Soc.*, **67**, 1854 (1945).
(23) Y. Liwschitz, Y. Edlitz-Pfefferman and Y. Shorr, *J. Chem. Soc.*, 4399 (1957).
(24) R. J. Cotter, C. K. Sauers, and J. M. Whelan, *J. Org. Chem.*, **26**, 10 (1961).
(25) N. Gravitz and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 489, 499, 507 (1974).
(26) A. J. Kresge, *Chem. Soc. Rev.*, **2**, 475 (1973).
(27) D. Bishop and K. J. Laidler, *J. Chem. Phys.*, **42**, 1668 (1965).
(28) G. M. Blackburn and W. P. Jencks, *J. Am. Chem. Soc.*, **90**, 2638 (1968).
(29) B. A. Cunningham and G. L. Schmir, *J. Am. Chem. Soc.*, **88**, 551 (1966).
(30) P. R. Rony, *J. Am. Chem. Soc.*, **91**, 6090 (1969).
(31) Detailed discussions of implications of this sort of behavior on the nature of proton transfer steps involving intermediates have been presented by Kershner and Schowen (ref 32) and by Jencks (ref 33).
(32) L. D. Kershner and R. L. Schowen, *J. Am. Chem. Soc.*, **93**, 2014 (1971).
(33) W. P. Jencks, *Chem. Rev.*, **72**, 705 (1972).

The Reversible Hydration of Pteridine. General Acid–Base Catalysis, Solvent Deuterium Isotope Effects, and Transition State Characterization[1]

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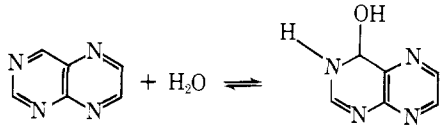
Abstract: The hydration of pteridine has been investigated over the pH range 1–10 at 25.0°, utilizing a spectrophotometric method. Above pH 7.5, the hydration of this heterocyclic Schiff base is catalyzed by hydroxide ions and by the basic component of a number of buffers (imidazole, 1,2-dimethylimidazole, borate, carbonate, triethylamine). The general base catalysis conforms to a Brønsted plot with an exponent $\beta = 0.64 \pm 0.03$. Below pH 7.5, the reaction is catalyzed predominantly by hydronium ions. The value of 0.50 ± 0.02 for the ratio $k_{H_3O^+}/k_{D_3O^+}$ indicates a preequilibrium protonation of pteridine. However, in a few instances, the acidic component of buffers capable of bifunctional catalysis ($H_2PO_4^-$, $H_2AsO_4^-$, and HCO_3^-) also exhibited an enhancement in the rate of hydration. The solvent isotope effect for water catalysis, k_{H_2O}/k_{D_2O}, has a value of 3.4 ± 0.4 which is similar to those of a number of other reactions involving water in a cyclic mechanism.

It is a well-documented experimental fact[2,3] that water adds across the N_3–C_4 double bond of pteridine (eq 1).



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Moreover, it has recently been demonstrated that this reversible hydration is catalyzed by the enzyme, adenosine deaminase.[4a,b] However, in order to fully characterize the enzymatic catalysis, it is first necessary to have a thorough knowledge of the enhancement afforded to the hydration by all other potential catalysts such as general acids and bases, water, and hydroxide and hydronium ions. In this paper, we examine the hydration of pteridine in a number of general acids and bases both in H_2O and D_2O. The data have important implications not only with respect to acid–base catalysis but also with regard to the reaction mechanisms of C=N hydrations.

Most of the kinetic work on C=N compounds which has been published to date has dealt with the hydrolysis of imines,[5-9] imidates,[10-14] iminolactones,[15-17] oximes,[18] and semicarbazones.[19,20] Although the mechanism has been fairly well established,[21,22] kinetic studies on these compounds have always been complex for two reasons. First, most of the compounds used were basic enough so that the true ground state changed from an iminium ion, $>C{=}N^+HR$, to the imine, $>C{=}\ddot{N}R$, somewhere along the pH range being investigated. Secondly, since the hydrolysis of such compounds to the corresponding >C=O and $R\ddot{N}H_2$ is known to proceed by addition of water across the double bond, then either the formation of the hydrate or its collapse to products can be rate determining. In contrast, pteridine offers a unique opportunity to study only the hydration of the double bond because the breakdown of the carbinol–amine intermediate to the ring-opened product occurs at a rate very much slower than the initial hydration step.[23] Furthermore, over the entire range examined in this paper, the ground state corresponds to pteridine because the pK_a of pteridine·H^+ is around −2.[24] Finally a thorough characterization of the hydration mechanism of pteridine seems timely in view of the fact that recent investigations have shown xanthopterine (a compound with a pteridine ring skeleton) to be an efficient antitumor agent.[25,26]

Experimental Section

Materials. Pteridine was synthesized according to the method of Albert and Yamamoto[27] and was purified by extensive sublimation

Table I. Various Spectral Properties of Pteridine and Its Hydrate

Species	pK_a	λ_{max}, nm	Log ϵ^a	λ_{max}, nm	Log ϵ^b	ϵ^c (λ 273 nm)
I		298, 308	3.875, 3.82	298, 309	3.90, 3.84	2.58×10^3
II	ca. −2					
III		325	3,97			
IV	11.2	269, 318	3.69, 3.89	269,317	3.69, 3.89	4.77×10^3
V	4.8	287, 300	3.92, 3.94	285, 300	3.90, 3.91	4.77×10^3

[a] Data taken from Perrin (ref 33a). [b] This work: ionic strength, $I = 0.1$, at 25.0°. [c] Spectral data at the isosbestic point for species IV and V.

