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Structure-Activity Correlations between Hydroxamic Acids and Their Inhibitory Powers on Urease Activity. I. A Quantitative Approach to the Effect of Hydrophobic Character of Acyl Residue

KENJI KUMAKI, SUICHI TOMIOKA, KYOICHI KOBASHI, and JUN'ICHI HASE

Research Laboratories, Chugai Pharmaceutical Co., Ltd. and Faculty of Pharmaceutical Sciences, University of Toyama

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Regression analyses were made in the study of structure-activity relationship between hydroxamic acids and their inhibitory powers on urease activity according to the Hansch and Fujita's method. It was found that the primary effect of acyl residues in altering the inhibitory power of hydroxamic acid is associated with the hydrophobic character of the acyl residues. That is, the inhibitory power, pI_{50}, of the series of congeners such as n-aliphatic, m-substituted benzo-, and p-substituted benzoylhydroxamic acids vary parabolically with the hydrophobic parameter, π, of acyl residues and substituents. The optimal hydrophobic parameter, π_{o}, for acyl residue of n-aliphatic hydroxamic acid, which gives theoretically the strongest inhibitory power, was calculated to be 2.543. The results presented here suggest that hydrophobic character of acyl residue of hydroxamic acids plays two kinds of roles in urease inhibition; the role in the random walk process of hydroxamic acids to the active site; the other in the stereospecific hydrophobic bonding at the active site.

On the other hand, electronic effect of acyl residues does not play a significant role in their inhibitory power on urease activity. Furthermore, it is assumed that in a series of araliphatic hydroxamic acids, the remarkable decrease in the inhibitory power of a-substituted derivatives is due to a steric effect of the bulk phenyl group in the a-position preventing the hydroxamic acid from proper fit on the active site of urease.

Introduction

It has been well established that the hydroxamic acids are the most potent inhibitors of urease activity (urea amidohydrolase, EC 3.5.1.5). Their inhibition also was found to

1) Location: a) 3-41-8 Takada, Toshima-ku, Tokyo; b) Gofuku, Toyama-shi, Toyama-ken.
be highly specific for urease activity of plant and bacterial origin. From comparison of the effect of hydroxamic acid and its various related compounds on urease activity, we concluded that -CONHOH is the main group absolutely necessary for inhibition of the enzyme activity.

In a previous report,\(^9\) we investigated the correlation between the chemical structure or physico-chemical properties of hydroxamic acids and their inhibitory effects on urease activity. More than sixty derivatives of hydroxamic acid were examined in detail for their inhibitory effect and we obtained the following conclusions in qualitative expression: 1) Increase of carbon number of \(n\)-aliphatic hydroxamic acid led to gradual increase in inhibitory power, reached a maximum in heptylhydroxamic acid and then led to a remarkable decrease in inhibitory power. Similar relations were obtained in the series of \(para\)-alkoxy benzo-hydroxamic acids. 2) Substitution at \(meta\)- or \(para\)-position of benzohydroxamic acid did not affect the inhibitory power, however, \(ortho\)-substituted derivatives were remarkably less inhibitory.

Recently structure-activity relationships have been evaluated,\(^{10,11}\) using the techniques of physical organic chemistry which have been proved to be useful in the study of organic reaction mechanisms. The present study was undertaken to establish quantitatively the structure-activity relationship between physico-chemical properties of hydroxamic acids and their inhibitory powers on urease activity by the method of Hansch.\(^{12,13}\)

**Method**

\(pK_{50}\) Values of Hydroxamic Acids—All the \(pK_{50}\) values used in the present study were reported in our previous paper.\(^9\) As a typical example, the measurement of inhibition by caprylhydroxamic acid on urease activity was shown in Fig. 1. The approximate \(I_{50}\) value of the compound was determined from the figure to be \(3.7 \times 10^{-7}\) in a final concentration, from which \(pK_{50}\) was calculated to be 6.34. The enzyme used for measuring the \(I_{50}\) value was a crude extract from sword bean powder but the \(I_{50}\) value has been found to be almost the same, regardless of the purity of urease preparation.\(^{5,6,7,9}\)

**Substituent Constants and Regression Analysis**—Using the substituent constant \(\sigma\), the inductive index \(I\), and the substituent constant \(\pi\), defined by Hansch, et al.,\(^{14,15}\) regression analyses were made according to Hansch’s equation,\(^{12,13}\) concerning the effects of acyl residue of aliphatic hydroxamic acids and the effects of substituent of \(meta\)- and \(para\)-substituted benzohydroxamic acids on urease inhibition. In this study, the substituent constants, \(\sigma\), are the Hammett substituent ones which were taken from the report of Brown\(^{16}\) and inductive indexes \(I\) were calculated, according to Chaing’s method.\(^{15}\) \(\pi\) values, defined by Hansch, et al.,\(^{14,15}\) are analogous constants representing the difference of the logarithms of the partition coefficients of the substituted compounds and the corresponding unsubstituted compound. These \(\pi\) values represent what we loosely term hydrophobic binding. The \(\pi\) values of acyl residues of aliphatic and araliphatic hydroxamic acids and those of substituents of \(meta\)- and \(para\)-alkoxybenzohydroxamic acids were calculated according to the Hansch’s method.\(^{16}\) Namely, these \(\pi\) values, which were calculated taking

advantage of the additive-constitutive nature of $\pi$, represent the total hydrophobic character of the hydrocarbon moiety of the acyl residues and the substituents. The values assigned to $\pi$ for the substituents of meta- and para-substituted benzohydroxamic acids were obtained from the phenoxyacetic acid system. These constants calculated are given in Table I—IV with the $pI_{50}$ values of a variety of hydroxamic acids.

