The Effects of Leaving Group Basicity on the Hydrolysis of Aryl-Substituted Maleanilinic Acids[1]

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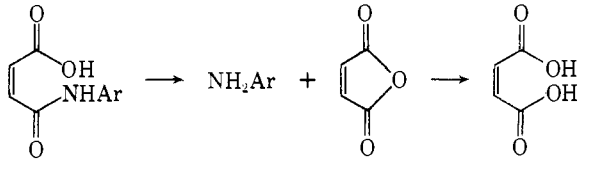
Abstract: The rates of hydrolysis of maleanilinic acid and aryl-substituted derivatives have been measured at 50° for hydrochloric, phosphoric, cyanoacetic, chloroacetic, and methoxyacetic acid buffers. The results have been analyzed in terms of processes involving specific acid, general acid, and water catalyzed processes. It was found that the rate constants for the acid catalyzed processes vary linearly with the basicity of the leaving group with approximately equal slopes, increasing with increasing basicity. The uncatalyzed (water) rate has a steeper dependence. Derivatives of maleanilinic acids, isomaleanilides, were found to proceed under these conditions initially to maleanilinic acids, suggesting existence of a common intermediate which decomposes slowly to anhydride and amine but rapidly to maleanilinic acid. A hydrolysis mechanism for maleanilinic acids is proposed which can account for the experimental observations. Since rate increases with increasing basicity of the leaving group, the strength of the C–N bond is less important in determining the hydrolysis rate than the basicity of the amine. This implies that the transition state for the rate-determining step involves considerably more proton transfer than it does heavy atom bond breakage. This supports the notion that in the decomposition of tetrahedral intermediates in enzymic reactions involving amide hydrolysis, facilitation of proton transfer by an internal catalyst should be an important aspect of the overall catalytic process.

The hydrolysis of amides has been studied extensively in order to obtain a basis for understanding the mechanism of enzymic hydrolysis of proteins.[2-4] In pioneering work on the hydrolysis of phthalamic acid, Bender observed that a carboxylic acid group adjacent to an amide increased the rate of hydrolysis of the amide dramatically.[5] Based on further experimental evidence, Bender suggested that this enhancement resulted from covalent participation of the un-ionized carboxyl group at the amide center, leading to formation of a tetrahedral addition intermediate of the amide.[6] Catalysis then results from lowering the barrier to conversion of the amide center to the destabilized ortho-amide derivative. Further work in this[7] and related systems[8-11] has led to modifications of some details of Bender's mechanism.[11] However, the essence of the original proposal[5,6] has been generally confirmed.

Kluger and Chan recently observed that a phosphate derivative could act as an internal catalyst for amide hydrolysis,[13] and a mechanism similar to that for carboxyl participation was proposed. In further studies on the participation of phosphates in hydrolysis, we observed that the participation reaction is subject to additional catalysis by external Brønsted acids.[14] At the time of our observation, general acid catalysis of carboxyl-promoted amide hydrolysis was reported to occur in a special circumstance involving rate-determining transfer of a proton to nitrogen.[15] Due to kinetic complications introduced by the presence of a second acidic proton in the phosphate derivative, we chose to examine the behavior of an analogous carboxylic acid system. We therefore studied catalytic patterns in the hydrolysis of aryl substituted derivatives of maleanilinic acid. These substrates contain only one acidic group and other derivatives involving different substitution in the aniline moiety are easily prepared. Our studies reveal the effects of varying basicity of the aromatic substituent on rate constants for different modes of catalyzed hydrolysis. We have been able to obtain an empirical basis for predicting the occurrence of general acid catalysis in this system. Furthermore, these results permit us to examine factors controlling the rate of decomposition of intermediates (or kinetically indistinguishable transition states) to yield the aromatic amine and anhydride. Since the decomposition of tetrahedral intermediates derived from additions to peptide amide functionalities is considered to be rate determining in some enzymic systems involving catalysis of the hydrolysis of proteins,[16-18] our results can provide a basis for elucidating the mechanism of the enzymic reaction.