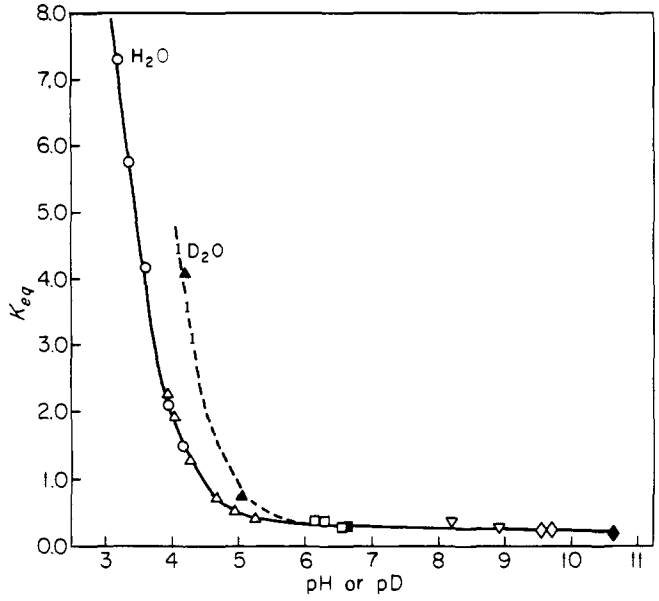


Figure 1. Plot of K_{eq} for pteridine hydration as a function of pH (open symbols) and pD (filled symbols) at 25.0°. Ionic strength was maintained at 0.10 with sodium sulfate. Data obtained in (○) formate; (△) acetate; (□) phosphate; (▽) 1,2-dimethylimidazole; and (◇) carbonate buffers.

(mp 138–138.5°). Imidazole was recrystallized three times from benzene, while 1,2-dimethylimidazole (Aldrich) was distilled under reduced pressure [bp 57° (2 Torr)]. Diethylmalonic acid was prepared by a procedure previously described.[12] All other buffer components were reagent grade and were used without further purification. Acetonitrile (Baker) was refluxed over triphenylmethyl fluoroborate, then fractionally distilled (bp 80–81°; $n^{21.5}D$ 1.3449) and stored under N_2 in an air-tight bottle. The heavy water containing at least 99.8% D_2O was purchased from Stholer.

The kinetics were followed at 325 nm on a Varian Techtron Model 635 spectrophotometer which was equipped with a Forma-Temp Jr. Model 2095 circulating bath to maintain the temperature at 25.00 ± 0.05°. Only rates that were reproducible to within 4% were used. The rates done in perchloric acid and in the formic acid-formate buffer system were followed on a Durrum-Gibson stopped-flow spectrophotometer with electronics and temperature control systems modified in our laboratory.

All buffers were made up to an ionic strength of 0.10 using sodium sulfate. Measurement of pH and pD (pD = pH reading + 0.41)[28] were made on a Beckman 101900 research pH meter. The pH readings were corrected for activity effects using eq 2 and 3 where I and Z stand for the ionic strength and charge, respectively:

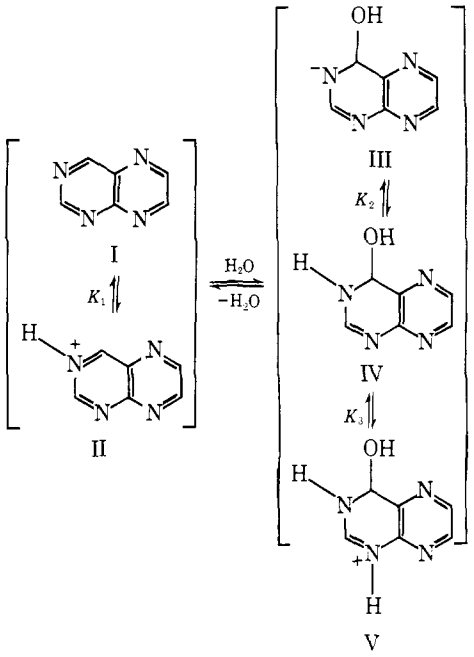
$$\text{pH} = -\log([H_3O^+]f\pm) \quad (2)$$

$$\log f\pm = (-0.51\ Z^2I^{0.5})/(1 + 1.5I^{0.5}) \quad (3)$$

Kinetics. A stock solution of pteridine free of its hydrate was prepared by dissolving freshly sublimed pteridine into dry acetonitrile. A kinetic run was initiated by injecting 15 μl of this stock solution from a calibrated Hamilton syringe into 3 ml of the appropriate buffer. The hydration was monitored spectrophotometrically by following the increase in absorbance as a function of time at 325 nm. However, a constant infinity value is not reached after 10 half-lives because the slow subsequent formation of a 5,6,7,8-dihydrated cation[29] leads to a gradual rise in absorbance. Extrapolated infinities were obtained by a technique reported by Pocker and Hill.[30] Using these extrapolated infinities, good pseudo-first-order kinetics were obtained for at least 2 half-lives when log $(A_\infty - A_t)$ was plotted against time. The absolute value of the slope of such a plot multiplied by 2.303 yields k_{obsd}, which at all pH values under investigation is equal to $(k_{hydration} + k_{dehydration})$, where $k_{hydration}$ is the rate constant for the hydration of pteridine and $k_{dehydration}$ is the rate constant for the dehydration of the conjugate hydrate. In order to obtain $k_{hydration}$ at any pH, it is necessary to multiply k_{obsd} by the fraction of hydration, $\chi = K_{eq}/(K_{eq} + 1)$, at this pH.

In order to determine the various catalytic coefficients, a matrix of 16 buffers per buffer system was generally employed.[31,32]

Equilibria. In an earlier investigation, Perrin studied the various equilibria involved in the hydration of pteridine (eq 4).[33] Although it is not possible to isolate crystals of pure hydrate, we have generated species IV and V in aqueous solution and recorded their uv



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spectra. These results along with some pertinent pK_a values are listed in Table I.

From the pK_a data, it is immediately clear that, over the pH range investigated in this paper, only species I, IV, and V are present in significant concentrations. One can define, therefore, an equilibrium constant (K_{eq}) in terms of stoichiometric pteridine hydrate and stoichiometric pteridine, as shown in eq 5. The equilibrium constant was evaluated at 25.0° as a function of pH by introducing 15 μl of the pteridine stock solution into the appropriate buffer. After allowing enough time to reach equilibrium (10 half-lives), the absorbance at 273 nm was recorded. These data, together with the known initial concentration of pteridine, provided enough information to calculate K_{eq} from the two simultaneous eq 6 and 7

$$K_{eq} = [\text{pteridine hydrate}]_s/[\text{pteridine}]_s = ([III] + [IV] + [V])/([I] + [II]) \simeq ([IV] + [V])/[I] \quad (5)$$

$$[\text{pteridine}]_0 = [I] + ([IV] + [V]) \quad (6)$$

$$A = \epsilon_I^{\lambda=273}[I] + \epsilon_{IV}^{\lambda=273}[IV] + \epsilon_V^{\lambda=273}[V] \quad (7)$$

