for $C_{13}H_{14}$: C, 91.76; H, 8.23. Found: C, 91.49; H, 8.46.

(1*S*,2*S*,3*S*,5*R*)-(+)-*trans*-Isopinocampheylethyne: yield 74%; bp 108–110 °C (70 Torr); IR (neat) 2104, 3305 cm^{-1}; ^1H NMR (CCl_4) 1.02 (d, 3 H), 1.22 (s, 6 H), 1.62–2.6 (m, 9 H); ^{13}C NMR ($CDCl_3$) 21.6, 23.0, 28.1, 28.4, 34.5, 35.4, 38.3, 41.4, 45.0, 47.7, 67.2, 91.5; α^{23}_D +43.10° ±0.01 (neat, *l* 1.0).

General Procedure for the Determination of Optical Purity of Acetylenes. The acetylene (7.8 mmol, 1.2 g) was dissolved in CH_2Cl_2 (25 mL) and a solution of methyltrialkylammonium chloride (Adogen 464) (0.8 g) in CH_2Cl_2 (25 mL) was added. To the mixture was added AcOH (5 mL) followed by a solution of $KMnO_4$ (17.7 mmol, 2.8 g) in water (50 mL). The mixture was refluxed with stirring for 6 h and then allowed to come to room temperature. Sodium bisulfite (solid) was added, in portions, until the mixture became colorless. The organic and aqueous layers were separated, and the aqueous layer was cooled to 0 °C and acidified with concentrated HCl. It was saturated with NaCl and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic portion was washed with brine (20 mL) and dried over $MgSO_4$. The solvent was removed to obtain the residue, which was dissolved in ether (15 mL) and extracted with 3 M NaOH (saturated aqueous $NaHCO_3$ in the case of chiral compounds). The aqueous portion was cooled to 0 °C, acidified with 3 M HCl, saturated with NaCl, and extracted with ether (3 × 20 mL). The combined ether portion was washed with brine (15 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure (25 °C, 12 Torr), and the residue was distilled to afford the α-chiral carboxylic acid. The carboxylic acids were obtained in yields of 65–70%. The acid (0.02 mmol) was coupled (16 h) to (*R*)-(+)-methylbenzylamine (0.02 mmol) in the presence of 1,1′-carbonyldiimidazole (0.02 mmol) in ether to give the desired amide. The crude amide was taken up in EE and analyzed on capillary GC.

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Supplementary Material Available: ^1H NMR, ^{13}C NMR, and IR spectra for **13**, **14**, **15**, **18**, **19**, and **20** (18 pages). Ordering information is given on any current masthead pages.

Boronic Acid Catalyzed Hydrolyses of Salicylaldehyde Imines

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The hydrolysis of salicylaldehyde imines is catalyzed by boric acid, substituted arylboronic acids, and diphenylborinic acid. These reactions show saturation kinetics, allowing the determination of first-order catalytic constants and dissociation constants. Dissociation constants reflect single-ionization p*K* values similar to the p*K* values of the boronic acids. Binding is best on the acid side of the p*K*. Use of the Brønsted and the Hammett relationships shows that the binding constants are improved by electron-withdrawing substituents on the catalysts. Catalytic constants are nearly independent of pH, of Hammett σ, and of the p*K* of the catalyst. The reactions display no solvent deuterium isotope effect.

Introduction

Enzyme models that show binding prior to chemical reaction steps are studied for various reasons; one of these is an effort to understand the mechanisms of enzyme-catalyzed reactions. Most of these systems have used oligomeric or polymeric substances[1] such as synthetic polymers,[2,3] micelles,[4] cyclodextrins,[5] macrocyclic ethers,[6,7] substituted oligonucleotides,[8] and antibodies.[9-11] Small molecules that exhibit reversible binding prior to the catalytic steps are less common. One such example is the boron family of acids, comprising boric, boronic, and borinic acids.

The chemistry of these compounds has long been of interest due to their Lewis acid character. Boron in trivalent compounds has one vacant orbital which is available for the formation of a fourth covalent bond with electron donor. Boric and boronic acids are trigonal about the boron atom. They ionize to become tetrahedral boronate anions by accepting an electron pair from OH^- as shown in eq 1.[12]

$$RB(OH)_2 + {}^-OH \rightleftharpoons RB(OH)^-_3 \qquad (1)$$

The ability of boronates to bind to polyols and various hydrolytic enzymes is well known. This binding depends on the ability of boronates to rapidly and reversibly esterify to alcohols.[13-19]

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This binding ability has acquired importance in chromatography, where boronic acids can be attached to cellulose derivatives and then used to separate sugars,[20] nucleotides,[21] and enzymes.[22] It is also important in studies on enzyme mechanisms due to boronates' ability to function as potent transition state analogue inhibitors of serine and metallohydrolases.[23-27]

Borates and boronates have also been used as enzyme models. Peer and coworkers[28,29] have found that phenol reacts with formaldehyde and gives *o*-(hydroxymethyl)-phenol only in the presence of boric acid. This is probably due to rapid and reversible formation of a complex of phenol, borate and formaldehyde.

Letsinger et al.[30] used 8-quinolineboronic acid as a catalyst in the hydrolysis of chloroethanol to ethylene glycol. It catalyzes the hydrolysis at least 80 times faster than a mixture of quinoline and benzeneboronic acid. 8-Quinolineboronic acid also shows stereoselectivity in the hydrolysis of chloro alcohols.[31] It hydrolyzes *trans*-2-chloro-1-indanol to product much more rapidly than it acts on the cis isomer.

Letsinger et al.[32] also used boronoarylbenzimidazole as a catalyst in the formation of ethers from chloroethanol in butanol solution. The borono group in boronoarylbenzimidazole apparently binds alcohol substrates and holds them in a position favorable for the reaction.

Capon and Ghosh[33] found that borate catalyzes the hydrolysis of phenyl salicylate more than 100-fold more rapidly than the hydrolysis of phenyl *o*-methoxybenzoate and phenyl benzoate. Borate forms a complex with phenyl salicylate, and the boron atom acts as a Lewis acid to accept a lone pair of electrons from the carbonyl oxygen atom. This leads to the formation of an intermediate that resembles the transition state of ester hydrolysis.[29]

Okuyama et al.[34] showed that borate accelerates the hydrolysis of *S*-butyl 2-hydroxy-2-phenylthioacetates 80-fold at pH 9. Butyl thioacetate is not hydrolyzed by borate since there is no hydroxyl group in this ester to form a complex with borate.