Experimental Section

Materials. Buffers were made from reagent grade materials where available. Cyanoacetic acid (Eastman) was purified by dissolving the material in benzene and decanting the solution from the insoluble oily precipitate. The solution was evaporated to dryness. Since benzene and water form an azeotrope, the residue was essentially anhydrous. The residue was taken up in anhydrous ether and evaporated leaving a white, crystalline residue of cyanoacetic acid. All inorganic materials were reagent grade.

Substituted maleanilinic acids were prepared by a general procedure which involved dissolving 1 equiv of maleic anhydride in benzene. One equivalent of the appropriate aniline derivative was dissolved in dioxane and added with stirring to the benzene solution of maleic anhydride. The resultant maleanilinic acid precipitated from solution and was recrystallized from ethanol. In this manner maleanilinic acid (mp 206 lit. 206–208[19]), 4′-nitromaleanilinic acid (mp 201–202, lit. 195,[20] 204[21]), 3′-nitromaleanilinic acid (mp 203–205, lit. 206[21]), 4′-chloromaleanilinic acid,[22] and 4′-methoxymaleanilinic acid (mp 195, lit. 200[23]) were prepared.

Isoimides were prepared from the corresponding maleanilinic acid by dehydration using dicyclohexylcarbodiimide (Eastman) in methylene chloride as the dehydrating agent, following the procedure devised by Cotter, Sauers, and Whelan.[24] Thus, 11.8 g of 3′-nitromaleanilinic acid was slurried in 100 ml of dichloromethane in a 250-ml round-bottom flask (magnetic stirrer, reflux condenser) to which was added 10.5 g of dicyclohexylcarbodiimide in 60 ml of dichloromethane. The reaction was slightly exothermic. The solution–suspension was stirred for 3 hr. The white powdery precipitate of dicyclohexylurea was filtered off and the filtrate solution was concentrated in vacuo. The residual oil was dissolved in benzene and filtered through a short column of alumina in benzene. Addition of pentane to the filtrate caused crystals to form (7.5 g), 3′-nitroisomaleanilide (**1**). The material was recrystallized from ethyl acetate (mp 55–57): ir 3100, 1700, 1725, 1680, 1520, 1375 cm^{-1}. Additional material was readily recovered from the solution. Mass spectrum parent peak was 218: exact mass of parent peak, calcd for $C_{10}H_6N_2O_4$, 218.0328; found, 218.0329 (on AEI

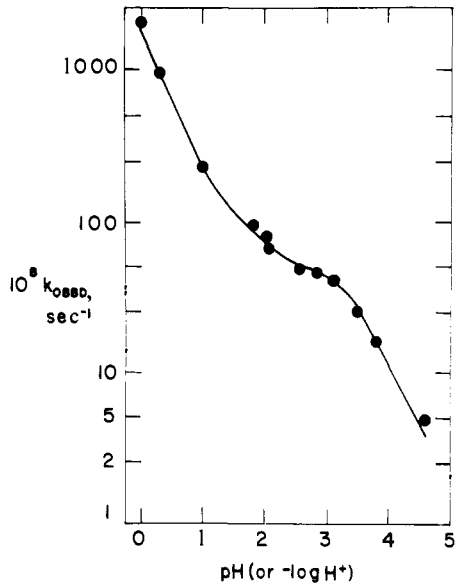
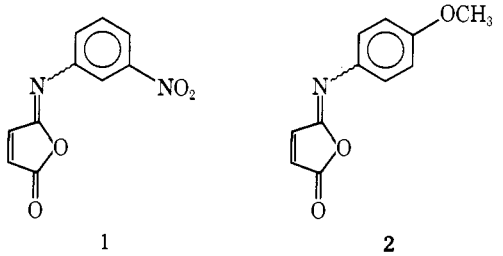


Figure 1. Observed rate constant for hydrolysis at 50° of 4′-nitromaleanilinic acid as a function of pH of acidic solutions. The curve has been generated from the rate constants in Table I and the assumption that the substrate has a pK_a of 3.4 with only the protonated form active toward hydrolysis.