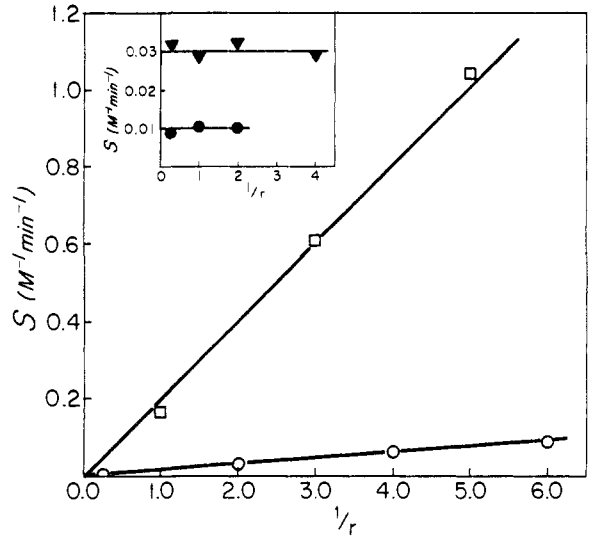


Figure 2. Determination of specific rate coefficients for the hydration of pteridine at 25.0° by the components of (□) arsenate; (○) diethyl malonate; (▼) 1,2-dimethylimidazole (insert); and (●) imidazole (insert) buffers in H_2O and ionic strength 0.10.

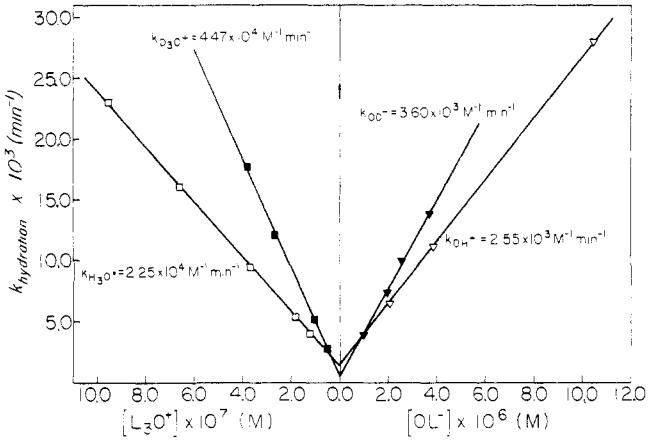


Figure 3. Catalysis of the hydration of pteridine by L_2O, L_3O^+ and OL^- at 25.0° and ionic strength 0.10. Filled symbols refer to the hydration in D_2O and open symbols to the hydration in H_2O; (▽) 1,2-dimethylimidazole buffers; (□) phosphate buffers.

where A is the optical density after 10 half-lives. The wavelength 273 nm was chosen because it is the isosbestic wavelength for IV and V. Since ϵ_{IV} equals ϵ_V at this wavelength, then eq 7 can be simplified to

$$A = \epsilon_I^{\lambda=273}[I] + \epsilon_{IV}^{\lambda=273}([IV] + [V]) \quad (8)$$

Solving eq 6 and 8 for the terms [I] and ([IV] + [V]) allows K_{eq} to be evaluated as shown in eq 9

$$K_{eq} = ([\text{pteridine}]_0 \epsilon_I^{\lambda=273} - A)/(A - [\text{pteridine}]_0 \epsilon_{IV}^{\lambda=273}) \quad (9)$$

Values for the equilibrium constant were determined as a function of pH in both H_2O and D_2O, and the results are shown in Figure 1.

Results

Since the presence of general catalysis was demonstrated for pteridine hydration, the first-order rate constants, $k_{hydration}$, are well correlated by eq 10 where k_0 is the spontaneous rate coefficient, $a_{H_3O^+}$ is the experimentally determined hydronium ion activity, and k_A and k_B are the rate coefficients for the acidic and basic components of the buffers. The mean activity coefficient, $f_\pm$ was calculated from eq 3.

Table II. Catalytic Rate Coefficients for Acids and Bases in the Hydration of Pteridine[a]

Catalyst	k_c,[b] l. mol^{-1} min^{-1}	pK_a[c]	p[e]	q[e]	k_{H_2O}/k_{D_2O}
H_3O^+	2.25×10^4	−1.74	3	1	0.50
$H_2AsO_4^-$	2.0×10^{-1}	6.73	2	3	
$HAsO_4^{2-}$	[d]				
$H_2PO_4^-$	2.3×10^{-1}	6.84	2	3	1.9
HPO_4^{2-}	[d]				
$HDEM^-$	1.4×10^{-2}	7.11	1	4	
DEM^{2-} [f]	[d]				
IMH^+	[d]	7.18	2	1	
IM	1.0×10^{-2}				
$DIMH^+$	[d]	8.2	1	1	
DMI[g]	3.0×10^{-2}				~3
H_3BO_3	[d]	9.2	3	4	
$B(OH)_4^-$	3.5×10^{-1}				
HCO_3^-	1.5	9.99	1	3	1.4
CO_3^{2-}	1.1				1
$HTEA^+$	[d]	10.85	1	1	
TEA[h]	4.0				
H_2O	$(1.2 \times 10^{-3})/55.5$	15.74	2	1	3.4
OH^-	2.55×10^3				0.70

[a] At 25.0° and ionic strength $I = 0.10$. [b] Catalytic coefficients for the forward rate (hydration) in H_2O. [c] Values of pK_a in both H_2O and D_2O were determined by titration at 25.0° and ionic strength, $I = 0.10$. At 25.0°, pK_W taken as 14.00 and pK_{D_2O} as 14.82: A. K. Covington, R. A. Robinson, and R. G. Bates, *J. Phys. Chem.*, 70, 3820 (1966). [d] Too small to obtain an accurate rate coefficient. [e] Taken from R. P. Bell and P. G. Evans, *Proc. R. Soc. Ser. A*, 291, 297 (1966). [f] DEM^{2-} = dianion of diethylmalonic acid. [g] DMI = 1,2-dimethylimidazole. [h] TEA = triethylamine.

$$k_{hydration} = \chi k_{obsd} = k_0 + k_{H_3O^+}(a_{H_3O^+}/f_\pm) + k_{OH^-}(K_w/a_{H_3O^+}f_\pm) + k_A[A] + k_B[B] \quad (10)$$

In order to evaluate the coefficients k_A and k_B for a particular buffer system, a set of rates were carried out varying the total buffer concentration while maintaining the same buffer ratio (r) defined as the ratio of the concentration of the conjugate base to that of the acid; i.e., $r = [B]/[A]$. Rearranging eq 10 gives eq 11.