Boric acid is also known to catalyze the formation and hydrolysis of hydroxyl group containing imines.[35-38] The reaction shows saturation kinetics, indicating the formation of a borate–imine complex. The suggested mechanism for this catalysis was an intramolecular transfer of a boron-coordinated hydroxide ion within a borate–substrate complex.

Boronic acids have been used less often in studies of boronate catalysis than boric acids, even though they have been observed to complex to alcohols[18] and various enzymes much more tightly. Boronic acids also provide the opportunity to study a reaction using linear free energy relationships, due to the availability of many substituted boronic acids.

In this study, we investigate the mechanism of the boronate–imine system by using boric, boronic, and borinic acids of differing pK as catalysts.

Experimental Section

Materials. Buffers of 0.1 M ionic strength[39] were made from reagent grade chemicals and deionized water. They were filtered through 0.45-μm Millipore membranes.

Benzeneboronic acid, 3-aminobenzeneboronic acid, 4-bromobenzeneboronic acid, diphenylborinic acid ethanolamine complex, D-fructose, and deuterium oxide were purchased from Aldrich.

3-Nitrobenzeneboronic acid was purchased from ICN Pharmaceuticals. 3-Carboxybenzeneboronic acid was purchased from Calbiochem. 3,5-Bis(trifluoromethyl)benzeneboronic acid was purchased from Alfa. Boric acid was purchased from Fisher Scientific.

Salicylaldehyde and 4-hydroxybenzaldehyde were purchased from Sigma. The salicylaldehyde was distilled before use. L-Isoleucine and L-isoleucinamide were obtained from U.S. Biochemicals.

4-Tolueneboronic acid was prepared by Mr. Roger Tietze using the procedure of Bean and Johnson.[40]

Preparation of the substrate imines relies on the spontaneous formation of imines from aldehydes and amines. The procedure used here is similar to that used earlier.[35-38] The progress of imine formation in each case was followed by observing the appearance of the characteristic imine absorbance maximum at 390 nm.[35-38]

Preparation of Salicylidene-L-isoleucine. A 12.6-mg (0.096-mmol) sample of L-isoleucine was dissolved in 7 mL of bicarbonate buffer, pH 10.0, and 0.01 mL of salicylaldehyde (0.096 mmol) was added. The reaction mixture was shaken for few minutes to dissolve salicylaldehyde and kept in the refrigerator overnight.

Preparation of Salicylidene-L-isoleucinamide. A 12.4-mg (0.096-mmol) sample of L-isoleucinamide were dissolved in 7 mL of bicarbonate buffer, pH 10.0, and 0.01 mL (0.096 mmol) of salicylaldehyde were added. The reaction mixture was kept in the refrigerator overnight and salicylidene-L-isoleucinamide was precipitated during this time. The precipitate was separated by filtration, washed with buffer, dissolved in ether, and then dried over anhydrous $MgSO_4$. Ether was evaporated under nitrogen at room temperature to get a solid imine. A stock solution of 0.011 M imine was made in acetonitrile (2.6 mg/mL).

Preparation of 4-Hydroxybenzylidene-L-isoleucine. A 0.136-mL portion of 0.1 M 4-hydroxybenzaldehyde from the stock solution was added to 1.0 mL of 0.013 M L-isoleucine stock solution in bicarbonate buffer, pH 10.0. The reaction mixture was mixed by shaking and kept in the refrigerator overnight.

Methods. Kinetic Measurements. The hydrolysis of an imine was carried out by following the decrease in 390-nm absorbance[35-38] on a McPherson double-beam spectrophotometer. Reactions were conducted at 30.0 ± 0.2 °C.

Typical reactions contained 1 mL of buffer, 5–30 μL of a boronic acid stock solution, and 10 μL of 13.6 mM salicylidene-L-isoleucine

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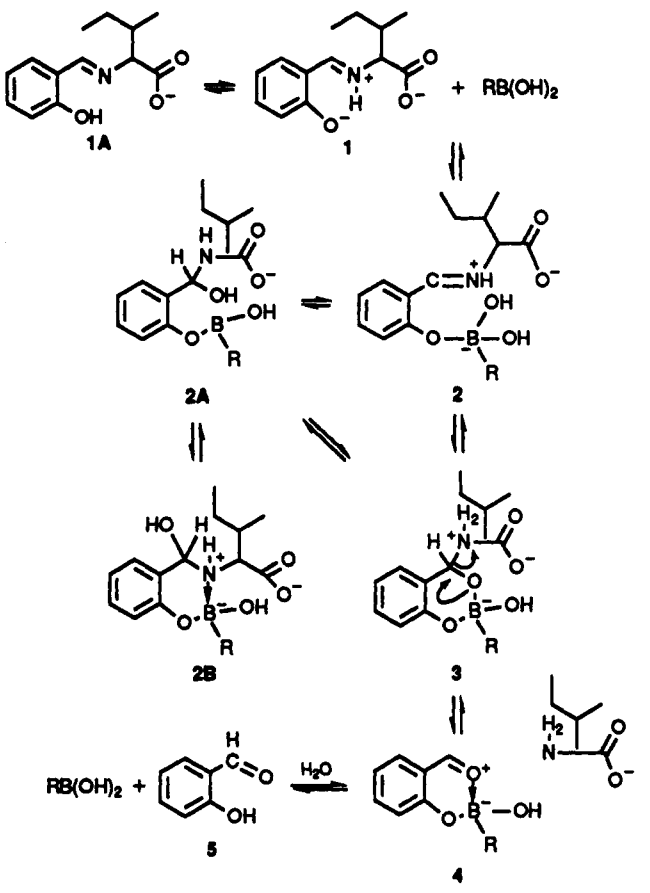
Scheme I



stock solution. In the case of (4-hydroxybenzylidene)-L-isoleucine, 0.2 mL of the 13.6 mM imine stock solution was used because it exhibits a smaller change in the absorbance on hydrolysis.