Table I. Rate Constants for Hydronium Ion and Water Catalyzed Hydrolysis of Aryl-Substituted Maleanilinic Acids at 50°

Substituent	k_{H^+}, M^{-1} sec^{-1}	k_{H_2O}, sec^{-1}
4′-Nitro	1.8×10^{-3}	6.0×10^{-5}
3′-Nitro	3.2×10^{-3}	1.3×10^{-4}
4′-Chloro	8.5×10^{-3}	6.0×10^{-4}
None	1.2×10^{-2}	1.3×10^{-3}
4′-Methoxy	1.6×10^{-2}	1.8×10^{-3}

MS 902 high resolution mass spectrometer). By an analogous procedure, 4′-methoxyisomaleanilide (**2**) was obtained from 4′-methoxymaleanilinic acid in 60% yield, mp 75°: mass spectrum parent peak 203; calcd for $C_{11}H_9NO_3$, 203.0582; found, 203.0582. The infrared spectrum again confirmed the existence of the C=N functionality.



Product Analysis. Spectra were determined using a Unicam SP1800A uv-vis spectrophotometer with an AR 25 recorder. All reactions were first studied by following the progress of hydrolysis of maleanilinic acids to maleic acid and amine by repetitive scanning at fixed time intervals of the 250–400-nm region. Final spectra thus obtained were compared with spectra obtained from equimolar solutions of maleic acid and the appropriate aniline derivative. In all cases involving the hydrolysis of maleanilinic acids, the observed spectra from hydrolysis reactions were identical with those obtained from mixing maleic acid and the amine. It was found that the spectra obtained when isomaleanilides were allowed to react with water were identical with those obtained from hydrolysis of the corresponding maleanilinic acids. However, initial spectra were first converted to those of the corresponding maleanilinic acids which at a much slower rate went on to the final spectra.

Kinetic Methods. Pseudo-first-order rate constants for the hydrolysis of the maleanilinic acids were determined by observing the decrease in amide absorbance in the 250–320-nm region depending on the particular compound studied. All hydrolyses were conducted in the thermostated sample holder of the spectrophotometer and temperatures were maintained by a circulating water bath to ±0.1°. Ionic strength was maintained at 0.5 with potassium chloride. Measurements of pH were performed after reactions had gone to completion using a Radiometer PHM 26 expanded scale pH meter. First-order rate constants were obtained from the slopes of plots of log [OD (infinite time) − OD (elapsed time)] vs. elapsed time. Straight lines were obtained for at least four half-times in all instances reported. For reactions at "pH 2" the meter was standardized with 0.01 *M* HCl containing 0.5 *M* KCl. At higher pH's, standard reference buffers were used. At higher acidities, −log acid concentration was used as the measure of solution acidity. Substrates were added to 3 ml of buffer solution either in 2-μl portions in alkaline solution in which hydrolysis is slow or in dioxane (2 μl also). The conversion of isomaleanilides to maleanilinic acids was followed by spectrophotometric procedures as well and also obeyed pseudo-first-order kinetics. In all cases where buffer catalysis was observed, rate constants for a particular pH in the absence of buffer were determined by extrapolation to zero buffer concentration on a plot of buffer concentration against rate constant observed.