$$\chi k_{obsd} = k_0 + k_{H_3O^+}(a_{H_3O^+}/f_\pm) + k_{OH^-}(K_w/a_{H_3O^+}f_\pm) + [B](k_A/r + k_B) \quad (11)$$

Plots of k_{obsd} against [B] were found to be linear with a slope $S_r = k_A/r + k_B$ and an intercept $I_r = k_0 + k_{H_3O^+}(a_{H_3O^+}/f_\pm) + k_{OH^-}(K_w/a_{H_3O^+}f_\pm)$. A plot of S_r against the reciprocal $(1/r)$ of several different buffer ratios allows k_A and k_B to be evaluated for any buffer. The plot for 1,2-dimethylimidazole and imidazole are shown in the insert to Figure 2. Similar results were obtained for the triethylamine and borate buffer systems. However, plots for the phosphate, arsenate, diethyl malonate, and carbonate buffer systems (Figure 2) were different since, in these cases, there was a substantial contribution to the rate by the acidic component of the buffer and, with the first three, a negligible contribution by the basic component.

The coefficients $k_{H_3O^+}$ and $k_0^{H_2O}$ were deduced from the data obtained in phosphate buffers by plotting I_r against the corresponding hydronium ion concentration. The slope of this plot gives $k_{H_3O^+}$ and the intercept $k_0^{H_2O}$. Similarly, k_{OH^-} was determined from the data collected in 1,2-dimethylimidazole buffers. In this case, the slope obtained by plotting I vs. the corresponding hydroxide concentration is equal to k_{OH^-} and the intercept to $k_0^{H_2O}$. As might be expected, the value obtained for $k_0^{H_2O}$ should be the same regardless of whether it was determined by extrapolation to zero hydroxide or zero hydronium ion concentration (Figure 3). The catalytic coefficients $k_0^{D_2O}$, $k_{D_3O^+}$, and k_{OD^-} were similarly evaluated, and the results for H_2O and D_2O are listed in Table II.

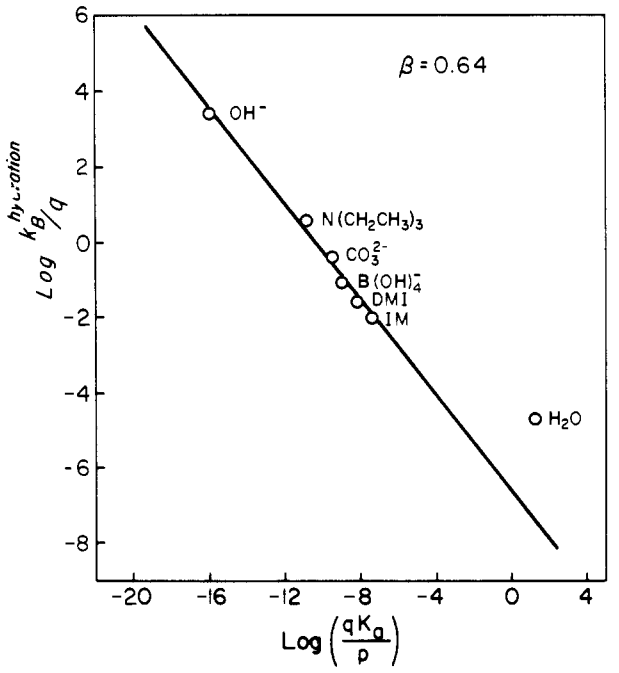


Figure 4. Brønsted plots of log (k_B/q) vs. log (qK_a/p) for the general base catalyzed hydration of pteridine: IM = imidazole; DMI = 1,2-dimethylimidazole; $B(OH)_4^-$ = borate. Statisticaal corrections have been made according to R. P. Bell and P. G. Evans, *Proc. R. Soc. London, Ser. A*, 291, 297 (1966).

In the basic region, pteridine hydration is subject to general base catalysis by imidazole, 1,2-dimethylimidazole, borate, carbonate, and triethylamine. These results are displayed in Figure 4 as a plot of the Brønsted relationship, eq 12,

$$\log k_B/q = \beta \log (qK_a/p) + G_B \qquad (12)$$

where k_B is the base catalytic coefficient, and K_a is the dissociation constant of the conjugate acid of the base B. The statistical factors as well as the catalytic coefficient for each general base are listed in Table II.

In the acidic region, pteridine hydration is subject to general catalysis by monohydrogen phosphate, monohydrogen arsenate, and the monoanion of diethylmalonic acid. It was found though, that the catalytic effectiveness of these species was not well correlated by the Brønsted relationship. In addition, no detectable catalysis by either buffer component, RCOOH or $RCOO^-$, was observed when the hydration was carried out in acetate or formate buffers maintained at an ionic strength of 0.10 *M*. However, when the ionic strength was increased tenfold and the total (stoichiometric) acetate concentration allowed to vary, a small rate enhancement was noticeable for the hydration. Unfortunately, under such extreme conditions, it is difficult to ascertain whether the rate increase should be ascribed to nonuniformity of salt effects at concentrations as high as 1 *M* or to a bona fide general acid catalysis. Indeed, even if all the additional catalysis is assumed to arise from HOAc, then a Brønsted plot utilizing the catalytic coefficients for H_3O^+ and HOAc gives a value for α of only 0.9.

Discussion

As can be seen from Figure 5, there are two distinct catalytic regions in the hydration of pteridine. First there is an acid catalyzed region in the pH range 1 to 7.5 and then, at higher values of pH, a base catalyzed region. Between these two regions, the velocity passes through a minimum at a hydrogen ion concentration given by eq 13.