The spontaneous hydrolysis of the imines was followed at various pH's. The reaction rate constants obtained with boronates were corrected for spontaneous hydrolysis by subtracting the spontaneous rate constant from the catalyzed rate constant.

Kinetics in Deuterium Oxide. I = 0.1 M phosphate buffers of measured pH 6.0 and 6.6 were prepared using small amounts of 0.5 M KH_2PO_4 and 0.5 M NaOH in D_2O. Buffers containing 0.02 M benzeneboronic acid were prepared by dissolving the boronic acid directly in these two buffers. Reactions were initiated by adding 0.01 mL of salicylidene-L-isoleucine from the stock solution to 1.0 mL of buffer that contains the boronic acid. The spontaneous reaction was followed without any added boronate.

The pH of reaction solutions was measured on a Radiometer PHM61 pH meter immediately after completion of each reaction. The pH of solutions containing deuterium oxide is reported as the value read on the pH meter, uncorrected for the deviation near 0.4 caused by deuterium oxide.[41,42] This procedure minimizes the change in observed pK values (5).

Determination of K_m and k_{cat}. The first-order rate constant for the hydrolysis of the imine was determined at different concentrations of boronic acid and fixed concentrations of imine. The reciprocal of this rate constant was plotted as an ordinate, while 1/[catalyst] was the abscissa. This yields a modified Lineweaver–Burk plot. Similar plots have been used previously.[43]

All linear plots were analyzed using a linear least-square fit, and the data were taken from those plots.

Results

The hydrolysis of salicylidene-L-isoleucine 1 (Scheme I) is accelerated by boric acid and a boronic acid. The product's spectrum agrees with that of salicylaldehyde (λ_{max} at 255 nm). The product spectra were the same for the catalyzed and the uncatalyzed reactions.

In work not shown, 3-nitrobenzeneboronic acid and diphenylborinic acid were shown to have no effect on the rate of hydrolysis of (4-hydroxybenzylidene)-L-isoleucine, the para isomer of the compound shown above.

The following experiments were done in order to understand the mechanism of the imine 1 hydrolysis catalyzed by boronic acids.

Effect of Boronic Acid Concentration on the Hydrolysis of the Imine. The hydrolysis of the imine was studied by varying the concentration of boronic acid and keeping the concentration of the imine constant. A hyperbolic curve was obtained which is similar to the one observed in the case of an enzyme-catalyzed reaction. Figure 1 shows a Lineweaver–Burk plot of (3-nitrobenzeneboronic acid concentration)$^{-1}$ vs (first-order rate constant)$^{-1}$. These results indicate the formation of a complex between the boronic acid and the imine.

Effect of pH on the Hydrolysis of the Imine by Boronic Acids. The second-order rate constants for the hydrolysis of the imine 1 by 3-nitrobenzeneboronic acid are plotted as a function of pH in Figure 2. All points on

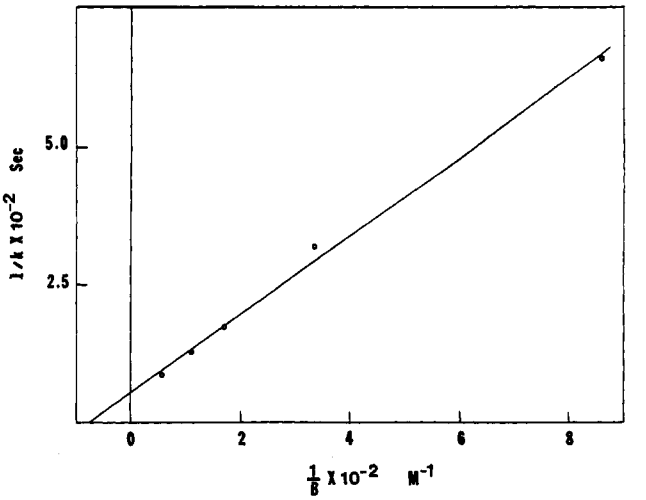


Figure 1. Lineweaver–Burk plot for the hydrolysis of 0.13 mM salicylidene-L-isoleucine catalyzed by 3-nitrobenzeneboronic acid in pH 6.0, 0.1 M phosphate buffer at 30 °C. B indicates the molar concentration of 3-nitrobenzeneboronic acid.

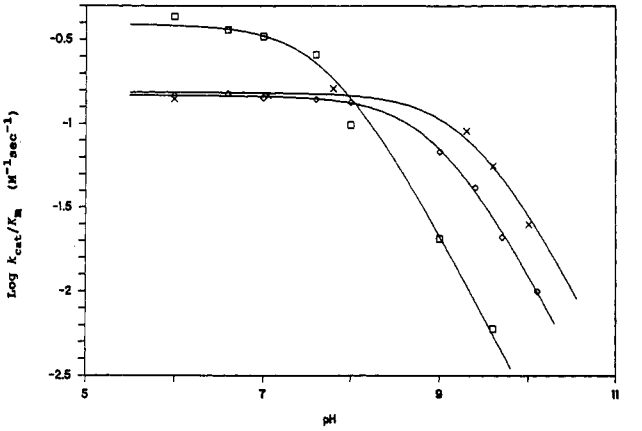


Figure 2. pH profiles for the hydrolysis of 0.13 mM salicylidene-L-isoleucine in 20 mM benzeneboronic acid (◇) [k(lim) = 0.147 ± 0.006 s^{-1}, pK = 8.96 ± 0.015]; 0.13 mM salicylidene-L-isoleucine in 20 mM 3-nitrobenzeneboronic acid (□) [k(lim) = 0.389 ± 0.064 s^{-1}, pK = 7.76 ± 0.07]; 0.13 mM salicylidene-L-isoleucinamide in 20 mM benzeneboronic acid (×) [k(lim) = 0.153 ± 0.016 s^{-1}, pK = 9.35 ± 0.016]. The k(lim) and the pK values are those of the best-fit theoretical curves (see Methods).