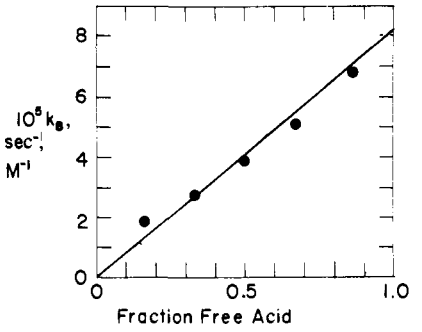


Figure 2. Variation of apparent "buffer" rate constant for hydrolysis of 4′-nitromaleanilinic acid at 50° in chloroacetic acid–potassium chloroacetate buffer with fraction of buffer in free acid form. Rate due to conjugate base of buffer is small or undetectable.

Results

pH–log k_{obsd} profiles for the series of maleanilinic acids we studied can be accommodated by fitting the experimental data to eq 1

$$k_{obsd} = [k_w + k_{H^+}(H^+)][AH/(AH + A^-)] \quad (1)$$

where AH is the undissociated maleanilinic acid and A^- its conjugate base. Empirical values for the dissociation constants of the maleanilinic acids give a reasonable fit of the experimental pH–log k_{obsd} profiles when the value pK_a = 3.4 was used in all cases. Spectrophotometric determination of the dissociation constant of 3′-nitromaleanilinic acid gave an experimental value of 3.3 ± 0.15, which encompasses the kinetically determined value. The pH–rate constant profile for 4′-nitromaleanilinic acid is presented in Figure 1. Other profiles have essentially the same shape but have necessary variation due to changes in component rate constants. Table I summarizes the values of k_w and k_{H^+} that were used to generate satisfactory profiles for the compounds indicated.

Buffer catalysis was investigated using a series of carboxylic acid buffers following the general procedure described by Gravitz and Jencks.[25] For maleanilinic acids derived from amines less basic than anisidine, general acid catalysis by some substituted carboxylic acids was observed. By observing the effect of the buffer ratio, it was determined that the acid portion of the buffer was responsible for catalysis in the hydrolysis of 4′-nitromaleanilinic acid (Figure 2). A Brønsted plot for that compound is presented in Figure 3. Since only a limited number of buffers is useful due to the complications introduced by ionization of the substrate to

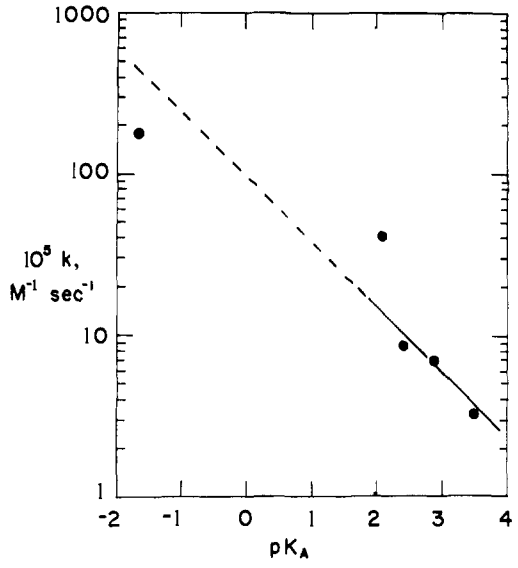


Figure 3. Brønsted plot for the hydrolysis of 4′-nitromaleanilinic acid at 50°. pK's (from left to right) are of hydronium ion, phosphoric acid, cyanoacetic acid, chloroacetic acid, and formic acid.

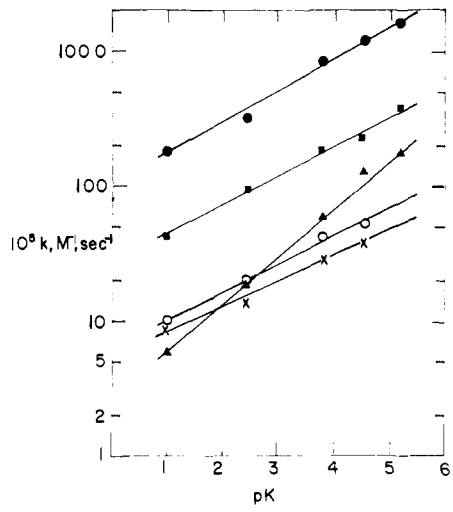
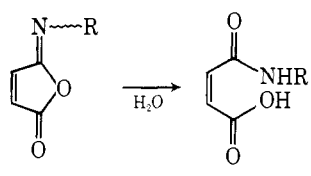