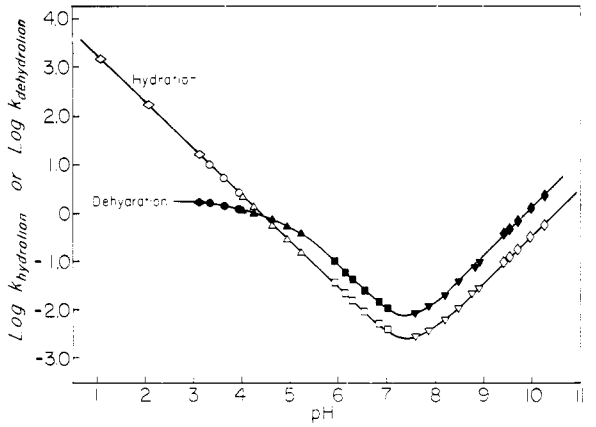


Figure 5. Plots of log of first-order rate constants (in min^{-1}; extrapolated to zero buffer concentration) for the hydration of pteridine (opened symbols) and the dehydration of pteridine hydrate (filled symbols) as a function of pH at 25.0° and ionic strength 0.10. Rate data were obtained in (◇) perchloric acid below pH 3.2 and in (○) formate; (△) acetate; (□) phosphate; (▽) 1,2-dimethylimidazole; and (◇) carbonate buffers.

$$[H^+]_{min} = (K_w k_{OH^-}/k_{H^+})^{0.5} \qquad (13)$$

However, even in this region of pH, the water rate contributes only 45%.

The Hydration Mechanism at Low pH. A plot of log $k_{hydration}$ vs. log $[H_3O^+]$ in this region has a slope of unity which indicates that the empirical formula of the transition state contains one more proton than that of the ground state. From this consideration, three possible mechanisms (i–iii) can be visualized as listed under Scheme I. It is difficult to accommodate mechanisms ii and iii with our data for two reasons. First when the hydration is carried out in acetic acid–acetate or formic acid–formate buffers, very little catalysis by the buffering system could be detected. A priori, acids of pK_a around 4 would be expected to function as general acid catalysts in both mechanisms ii and iii. In fact, the hydrolysis of more basic imines is clearly subject to general acid catalysis and is generally believed to be a consequence of a reaction between the protonated Schiff base and the basic form of the buffer, mechanism iii.[18] This mechanism is favored over its kinetically equivalent counterpart ii from the results of the hydrolysis of benzhydrilidenedimethylammonium iodide[8] and phthalimidium perchlorate.[34] It is interesting to note that both of these compounds are subject to general base catalysis and have β values of 0.27 and 0.26, respectively. In contrast, an upper limit for the value of "β" in the hydration of pteridine is only 0.1 (i.e., $\alpha_{obsd} \geq 0.9$). Clearly this low value of "β" indicates that the attack of water on pteridine·H^+ requires little or no assistance from the basic component of the buffer (mechanism i). Presumably this unique behavior, which sets pteridine apart from all Schiff bases studied to date, can be explained in terms of the poor selectivity of the energetically unstable intermediate, pteridine·H^+.

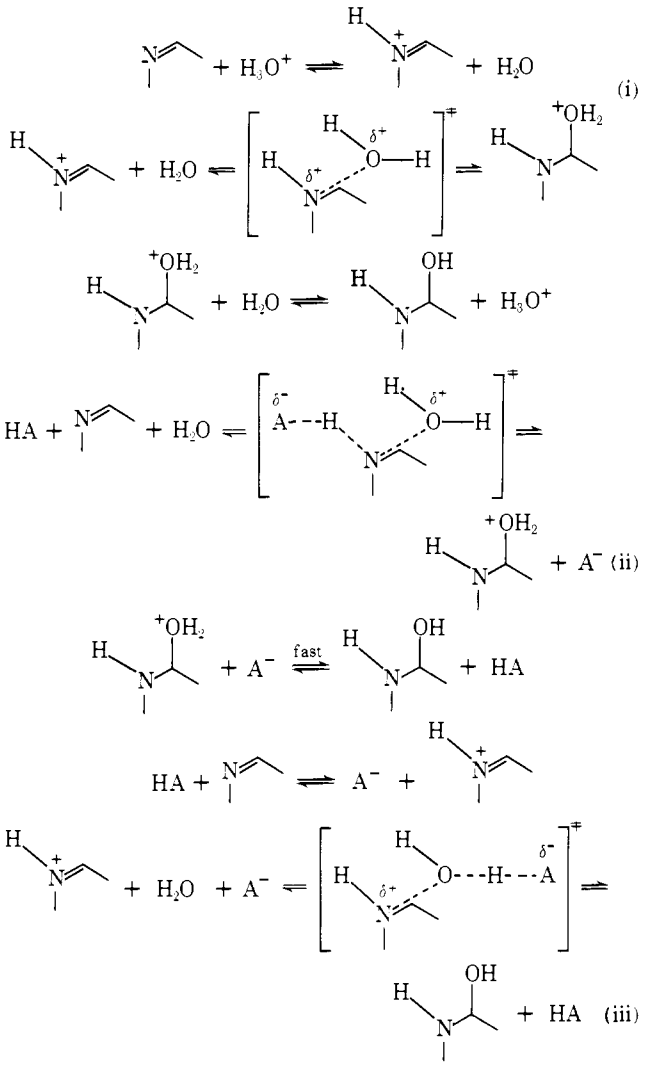
A second experimental fact which favors mechanism i is the solvent deuterium isotope effect.[32,35-41] As can be seen from Table II, the ratio $k_{H_3O^+}/k_{D_3O^+}$ is 0.5. Such inverse isotope effects have been observed with substrates (S) that undergo a preequilibrium transformation into SH^+ and SD^+, respectively, followed by a rate-determining step *not* involving a proton vs. a deuteron transfer to a general base (Table III). In contrast to the inverse isotope effect typical of (i), the value of $k_{H_3O^+}/k_{D_3O^+}$ would be expected to be larger than unity if H_3O^+ or HA were functioning as a proton donor in the rate-determining step. Of course, the exact magnitude would be determined by the extent of proton