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the pH–rate profile were corrected for the spontaneous reaction at the same buffer concentration. The rate of imine hydrolysis obtained with the boronic acid is maximum at lower pH's and decreases with increasing pH. The pH profile reflects a single-proton ionization with a pK of 7.76. This is not far from the literature pK of 7.23 for 3-nitrobenzeneboronic acid[44,45] and may also be close to the pK of the phenolic hydroxyl group in the substrate.

In order to determine the origin of this pK, another boronic acid that has a different pK was chosen for the pH study. Benzeneboronic acid was chosen as its pK of 8.83[44,45] is nearly 1.5 pH units higher than that of 3-nitrobenzeneboronic acid. Figure 2 also shows higher than that of 3-nitrobenzeneboronic acid. Figure 2 also shows the pH profile of the hydrolysis of the imine in the presence of benzeneboronic acid. This pH profile also shows a single ionization. The pK of 8.96 for the theoretical curve is close to the pK of 8.8 of benzeneboronic acid. These results show that the pK observed in the pH profile varies with the boronic acid used in the study. This suggests that only the acidic forms of boronic acids are catalytically active.

In another experiment, we examined the effect of the carboxyl group, present in the amino acid part of the imine, on the pH profile of these boronic acid catalyzed hydrolyses. A different imine, salicylidene-L-isoleucinamide was prepared, using isoleucinamide instead of isoleucine.

The pH dependence of the benzeneboronic acid catalyzed hydrolysis of salicylidene-L-isoleucinamide was compared to that of salicylidene-L-isoleucine. The pH profile for this hydrolysis is also shown in Figure 2. The pK used to draw the theoretical curve is 9.35. This is 0.4 pK units higher than that obtained from salicylidene-L-isoleucine hydrolysis.

The pH Dependence of k_{cat} and K_m in the Hydrolysis of the Imine by the Boronic Acid. The pH profiles obtained earlier do not explain the reason for the decrease in imine hydrolysis with increasing pH. It is not known whether it is due to poor binding or lower catalytic rate constants at higher pH's. The kinetic constants, k_{cat} and K_m, were determined at pH 6.0 and 7.8 for the hydrolysis of the imine with 3-nitrobenzeneboronic acid. Conditions were as in Figure 2. The k_{cat} values are nearly the same at both pH's (0.018 s^{-1} at pH 6.0, 0.023 s^{-1} at pH 7.8). K_m values were radically different and followed the trend shown by k_{cat}/K_m. The value of K_m at pH 6.0 is 0.013 M and is 0.49 M at pH 7.8. Thus, K_m at pH 7.8 is approximately 4-fold higher than at pH 6.0.

The Solvent Deuterium Isotope Effect on the Hydrolysis of the Imine by Boronic Acids. The solvent deuterium isotope effect was studied in order to determine if proton transfer is involved in the rate-determining step of the catalysis. The reaction would be expected to be slower in D_2O if such transfer takes place in the rate-determining step of the reaction. The hydrolysis of the imine catalyzed by benzeneboronic acid was observed at pH 6.0 and 6.6 in D_2O and in H_2O. This is a region where the observed rate is independent of pH, allowing the determination of the isotope effect without interference by effects resulting from pH–pD differences. The kinetics in D_2O were determined to be in the pD-independent region since the observed rates at $pH_{measured}$ 6.0 and 6.6 were the same.

The ratio of k_{H_2O}/k_{D_2O} at both pH's is close to unity; each rate constant is 0.003 s^{-1} in 0.20 M benzeneboronic acid, I = 0.1 M phosphate at 30 °C. This suggests that no proton transfer is involved in the rate-determining step.

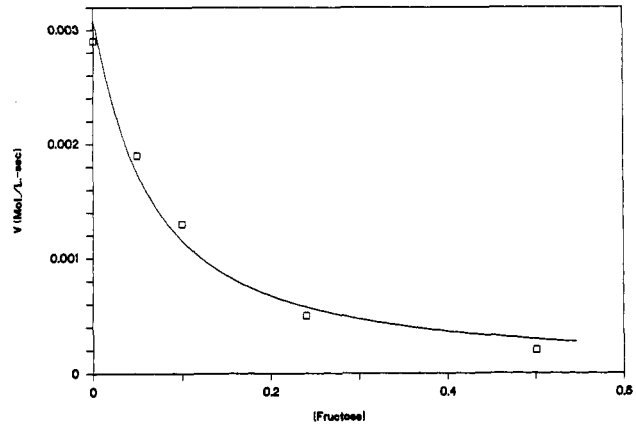

Figure 3. The effect of fructose on the hydrolysis of 0.13 mM salicylidene-L-isoleucine catalyzed by 25 mM benzeneboronic acid in pH 6.0, 0.1 M phosphate buffer at 30 °C. The curve is drawn using a fructose–benzeneboronic acid dissociation constant of 0.05 M.

Table I. Kinetic Constants for the Hydrolysis of Salicylidene-L-isoleucine with Boric Acid, Substituted Benzeneboronic Acids, and Diphenylborinic Acid[a]

boron acid	K_m (M × 10^2)	k_{cat} (s^{-1} × 10^2)	k_{cat}/K_m (M^{-1} s^{-1})	pK (lit.)
boric acid	435	12.8	0.029	8.98 (19)
3-aminobenzeneboronic acid	19.5	21.7	1.11	
benzeneboronic acid	17.0	2.50	0.147	8.8 (45)
3-carboxybenzeneboronic acid	21.7	4.34	0.20	
4-tolueneboronic acid	15.0	1.47	0.09	8.95 (60)
4-bromobenzeneboronic acid	3.1	1.16	0.37	8.06 (45)
3-nitrobenzeneboronic acid	1.3	1.81	1.39	7.29 (45)
3,5-bis(trifluoromethyl)benzeneboronic acid	0.56	1.35	2.38	
diphenylborinic acid	0.10	7.7	77.0	6.2 (59)

[a] Conditions: 0.1 M phosphate buffer, pH 6.0, 30 °C.