Figure 4. Variation of rate of hydrolysis of aryl-substituted maleanilinic acids at 50° with changes in substituents. pK represents that of aniline from which anilinic acid is derived. Lines are for hydronium ion (●), phosphoric acid (■), cyanoacetic acid (○), chloroacetic acid (×), and water (▲). The units for the water reaction rate constants are in sec^{-1} × 10^5. Other rate constants are as indicated.

an inactive form and the presence of a large background rate due to hydronium ion and water, an accurate plot is difficult to obtain. However, it appears that extension of the line to the pK_a of hydronium ion ($\simeq$−log 55[26]) will place the rate constant for hydronium significantly below the "expected" value. Whether this is interpretable is not ascertainable from our data since one cannot even be sure that the Brønsted line itself does not curve. The point for phosphoric acid is above the extended line, even if statistical corrections[27] are made. Since concerted general acid–base catalysis[28-30] by carboxylic and phosphoric acid buffers is possible and any proposed intermediate may respond differently to tetrahedral tautomeric catalysts[30] (phosphoric acid) vs. trigonal tautomeric catalysts (carboxylic acids), the results may have general implications. Nonetheless, since pH measurements are subject to great uncertainty in the region in question and since the Brønsted line is considerably uncertain, we prefer to draw no conclusions at this time. Leaving group effects were determined for the hydrolysis reaction. Plots of the effect of the basicity of the leaving amine on various empirical rate constants are given in Figure 4. Observed rate constants increase with increasing basicity of the leaving amine; the slopes are of two types. The "water" reaction is subject to a steeper slope (~0.4) than are the acid reactions which are approximately parallel with slopes for Δ log k/ΔpK_a of about 0.3, where pK_a is that of the conjugate acid of the leaving group.



The hydrolysis of isoimides (R = *m*-nitrophenyl, *p*-methoxyphenyl) was followed spectrophotometrically using a reaction temperature of 30° (see Experimental Section). An initial reaction to give a spectrum of the maleanilinic acids was observed in the absence of buffer (0.01 *M* HCl) and in the presence of buffer (chloroacetic acid 0.1–1 *M*). The maleanilinic acid spectrum then went on slowly (~1/10 first reaction rate) to that of maleic acid and amine.

Under the conditions of our studies it was observed that maleic anhydride hydrolyzed rapidly to maleic acid (compared to hydrolysis of maleanilinic acids).

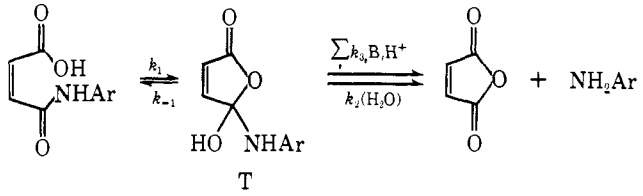
An experiment was performed in which maleic anhydride and anisidine were added at the same time to buffered solution (pH 3.5, methoxyacetate) at 30°. The initial spectrum indicated some maleanilinic acid formed and hydrolyzed slowly. However, when maleic acid and anisidine were combined a spectrum identical with the final spectrum obtained in the hydrolysis of *p*-methoxymaleanilinic acid was obtained.