Table III. Solvent Deuterium Isotope Effects, k_{H_2O}/k_{D_2O}

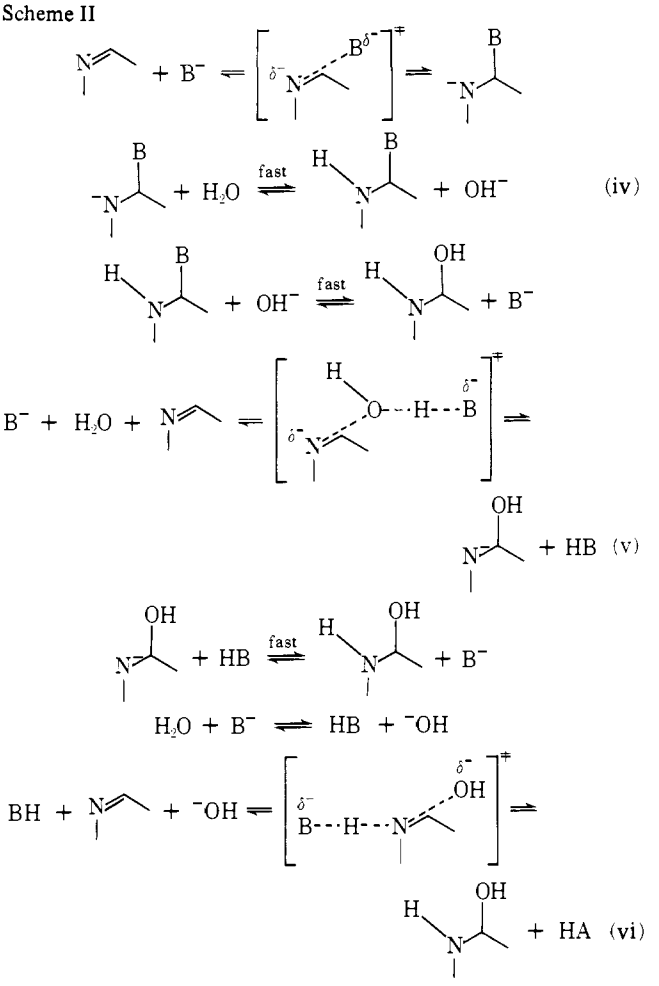
Catalyst	Hydration of pteridine	Hydration of acetaldehyde	Mutarotation of glucose	Inversion of sucrose	Hydrolysis of ethyl orthoformate	Hydrolysis of *N*-benzoylimidazole	Hydrolysis of D-glucono-δ-lactone
H_2O-D_2O	3.4	3.9[a]	3.8[c]			2.28[g]	3.9[h]
OH^--OD^-	0.70	~1[b]	~1[c]			1.03[g]	0.8[h]
$H_3O^+-D_3O^+$	0.50	1.3[a]	1.4[c,d]	0.5[e]	0.4[f]	1.32[g]	1.1[h]

[a] Reference 35. [b] Reference 36. [c] Reference 37. [d] Reference 38. [e] Reference 39. [f] Reference 40. [g] Reference 41. [h] Reference 32.

Scheme I



Scheme II



transfer in the transition state, but numerous examples of this case with $k_{H_3O^+}/k_{D_3O^+} > 1$ have been documented in the literature (Table III).

An interesting consequence of assigning mechanism i as the route of hydration for pteridine is to use the mechanism to predict the shape of the pH profile for the dehydration. By the principle of microscopic reversibility, the step involving the dehydration of the protonated hydrate must be the rate-determining one. It is also known that the pK_a of the hydrate is 4.8. Therefore, in the pH range above 5.5, the neutral hydrate will be the ground state, and one would predict using mechanism i, that a plot of log $k_{dehydration}$ vs. log $[H_3O^+]$ would give a straight line with a slope of unity since the transition state contains one more proton than the ground state. However, as the pH is lowered the protonated hydrate will eventually become the ground state. Hence at pH values slightly below 4.8, there will be no difference in proton composition between the ground state and transition state. In this pH region, one would expect a plot of log $k_{dehydration}$ vs. log $[H_3O^+]$ to be linear with zero slope. As can be seen in Figure 5, both of these predictions are borne out.

The Hydration Mechanism at High pH. In this region, the rate of hydration is directly proportional to the hydroxide ion concentration. Consequently, the empirical formula of the transition state must contain one less proton than that of the ground state plus or minus any number of water molecules. Three possible reaction mechanisms based on this fact, iv-vi, are shown in Scheme II.

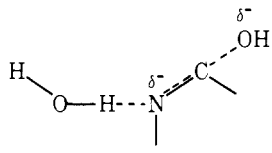
Since general base catalysis was observed over the pH range 7.5-10.5, mechanism iv can immediately be ruled out. The only way in which a buffer could be involved in the catalysis for this mechanism would be as a nucleophile, followed by the rapid hydrolysis of the intermediate. However, most of the buffers used in this pH interval were deliberately chosen because of their poor nucleophilicity. For example, 1,2-dimethylimidazole was shown to be a catalyst for the hydration (Figure 2). Yet being a tertiary amine, it would be very improbable for this base to be acting as a nucleophile, and it is much more likely for it to be participating as a general base. It is, of course, still possible that the basic component of the buffering system is functioning as a

general base while hydroxide as a nucleophile. This situation has been demonstrated in the hydrolysis of ethyl dichloroacetate[42] and of D-glucono-δ-lactone.[32] However, in these examples, it has always been found that the catalytic activity of the hydroxide ion is greater than that predicted from a Brønsted plot by roughly two orders of magnitude. In the hydration of pteridine, it is seen (Figure 4) that hydroxide falls on the Brønsted line. For this reason, it seems fairly safe to exclude mechanism iv, but there still remains the difficult task of choosing between the two kinetically equivalent mechanisms v and vi.

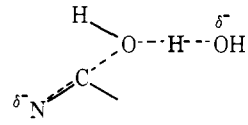
In an earlier study on the base catalyzed addition of water to substituted hydrazones,[19] a mechanism equivalent to that shown in (v) was excluded on the basis of the instability of the N anion. Unfortunately the same argument cannot be applied to the hydration of pteridine. As shown in Table I, the pK_a of pteridine hydrate is 11.2. Consequently a mechanism for the dehydration of pteridine hydrate involving a preequilibrium formation of the N anion (i.e., mechanism v) would not lead to a rate constant exceeding the diffusion-controlled limit. Therefore, route vi can be thought of as the mechanism in basic solution only inasmuch as the substituted hydrazone serves as an appropriate model for pteridine.

There is other evidence, however, that mechanism vi is operating for the hydration of pteridine and that comes from the solvent deuterium isotope effect.

The observed value of 0.7 for the ratio k_{OH^-}/k_{OD^-} is in the range expected for nucleophilic attack by hydroxide provided that the transfer of the proton from water to the nitrogen is not well developed in the transition state VI. If the mechanism outlined in (v) were correct, it might be expected from VII that the ratio k_{OH^-}/k_{OD^-} would be slightly greater than unity[36,37,41] (see also Table II). Clearly these arguments are not compelling since the exact magnitude of such isotope effects depends on the degree of proton transfer in the transition state.