The Effect of Fructose on the Hydrolysis of the Imine Catalyzed by the Boronic Acid. It is known that boronic acids form complexes with sugars and become tetrahedral upon complexation.[46] It is of interest to know whether boronic acids act as catalysts while complexed with sugars. Figure 3 shows the effect of fructose on the hydrolysis of the imine catalyzed by benzeneboronic acid at pH 6.0. The rate of hydrolysis decreases with increasing concentration of fructose and is completely inhibited at higher concentration of fructose. It clearly shows that boronic acids do not act as catalysts in this system when saturated with fructose. The curve in Figure 3 is calculated using a K_{diss} of 0.05 M for the fructose–benzeneboronic acid dissociation constant. This value is close to the calculated benzeneboronic acid–fructose dissociation constant of 0.14 M, which is obtained from the literature value[46] when corrected for pH using the pK of benzeneboronic acid.

Effect of Substituents of Benzeneboronic Acid on the Hydrolysis of the Imine. This experiment has been done in order to determine the Hammett ρ value for these catalyses. The effect of various substituted benzeneboronic acids on the hydrolyses was studied at pH 6.0. The kinetic

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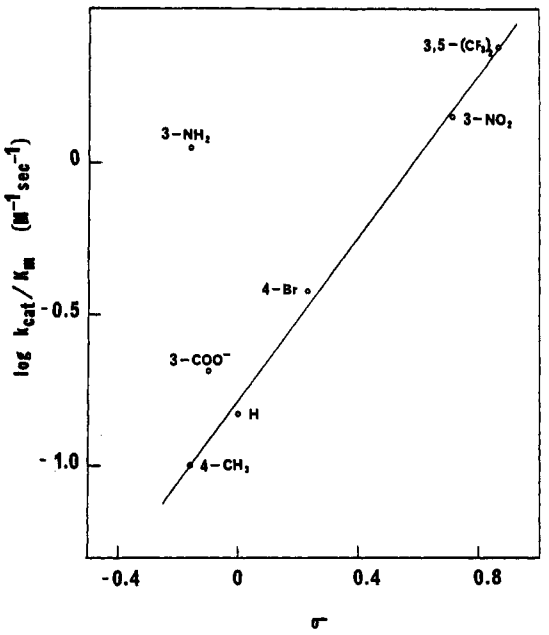

Figure 4. A Hammett plot relating log k_{cat}/K_m and σ for the hydrolysis of the 0.13 mM salicylidene-L-isoleucine by substituted benzeneboronic acids in pH 6.0, 0.1 M phosphate buffer at 30 °C. The 3-carboxy- and 3-aminobenzeneboronic acids, showing a systemic deviation from the others, were not included in the regression analysis. The value of ρ is +1.35 ± 0.04.

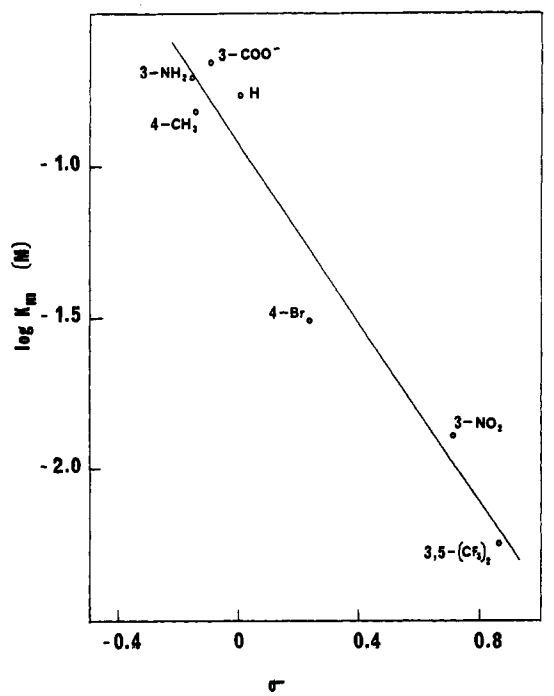

Figure 5. A Hammett plot relating log K_m and σ for the hydrolysis of the 0.13 mM salicylidene-L-isoleucine by substituted benzeneboronic acids in pH 6.0, 0.1 M phosphate buffer at 30 °C. The value of ρ is −1.48 ± 0.15.

constants, k_{cat} and K_m, were determined for all boronic acids using Lineweaver–Burk plots, and the results are given in Table I. The second-order rate constants, k_{cat}/K_m, are higher in the cases of benzeneboronic acids with electron-withdrawing substituents as compared to benzeneboronic acids with electron-donating substituents. The highest value obtained with 3,5-bis(trifluoromethyl)benzeneboronic acid is 2.38 M^{-1} s^{-1} and the lowest value is 0.09 M^{-1} s^{-1} with 4-tolueneboronic acid.

Figure 4 shows a Hammett plot of log (k_{cat}/K_m) of the hydrolysis of the imine by boronic acids vs substituent constants. The values of k_{cat}/K_m for all boronic acids fall on a straight line with a slope of 1.35. 3-Amino- and 3-carboxybenzeneboronic acids deviate systematically from the plot and were not included in the calculation.

When log K_m values for the hydrolysis of the imine by boronic acids are plotted as a function of substituent constants, all boronic acids, including the two that deviate in the plot of log (k_{cat}/K_m) vs σ fall on a straight line with a slope of −1.48 (Figure 5). This suggests that benzeneboronic acids with electron-withdrawing substituents bind the imine more tightly than do boronic acids with electron-donating substituents. These results are similar to the pattern observed in the binding of substituted benzeneboronic acids to serine proteases, where electron-withdrawing substituent groups induce tighter binding by forming a strong complex with the active site serine hydroxyl group.[24,47]

When log k_{cat} values for imine hydrolyses catalyzed by various boronic acids are plotted as a function of Hammett σ, the result is a linear plot with a slope close to zero (−0.064). This is seen in Figure 6. The exceptions are values for 3-amino- and 3-carboxybenzeneboronic acids, which reacted faster than would be expected from their substituent constants. This plot shows that the catalytic rate constant, k_{cat}, is independent of electronic effects due to substituents on the benzeneboronic acid and suggests