Discussion

The catalytic patterns for the hydrolysis of maleanilinic acids in solutions of pH below neutrality contrast with those known for amides which hydrolyze without nucleophilic assistance from a neutral carboxyl group[4] and with the reported patterns for maleamides derived from alkylamines.[10,12] The most striking contrast is the susceptibility which maleanilinic acids show toward general acid catalysis compared to the limited accessibility of the hydrolysis of other amides to this sort of catalysis.[10,12] By way of comparison, maleamic acids rarely are susceptible to general acid catalyzed hydrolysis. In the cases where general acid catalysis of maleamic acid hydrolysis is observed, limiting Brønsted slopes of 1 and zero only are observed (presumably due to control by rate-limiting proton transfer).[12] Furthermore, the hydrolysis rate for alkyl maleamides is independent of leaving group basicity. These contrasts suggest that the hydrolyses of maleanilinic acids and alkyl maleamic acids occur by somewhat different mechanisms. A major difference that is subject to analysis is the difference in response of the two classes of compound to changes in leaving group basicity.

The rate constants we observed for acid catalysis of maleanilinic acids show an increasing trend with basicity (Figure 4) of the leaving group. It therefore must be assumed that of the opposing factors (in terms of effect on observed rate constants) of C–N bond strength and basicity of the amino group, the latter is dominant. If all the amines of the intermediates were fully protonated under reaction conditions, it would be expected that the reverse situation would dominate since basicity as a virtue could not be expressed in the rate constant. The monotonic increase in rate

Scheme I



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constant with pK_a indicates that proton saturating effects for anilides cannot be achieved with the derivatives used. The behavior of alkyl maleanides contrasts with the behavior of the aryl compounds. In the alkyl systems, leaving group basicity is of little importance in affecting overall hydrolysis rates. Presumably in those systems C–N bond strength and basicity are nearly balanced in determining the observed rate. The expected decrease in rate with increasing basicity of the leaving group in those systems has not been attained.

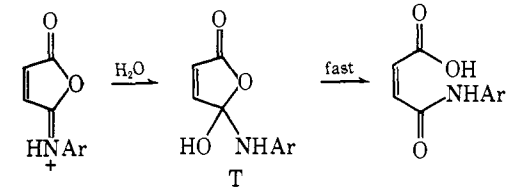
Empirically, the observation of general acid catalysis is related to the size of water and hydronium ion rates. Since the slope for the water rate in Figure 4 is steeper than for general acid rates, general acid catalysis is most difficult to observe with the most basic leaving groups and weakest acid buffers. It is likely that water may be involved in a special mechanism involving concerted general acid base catalysis utilizing hydrogen bonding at all necessary sites.

Kinetic Equations. Although our results do not require assuming the existence of an intermediate in any hydrolysis reaction we have so far examined, the large body of evidence which suggests the existence of tetrahedral intermediates in related systems makes the existence of the analogous species likely here as well. Scheme I proposes a mechanism which can account for our observations. However, variations involving different protonation states of the intermediates and slow proton transfer leading to kinetically equivalent general catalysis terms are not ruled out. We emphasize here that interpretation of our results can be adjusted to accommodate additional refinements as further data become available. Applying the steady state approximation for T:

$$k_{obsd} = \frac{(k_1k_2 + k_1\sum_i k_{3_i}(B_iH^+))}{k_{-1} + k_2 + \sum_i k_{3_i}(B_iH^+)} \qquad (2)$$

The term $\sum_i k_{3_i}(B_iH^+)$ includes hydronium ion. The step associated with k_{3_i} is assumed to involve carboxylic acids or phosphoric acid as tautomeric catalysts in the manner suggested by Blackburn and Jencks[28] and by Cunningham and Schmir.[29] Other Brønsted acids, including hydronium ion are incorporated in this term. Our observation that corresponding isoimides are converted exclusively to maleanilinic acids in the presence and absence of buffers assures that $k_{-1} \gg (k_2 + \sum_i k_{3_i}B_iH^+)$ if proton transfers are rapid compared to heavy atom bond formation and cleavage (Scheme II). This result is consistent with our observation of a linear relationship between buffer concentration and observed rate constant for the hydrolysis of maleanilinic acids. Analysis of the kinetic equation (2) proposed for Scheme I indicates that if addition of water to an isoimide leads to intermediate T (Schemes I and II), the chances for producing maleic anhydride and the substituted aniline rather than the maleanilinic acid are highest in the presence of high acid or buffer concentrations, with the most basic amine as leaving group. Since we observe that maleanilinic acids are the only detectable-initial products of isoimide hydrolysis under the experimental conditions and we observe linear buffer plots this confirms that $\sum_i k_{3_i}(B_iH^+)$ is small compared to other denominator terms that $k_{-1} \gg (k_2 + \sum_i k_{3_i}B_iH^+)$. By contrast Kirby et al. observe nonlinear