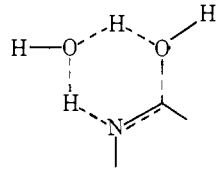


VI



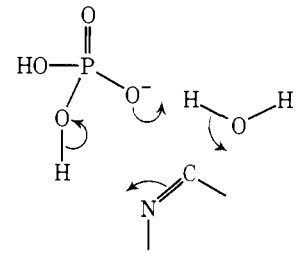
VII

The Hydration Mechanism. Catalysis by H_2O and Other Bifunctional Catalysts. As seen in Figure 5, there is no region of the pH profile where water is the dominant catalyst. In fact, even at the reaction minimum the contribution by water accounts for only 45% of the rate. Yet in spite of its poor catalytic effectiveness, there are two pieces of evidence that indicate water functions in a very efficient cyclic mechanism.[43] First, the catalytic coefficient of water is nearly a thousand times greater than that predicted from the Brønsted plot, suggesting that water is not acting solely as a general base, but in some additional capacity as well. Secondly, the solvent deuterium isotope effect, k_{H_2O}/k_{D_2O}, is 3.4. An isotope effect of this magnitude usually indicates that at least two water molecules must be tightly bound in the transition state.[32,36,38,44,45] A likely depiction of the transition state VIII is shown:



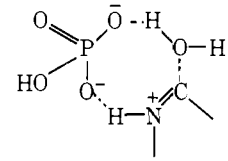
VIII

As seen in VIII, water is acting in the dual capacity of a base as well as of an acid. This behavior may explain why bifunctional catalysts such as $H_2PO_4^-$, $H_2AsO_4^-$, and HCO_3^- are particularly efficient[46] in catalyzing the hydration of pteridine. Indeed, these monoanions have the added advantage in that they possess both moderately acidic and basic groups situated on well separated oxygen atoms. Clearly, for these catalysts, proton donation and proton removal are greatly facilitated via acid–base bifunctional catalysis as depicted in IX. The observed isotope effect of 1.9

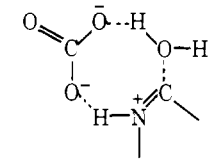


IX

for $k_{H_2PO_4^-}$ is in good agreement with that noted for other reactions utilizing $H_2PO_4^-$ in this manner.[32,36,37] Of course, from the preceding evidence, it is impossible to dismiss the kinetically equivalent mechanism of specific acid–general base catalysis (structures X and XI). Since according to this mechanism the substrate is already fully protonated, the role of the conjugate dianions, HPO_4^{2-}, $HAsO_4^{2-}$, and CO_3^{2-}, respectively, must be the removal of a proton from an attacking water molecule. The amount of stabilization of such transition states would presumably be due to highly favorable electrostatic interactions. However, calculations based on this electrostatic model show that certain rate constants approach the limiting value for diffusion-controlled reactions[47] which is in contradiction to what has been found for other related processes.[48]



X



XI

Our work also shows that diethyl malonic acid monoanion [$HO_2CC(C_2H_5)_2CO_2^-$], while significantly less effective than $H_2AsO_4^-$, ($k_{H_2AsO_4^-}/k_{HDEM^-} \simeq 14$; Figure 2) is nevertheless a much more powerful catalyst in regard to pteridine hydration than either HCO_2H or CH_3CO_2H. Clearly the presence of a significant stabilization of the transition state by a sort of cyclic process involving concerted catalysis by well separated acidic and basic groups of moderate strength is particularly noticeable in the hydration of pteridine and in its microscopic reverse, the dehydration of pteridine hydrate. This reversible reaction provides the first known example of effective bifunctional catalysis in water by the monoanion of a dicarboxylic acid. In contrast, the catalytic effects of such bifunctional carboxylate acid–bases are merely additive in the mutarotation of glucose, in the hydration of acetaldehyde, and in the iodination of acetone.[49]

References and Notes

(1) (a) Support of this work by U.S. Public Health Service Grant AM 09221 from the National Institutes of Arthritis and Metabolic Diseases is gratefully acknowledged. (b) Taken in part from the dissertation of David Bjorkquist to be submitted to the University of Washington in partial fulfillment of the Ph.D. degree. (c) D.B. was an NDEA Title IV Fellow, 1971–1974.

(2) A. Albert, *J. Chem. Soc.*, 2690 (1955).