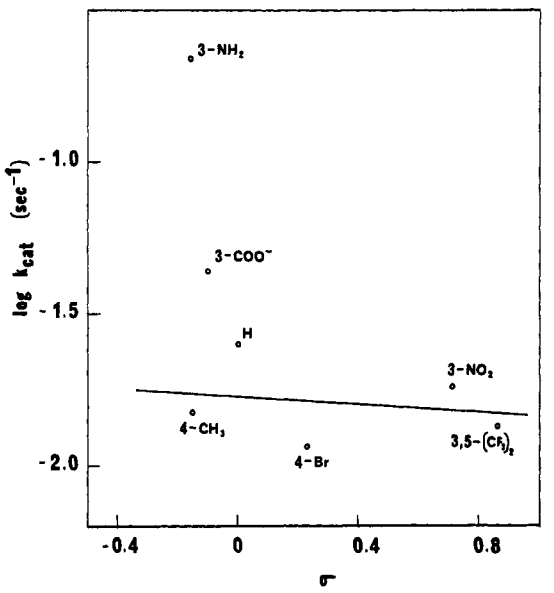

Figure 6. A Hammett plot relating log k_{cat} values and σ for the hydrolysis of 0.13 mM salicylidene-L-isoleucine by substituted benzeneboronic acids in pH 6.0, 0.1 M phosphate buffer at 30 °C. The 3-carboxy- and 3-aminobenzeneboronic acids, showing a systemic deviation from the others, were not included in the regression analysis. The value of ρ is −0.064 ± 0.16.

that the rate-determining step is common with all boronic acids. These data confirm that 3-amino- and 3-carboxybenzeneboronic acid exhibit unusually high reaction rates.

Comparison of Boric, Boronic, and Borinic Acids. The effects of boric acid, benzeneboronic acid, and diphenylborinic acid on the hydrolysis of the imine were also studied at pH 6.0. The kinetic constants, k_{cat} and K_m, were determined for all three acids. The results are listed in Table I which show that the dissociation constant K_m decreases as the hydroxyl group of boric acid is replaced by benzene ring. k_{cat} does not change significantly when compared to K_m. Benzeneboronic acid and diphenylborinic

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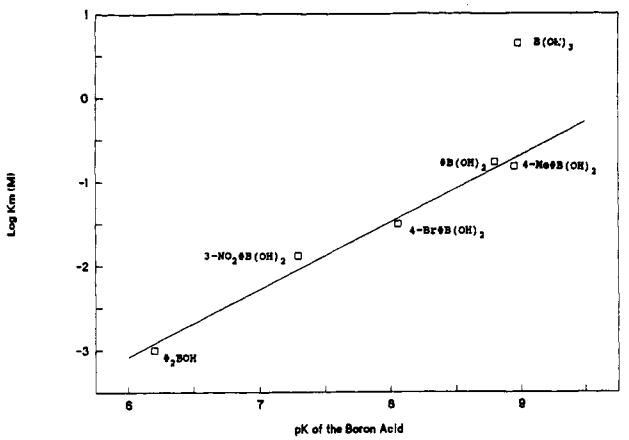

Figure 7. A Brønsted plot relating boron acid pK values to log K_m for the hydrolysis of salicylidene-L-isoleucine. pH 6.0, 0.1 M phosphate buffer at 30 °C. pK values reflect the known pK values of the boron acids. The slope, calculated using boronic and borinic acid data, is +0.80 ± 0.06.

acid bind the imine better than boric acid by nearly 25-fold and 4350-fold, respectively.

A Brønsted plot relating the boronic acid pK values to log K_m for the hydrolysis of the imine is shown in Figure 7. The boronic acids and diphenylborinic acid fall on a single straight line with a slope of +0.80. Boronic acids with low pK values show better binding. The reason phenylboronic and diphenylborinic acids exhibit different reaction rates is thus related to the different pK's of the acids and is not directly due to the different number of aryl groups attached to the boron atom. Boric acid binds about 30-fold less well than boronic acids of similar pK.

Discussion

Benzeneboronic acids accelerate the hydrolysis of salicylidene-L-isoleucine. The hyperbolic dependence of first-order rate constants on 3-nitrobenzeneboronic acid concentration (shown as a Lineweaver–Burk plot in Figure 1) indicates that this system follows Michaelis–Menten kinetics by the formation of a boronic acid–imine complex. This suggests that the acceleration of imine hydrolysis by the boronic acid must be due to the complex formation between the boronic acid and the imine followed by the breakdown of the complex to products.

boronic acid + imine ⇄ boronic acid–imine complex → products

In work not shown, the boronic acid has no effect on the hydrolysis of the imine derived from 4-hydroxybenzaldehyde and L-isoleucine. These results support the idea that boronic acid catalysis depends on the formation of a boronic acid complex with the phenolic OH group in the ortho position of the imine.

It has been known that boric acid esterifies with the phenolic OH group of salicylaldehyde, salicylamide,[48] and salicylaldehyde oxime.[49] It may be concluded that the boronic acid complexes with the imine through esterification of phenolic OH group of the imine.

The boronic acid group in the complex facilitates the hydrolysis of the imine by forming a six-membered ring involving either imine carbon or nitrogen. In the case of (4-hydroxybenzylidene)-L-isoleucine, the boronic group is far away from the imine nitrogen or carbon in the complex and it may not be possible for the boronic group to form a cyclic structure like the one possible in the case of salicylidene-L-isoleucine.

pH Effects on Catalytic Constants. The pH dependence of the hydrolysis of the imine catalyzed by 3-nitrobenzeneboronic acid (Figure 2) shows that the rate constants are maximal at lower pH's and decrease with increasing pH. The pH profile observed may be due to the ionization of 3-nitrobenzeneboronic acid as the pK obtained from the theoretical curve is close to the pK of 3-nitrobenzeneboronic acid. This is consistent with the pH profile observed for the boric acid-catalyzed hydrolysis of a hydroxy imine.[36] It is also possible that the pH profile observed is due to the ionization of some group in salicylideneisoleucine, such as the phenolic OH group.

The pH profile for the catalysis of salicylideneisoleucine by unsubstituted benzeneboronic acid was done in order to determine the origin of this pK. Figure 2 shows that the pH profile is shifted, to give a calculated pK of 8.96, nearly identical with the literature value of 8.8[44] for benzeneboronic acid. The shift in reaction pK that results from exchange of catalyst shows that ionizeable groups in salicylideneisoleucine are not responsible for the pK values seen in catalysis.