Scheme II



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buffer dependence flattening to a maximum in the few cases involving hydrolysis of alkyl maleamides which show buffer catalysis.[12] This behavior requires $\sum_i k_{3_i}(B_iH^+) \gg k_2 + k_{-1}$. We would predict that the corresponding alkyl isoimides should hydrolyze to yield anhydride and amine, rather than alkyl maleamic acids, under conditions leading to leveling of buffer catalysis.

For our system then

$$k_{obsd} = [k_1k_2 + k_1k_3\sum_i(B_iH^+)]/k_{-1} \qquad (3)$$

Therefore in the absence of added buffer (or significant hydronium ion concentration)

$$k_{obsd} = k_1k_2/k_{-1} \qquad (4)$$

Since all the substrates should have roughly the same relationship of k_1 and k_{-1}, variations in "water rate" must be due to factors affecting k_2, the rate constant for decomposition of the intermediate T. These differences must result from differences in the departing amines, presumably due to basicities. In the presence of Brønsted acids, again

$$k_{obsd} - k_{H_2O} = (k_1\sum k_{3_i}B_iH^+)/k_{-1} \qquad (5)$$

The observed rate constant is a function of the product of (k_1/k_{-1}) and rate constants which vary with the leaving group.

Since in Figure 4 we observe increases in observed rate constant with increasing basicity of the leaving group, the facility of transfer of proton to the leaving group (or a kinetic equivalent) is the controlling feature of the C–N bond cleavage steps, k_{3_i} and k_2.[31]

In summary, we find that general acid catalyzed hydrolysis can be an important factor in the behavior of maleanilinic acids derived from amines less basic than aniline. Reactions of maleanilinic acid and *p*-methoxymaleanilinic acid are dominated by background water rates. All observed rate constants for maleanilinic acid derivatives appear to be linearly related (positive slope) to the pK_a of the conjugate acid of the aromatic amine leaving group. This indicates that C–N bond strength is less of a rate-controlling feature than is amine basicity. The contrasting behavior of alkyl amine derivatives suggests that the nature of the C–N bond cleavage step in the two systems is different. The susceptibility of maleanilinic acids to general acid catalysis appears to be a direct consequence of the greater facility of C–N bond cleavage in the aromatic series.

The nature of this facility is perhaps analogous to that observed for alkyl amides with strong steric interactions[12] which also would tend to promote C–N bond cleavage but leave the requirement for proton addition to an external source. It is therefore a likely possibility that through interactive forces, an enzyme could promote cleavage of a C–N bond of an addition intermediate to drive reaction toward the transition state for hydrolysis. However, efficient catalysis will best occur if in addition an acid source is available to protonate the departing amine, the role we have ascribed to the general acid in the systems we have so far examined.

References and Notes

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

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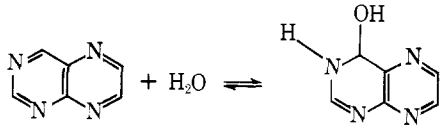
Abstract: The hydration of pteridine has been investigated over the pH range 1–10 at 25.0°, utilizing a spectrophotometric method. Above pH 7.5, the hydration of this heterocyclic Schiff base is catalyzed by hydroxide ions and by the basic component of a number of buffers (imidazole, 1,2-dimethylimidazole, 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).



(1)

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