(3) A. Albert, D. J. Brown, and H. C. S. Wood, *J. Chem. Soc.*, 2066 (1956).
(4) (a) B. E. Evans and R. V. Wolfenden, *Biochemistry*, **12**, 392 (1973); (b) Y. Pocker and D. Bjorkquist, unpublished results from this laboratory.
(5) R. L. Reeves, *J. Am. Chem. Soc.*, **84**, 3332 (1962).
(6) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 832 (1962).
(7) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **85**, 2843 (1963).
(8) K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **86**, 2413 (1964).
(9) R. L. Reeves, *J. Org. Chem.*, **30**, 3129 (1965).
(10) T. C. Pletcher, S. Koehler, and E. H. Cordes, *J. Am. Chem. Soc.*, **90**, 7072 (1968).
(11) Marjorie Kandel and E. H. Cordes, *J. Org. Chem.*, **32**, 3061 (1967).
(12) Y. Pocker, M. W. Beug, and K. L. Stephens, *J. Am. Chem. Soc.*, **96**, 174 (1974).
(13) A. C. Satterthwait and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 7018, 7031, 7045 (1974).
(14) R. K. Chaturvedi and G. L. Schmir, *J. Am. Chem. Soc.*, **90**, 4413 (1968).
(15) G. L. Schmir and B. A. Cunningham, *J. Am. Chem. Soc.*, **87**, 5692 (1965).
(16) B. A. Cunningham and G. L. Schmir, *J. Am. Chem. Soc.*, **88**, 551 (1966).
(17) B. A. Cunningham and G. L. Schmir, *J. Org. Chem.*, **31**, 3751 (1966).
(18) S. M. Silver and J. M. Sayer, *J. Am. Chem. Soc.*, **95**, 5073 (1973).
(19) J. M. Sayer, M. Peskin, and W. P. Jencks, *J. Am. Chem. Soc.*, **95**, 4277 (1973).
(20) J. M. Sayer and W. P. Jencks, *J. Am. Chem. Soc.*, **95**, 5673 (1973).
(21) Albert Bruylants and Mrs. E. Feytmants-De Medicis in "The Chemistry of The Carbon-Nitrogen Double Bond", Saul Patai, Ed., Interscience, New York, N.Y., 1970, pp 465–504.
(22) W. P. Jencks in "Progress in Physical Organic Chemistry", S. G. Cohen, A. Streitwiesser, Jr., and R. W. Taft, Ed., Interscience, New York, N.Y., 1964, pp 63–128.
(23) Y. Inoue and D. D. Perrin, *J. Chem. Soc.*, 2648 (1963).
(24) A. Albert in "Pteridine Chemistry", W. Pfleiderer and E. C. Taylor, Ed., Macmillan, New York, N.Y., 1964, pp 111–128.
(25) G. R. Pettit, L. E. Houghton, N. H. Rogers, R. M. Coomes, D. F. Berger, P. R. Rencroft, J. F. Day, J. L. Hartwell, and H. B. Wood, Abstracts, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept 1971, MEDI-37.
(26) E. C. Taylor and P. A. Jacobi, *J. Am. Chem. Soc.*, **95**, 4455 (1973).
(27) A. Albert and H. Yamamoto, *J. Chem. Soc. C*, 2289 (1968).
(28) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).
(29) A. Albert, T. J. Batterham, and J. J. McCormack, *J. Chem. Soc. B*, 1105 (1966).
(30) Y. Pocker and M. J. Hill, *J. Am. Chem. Soc.*, **91**, 3243 (1969).
(31) R. P. Bell and B. Darwent, *Trans. Faraday Soc.*, **46**, 34 (1950).
(32) Y. Pocker and Edmond Green, *J. Am. Chem. Soc.*, **95**, 113 (1973).
(33) (a) D. D. Perrin, *J. Chem. Soc.*, 645 (1962); (b) F. A. Long, F. B. Dunkle, and W. F. McDevit, *J. Phys. Colloid Chem.*, **55**, 829 (1951).
(34) N. Gravitz and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 489 (1974).
(35) Y. Pocker, *Proc. Chem. Soc., London*, 17 (1960).
(36) Y. Pocker and J. E. Meany, *J. Phys. Chem.*, 71, 3113 (1967); also unpublished observations.
(37) Y. Pocker, J. W. Long, and D. B. Dahlberg, unpublished results from this laboratory.
(38) B. C. Challis, F. A. Long, and Y. Pocker, *J. Chem. Soc.*, 4679 (1957).
(39) W. H. Hamill and V. K. LaMer, *J. Chem. Phys.*, **4**, 294 (1936).
(40) F. Brescia and V. K. LaMer, *J. Am. Chem. Soc.*, **60**, 1962 (1938).
(41) M. Choi and E. R. Thornton, *J. Am. Chem. Soc.*, **96**, 1428 (1974).
(42) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **83**, 1743 (1961).
(43) Calculations show that the rate constant k_2' would exceed diffusion control for the kinetically equivalent mechanism involving a hydroxide ion attack on protonated pteridine; $V = k_2'[\text{pteridine}\cdot H^+][OH^-] = (k_2'/K_1)[\text{pteridine}][H_3O^+][OH^-]$.
(44) H. H. Huang, R. R. Robinson, and F. A. Long, *J. Am. Chem. Soc.*, **88**, 1866 (1966).
(45) S. S. Minor and R. L. Schowen, *J. Am. Chem. Soc.*, **95**, 2279 (1973).
(46) Another reaction in which concerted acid–base catalysis of proton transfer in aqueous solution gives a large rate acceleration is the hydrolysis of *N*-phenyliminolactone, studied by B. A. Cuningham and G. L. Schmir, *J. Am. Chem. Soc.*, **88**, 551 (1966).
(47) For the electrostatic model to be applicable, $k_{HCO_3^-}[\text{pteridine}][HCO_3^-]$ must equal $k_{CO_3^{2-}}[\text{pteridine}\cdot H^+][CO_3^{2-}]$. However, this necessitates the value for $k_{CO_3^{2-}}$ to be as large as diffusion-controlled processes:

$$k_{CO_3^{2-}} = k_{HCO_3^-}\left(\frac{[\text{pteridine}]}{[\text{pteridine}\cdot H^+]}\right)\left(\frac{[HCO_3^-]}{[CO_3^{2-}]}\right) = k_{HCO_3^-}\left(\frac{K_1}{[H^+]}\right)\left(\frac{[H^+]}{K_{HCO_3^-}}\right)$$

$$= \frac{(k_{HCO_3^-})(K_1)}{K_{HCO_3^-}} = \frac{(1.5\ 1\ \text{mol}^{-1}\text{min}^{-1})(10^2)}{(60\ \text{sec/min})10^{-10}}$$

$$= 2.5 \times 10^{10}\ 1\ \text{mol}^{-1}\ \text{sec}^{-1}$$

(48) M. Eigen, *Discuss. Faraday Soc.*, **39**, 7 (1965).
(49) G. E. Lienhard and F. H. Anderson, *J. Org. Chem.*, **32**, 229 (1967).

The Alcohol–Bicarbonate–Water System. Structure–Reactivity Studies on the Equilibria for Formation of Alkyl Monocarbonates and on the Rates of Their Decomposition in Aqueous Alkali[1]

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Abstract: The decomposition rates for the alkyl monocarbonates in aqueous alkaline solutions have been measured, and it has been shown that these reactions are characterized by a Bronsted β value for the leaving group of -1.1. This correlation provides a method for estimating the pK_a of weakly acidic alcohols. The equilibria for the reaction, $ROH + HCO_3^- \rightleftharpoons ROCO_2^- + H_2O$, have been measured for a smaller series of alcohols. The data thus obtained were used to calculate a Bronsted β value of 1.4 for the reaction, $RO^- + CO_2 \rightleftharpoons ROCO_2^-$, which shows that the carboxylate group is significantly more electron withdrawing than hydrogen in this reaction. The β value for nucleophilic attack of alkoxide ion on carbon dioxide was found to be 0.3. These results may be used to predict that carbonyl phosphate can exist only in very small amounts in aqueous solutions of bicarbonate and hydrogen phosphate dianion and that its rate of decomposition would be on the order of 10 sec^{-1}. It is suggested that "activated CO_2" represents low-entropy CO_2 fixed at the active site of carboxylating enzymes and made available by the ATP-mediated dehydration of bicarbonate or the decarboxylation of carboxybiotin in situ.

The available evidence derived from studies of a number of enzymic carboxylation reactions has led investigators to propose that enzyme-bound carbonyl phosphate I is an intermediate in these reactions. The existence of this interme-