This conclusion is supported by the low pK of 4.70[50] determined for the phenolic hydroxyl group in salicylidene-2-aminopropane. Based on this value, it may be assumed that the phenolic OH group of salicylideneisoleucine is ionized in the entire pH range (6.0–10.4) used in this study.

The benzeneboronic acid catalyzed hydrolysis of salicylidene-L-isoleucinamide exhibited rate constants similar to those found for the analogous isoleucine-containing system (Figure 2).

The pK of k_{cat}/K_m for the salicylidene-L-isoleucine system was 0.4 units lower than that seen using salicylidene-L-isoleucinamide. This effect (Figure 2) is the opposite of that expected by charge repulsion effects alone. The presence of isoleucine's carboxylic acid anion adjacent to the boronic acid should, by charge repulsion effects, favor the neutral boronic acid in comparison with the boronate anion, and thus raise the boronic acid pK. The small decrease in pK seen in the presence of the carboxylate anion is possibly due to the anion's effect on the local ionic strength seen by the boronic acid–imine complex. An increased local ionic strength might facilitate boronic acid ionization, raising the boronic acid pK. The differences in pK seen are greater than the estimated experimental error.

The pH dependencies shown in Figure 2 indicate that un-ionized boronic acids are effective catalysts in this system. These studies were done using the composite second-order rate constant k_{cat}/K_m. The decrease in the imine hydrolysis at higher pH's could be due to poorer binding of substrate or to lower catalytic rate constants.

It was observed that the catalytic rate constant, k_{cat}, observed during the hydrolysis of the imine by 3-nitrobenzeneboronic acid is the same at pH 6.0 and 7.8. The value at pH 7.8 is 0.0232 s^{-1}, compared to the value of 0.0181 at pH 6.0 seen in Table I. This suggests that k_{cat} is independent of pH and indicates the absence of hydroxide ion involvement in the hydrolysis.

The dissociation constant, K_m, is pH dependent and is approximately 4-fold higher at pH 7.8 than at pH 6.0. The value of K_m at pH 7.8 is 0.0487 M, compared to the value of 0.0129 observed at pH 6.0 seen in Table I. Binding becomes poorer with increasing pH, indicating that ionized

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boronic acids do not form a complex with the imine.

Figure 3 shows that the rate of the boronic acid catalyzed imine hydrolysis decreases in the presence of fructose and is completely inhibited at higher concentration of fructose. It has been known that boric and boronic acids form complexes with fructose and become tetrahedral after complexation.[46] These results suggest that fructose can prevent boronic acids from complexing with the imine. This may be related to the fact that the tetrahedral boronate anion is an ineffective catalyst.

Solvent Deuterium Isotope Effects on Catalytic Constants. It is known that imines are susceptible to hydrolysis mediated by general acid catalysis at lower pH's.[51] It may be possible that the boronic acid acts as a general acid in the hydrolysis of the imine and that a proton transfer is involved in the rate-determining step.

Data gathered in this study show that there is no solvent deuterium isotope effect on the hydrolysis of the imine when catalyzed by benzeneboronic acid. The rate constants obtained in D_2O as well as in H_2O at pH 6.0 and 6.6 are the same. The ratio of k_{H_2O}/k_{D_2O} shows that proton transfer is not involved in the transition state and suggests that the boronic acid does not act as a general acid.

Linear Free Energy Relationships. The Hammett plot of log K_m vs substituent constants (Figure 5) shows that K_m is dependent on electronically important substituents on the catalyst. Electron-withdrawing substituents are expected to stabilize an anionic tetrahedral boronic acid–imine complex, whereas boronic acids with electron-donating substituents would destabilize such a complex. This suggests that the complex that must form prior to the slow chemical steps of the reaction must contain the tetrahedral boronate anion, even though the effective catalyst is the neutral trigonal boronic acid.

These results are consistent with the results observed by Torssell et al.[52] who found that electron-withdrawing groups on benzene ring increase the stability of the complexes of benzeneboronic acid with sugars compared to electron-donating substituents. Similar results were observed when boronic acids were studied as inhibitors of enzyme-catalyzed reactions.[24,47]

Figure 6 shows that k_{cat} is independent of the electronic effects that arise when substituent group present in the benzeneboronic acid are varied. The linearity of the plot suggests that the rate-determining step is common for all boronic acids with nucleophilic side chain groups. These plots also show that the substituent groups have an effect only on binding and not on catalytic steps.

3-Amino- and 3-carboxybenzeneboronic acids deviate from the other boronic acids in catalytic rate constants as they react faster than expected from the substituent constants of 3-amino and 3-carboxyl groups (Figure 6). Their binding constants do not deviate from the expected values. These two boronic acids differ from the other boronic acids by having nucleophilic substituents on the benzene ring.

That such substituents can accelerate imine hydrolysis is clear from the literature. The experiments of Cordes and Jencks on aniline-catalyzed semicarbazide formation[53] also show acceleration of *N*-(4-chlorobenzilidene)aniline hydrolysis by cyanoacetate and glycine at a wide variety of pH values. In a separate study, Hoffmann and Sterba[54] state that chloroacetate anion catalyzes salicylaldehyde imine hydrolysis.

The amino and carboxyl groups in boronic acids may act as nucleophiles to catalyze the hydrolysis of the imine. Since the effects of the nucleophilic substituents are on k_{cat}, which is observed under conditions where the catalyst saturates the substrate, the side chain groups must act on the boronic acid–imine complex. This separate catalysis will be the subject of a future study.

Earlier studies have demonstrated that boric acid catalyzes the hydrolysis of imines.[35-37] It was expected that boronic acids would be better catalysts compared to boric acid based on the binding constants observed in earlier studies.[18,19,55] This is confirmed by the data presented in Table I, showing that the second-order rate constants, k_{cat}/K_m, obtained in the hydrolysis of the imine **1**, are higher in the cases of boronic and borinic acids when compared to boric acid. The highest second-order rate constant obtained with diphenylborinic acid is due almost totally to better binding.

The catalytic rate constants do not change significantly when compared to changes in dissociation constants. Benzeneboronic acid binds the imine nearly 25 times better than boric acid, whereas diphenylborinic acid binds nearly 4350 times better than boric acid. These results suggest that the dissociation constant decreases as the hydroxyl groups of boric acid are replaced by benzene rings. This is consistent with the results observed by Babcock and Pizer,[18,19] where stability constants for benzeneboronic acid complexes were higher than those for boric acid complexes.

The Brønsted plot (Figure 7) shows that dissociation constants are controlled by the pK of the boron acid. The differences between boronic acids and diphenylborinic acid result from their different dissociation constants, and are not directly due to the different number of groups on the boron atom. The slope of 0.80 shows that electron-withdrawing groups on benzeneboronic acids have a slightly lower effect on dissociation constants than on boronic acid pK values. The data discussed above leads us to conclude that: (1) The un-ionized boronic acid reversibly esterifies with the phenolic hydroxyl group of the imine in the first step of the reaction. (2) The boronic acid complexes to the imine when the acid is in the trigonal, neutral configuration. (3) While binding is controlled by the boronic acid ionization, catalysis is independent of pH. (4) There is no proton transfer in the rate-determining step. (5) There is no substituent effect (electronic effect) on k_{cat} and the rate-determining step is common for all boronic acids.

Mechanism. The first step in the reaction must be the esterification of un-ionized boronic acid with the ionized phenolic hydroxyl group in the imine. This may be as rapid as the esterification of boric acid with polyols.[56]

In the second step, the nucleophilic oxygen of the hydroxyl group, attached to the boron, attacks the imine carbon and forms a six-membered ring as shown in **3**. It is also possible that six-membered ring **3** forms through a carbinolamine intermediate **2a**, which in turn forms from **2** by intramolecular transfer of hydroxyl group of the boronic group to the imine carbon. The six-membered ring **3** has a negative charge on the boron which is stabilized by electron-withdrawing substituents on benzene ring. This could explain the better binding observed in the cases of benzeneboronic acids with electron-withdrawing substituents compared to electron-donating substituents. In subsequent steps, the six-membered ring breaks down followed by the hydrolysis to give products.

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It is also possible that a different six-membered ring is formed, which is shown as structure **2b**. In **2b** the imine nitrogen from the carbinolamine intermediate **2a** is complexed to the boron atom. This type of bicyclic structure is believed to be present in the borate–salicylamine complex.[48] For diphenylborinic acid, structure **2b** is analogous to its crystallizable ethanolamine complex.[57] The similar diethanolamine–boronic acid complex is observable in solution using ^{11}B NMR spectroscopy.[24] The stability of this type of structure suggests that it participates in the binding term. On the other hand, structure **2b** does not provide a pathway for carbon–nitrogen bond scission, as does structure **3**. Thus, **2b** contributes to binding but not to catalysis.

The rate-determining step is likely to be the breakdown of six-membered ring **3** to another intermediate **4** as it follows all the observed results. There was no solvent deuterium–isotope effect in the experiment as there is no proton transfer in this step. The boron atom does not change much electronically going from six-membered ring **3** to another intermediate **4**, as it carries negative charge in both structures. That is why there is no electronic substituent effect on this step. The catalytic rate constant is independent of pH as this step does not require any hydrogen or hydroxide ions. If the hydrolysis of intermediate **4** is the rate-determining step, then it would be expected that pH and substituent groups will have an effect on the catalytic rate constant. But there were no such effects observed in the experiments, and it excludes the possibility of the hydrolysis of intermediate **4** is the rate-determining step. Based on the above results, it may be concluded that the rate-determining step is the breakdown of the six-membered ring **3**.

This mechanism is also consistent with reversibility of the catalyzed reaction. It is known that boric acid catalyzes the formation of an imine.[38] In the reverse reaction, the boronic acid must form a complex with salicylaldehyde, the boron in the complex acting as a Lewis acid and coordinating to the carbonyl oxygen. This makes the carbonyl carbon atom more electron deficient and facilitates nucleophilic attack by the amine.

The action of the boron atom resembles that of metal ions in the hydrolysis of esters. In the hydrolysis of amino acid esters by metal ions, the metal ion coordinates to the carbonyl oxygen and facilitates nucleophilic hydroxide attack on the carbonyl carbon.[58] It has been shown that borate also acts in the same way in the hydrolysis of phenyl salicylate.[33] Thus, rate-determining step in the formation of an imine may be the attack of an amine on the carbonyl carbon.

Finally, the reason for the enhanced effectiveness of the 3-carboxy- and 3-aminobenzeneboronic acids is yet unknown; this will be the subject of a future study.

Conclusions

The results presented here clearly indicate that boric, boronic, and borinic acids share a common mechanism of action in their reactions with salicylideneisoleucine.[35] These results serve to emphasize the importance of substrate–catalyst association prior to the catalytic steps, since the main difference between the best and the poorest catalysts studied here is in their dissociation constants. Boron acids are unusual in that they form readily reversible complexes to a number of simple alcohols in aqueous solution. The dissociation constants of these complexes are in the same range as seen in enzymatic reactions. The combination of such reversible complexes with catalytic activity provides an interesting system for the study of catalysis.

Acknowledgment. A preliminary part of this study was done by Ms. Liliah Anand, who was supported by a grant from Project SEED of the American Chemical Society. This work was supported by grants from the PSC–CUNY Research Foundation.

Registry No. H-Ile-OH, 73-32-5; H-Ile-NH_2, 90-02-8; 4-HOC_6H_4CHO, 123-08-0; $B(OH)_3$, 10043-35-3; 3-$H_2NC_6H_4B(OH)_2$, 30418-59-8; $PhB(OH)_2$, 98-80-6; 3-$HOOCC_6H_4B(OH)_2$, 25487-66-5; 4-$MeC_6H_4B(OH)_2$, 5720-05-8; 4-$BrC_6H_4B(OH)_2$, 5467-74-3; 3-$O_2NC_6H_4B(OH)_2$, 13331-27-6; 3,5-$(F_3C)_2C_6H_3B(OH)_2$, 73852-19-4; Ph_2BOH, 2622-89-1; salicylidene-L-isoleucine, 63546-29-2; salicylidene-L-isoleucinamide, 130985-91-0; 4-hydroxybenzylidene-L-isoleucine, 130985-92-1; D-fructose, 57-48-7.

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