## Result

### 1) Effect of Acyl Residues of $n$-Aliphatic Hydroxamic Acids on Urease Inhibition

In the previous paper, we reported that the $pI_{50}$ values of $n$-aliphatic hydroxamic acids were remarkably affected by the carbon number of their acyl moieties. An increase in the carbon number from two to six led to a gradual increase in $pI_{50}$ value, and more than nine in the carbon number, to the contrary, led to a marked decrease in $pI_{50}$ value. Among the eleven derivatives tested, heptylohydroxamic acid was the most powerful inhibitor.

The $pI_{50}$ values observed, $\pi$, and $I$ values for each acyl residue of $n$-aliphatic hydroxamic acids are given in Table I.

From these data, we derived Eq. 1—3 via the method of least squares. In these equations, $C$ is the molar concentration of hydroxamic acid giving 50% inhibition of urease activity, that is, $I_{50}$ value, $n$ is the number of points used in the regression, $r$ is the correlation coefficient, and $s$ is the standard deviation.

$$
\begin{align*}
\log 1/C &= pI_{50} = -0.213\pi + 6.848 \\
\log 1/C &= pI_{50} = 6.196I + 11.876 \\
\log 1/C &= pI_{50} = -0.080\pi^2 + 0.407\pi + 5.975
\end{align*}
$$

The $pI_{50}$ values calculated according to Eq. 3 and the differences between $pI_{50}$ values observed and calculated ($\Delta pI_{50}$) are also shown in Table I. From Eq. 1—3, it is obvious that the primary effect of acyl residues in affecting the inhibitory power of $n$-aliphatic hydroxamic acid is associated with the hydrophobic character of the acyl residue. Eq. 2, in which $I$ values represent the inductive effect on the carbon atom holding acyl residue, however shows that electronic effects of the acyl residues do not play a significant role, because in Eq. 2

### Table I. Effect of Acyl Residue of $n$-Aliphatic Hydroxamic Acid on Urease Inhibition

<table>
<thead>
<tr>
<th>Acid Residue (R)</th>
<th>$\pi$</th>
<th>$I$</th>
<th>$pI_{50}$</th>
<th>$\Delta pI_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_2$-</td>
<td>0.50</td>
<td>-0.902</td>
<td>6.22</td>
<td>6.16</td>
</tr>
<tr>
<td>n-C$_3$H$_7$-</td>
<td>1.00</td>
<td>-0.935</td>
<td>6.25</td>
<td>6.30</td>
</tr>
<tr>
<td>n-C$_4$H$_9$-</td>
<td>2.50</td>
<td>-0.955</td>
<td>6.34</td>
<td>6.49</td>
</tr>
<tr>
<td>n-C$<em>5$H$</em>{11}$-</td>
<td>3.00</td>
<td>-0.955</td>
<td>6.50</td>
<td>6.48</td>
</tr>
<tr>
<td>n-C$<em>6$H$</em>{13}$-</td>
<td>3.50</td>
<td>-0.955</td>
<td>6.60</td>
<td>6.42</td>
</tr>
<tr>
<td>n-C$<em>7$H$</em>{15}$-</td>
<td>4.00</td>
<td>-0.955</td>
<td>6.40</td>
<td>6.33</td>
</tr>
<tr>
<td>n-C$<em>8$H$</em>{17}$-</td>
<td>4.50</td>
<td>-0.955</td>
<td>6.26</td>
<td>6.19</td>
</tr>
<tr>
<td>n-C$<em>9$H$</em>{21}$-</td>
<td>5.00</td>
<td>-0.955</td>
<td>6.08</td>
<td>6.02</td>
</tr>
<tr>
<td>n-C$<em>{10}$H$</em>{23}$-</td>
<td>5.50</td>
<td>-0.955</td>
<td>5.77</td>
<td>5.81</td>
</tr>
<tr>
<td>n-C$<em>{11}$H$</em>{25}$-</td>
<td>6.00</td>
<td>-0.955</td>
<td>5.54</td>
<td>5.26</td>
</tr>
<tr>
<td>n-C$<em>{12}$H$</em>{27}$-</td>
<td>6.50</td>
<td>-0.955</td>
<td>4.67</td>
<td>4.55</td>
</tr>
</tbody>
</table>

$a$) $\pi$ values were obtained according to Hansch’s method described in the section of method.  
$b$) $I$ values were calculated according to Chiang, et al’s method.  
$c$) calculated from Eq. 3.  
$d$) The optimal value which gives theoretically the strongest inhibitory power in the series of $n$-aliphatic hydroxamic acids, obtained by setting ($d$ log 1/C)(d$\pi$) in Eq. 3 to zero.
the correlation was poor \((r=0.168)\) and the standard deviation was considerably large \((s=0.630)\). Comparison between Eq. 1 and 3 indicates that adding a \(\pi^2\) term to Eq. 1 improved significantly the correlation and resulted in a considerable reduction in the variance.

An F test indicates that the \(\pi^2\) term in Eq. 3 is justified at better than 0.995 level of significance when compared with Eq. 1 \((F_{1.8}=102, F_{1.6.0.05}=14.7)\). Therefore, it is clear that the \(pI_{50}\) values of \(n\)-aliphatic hydroxamic acids on urease inhibition vary parabolically with \(\pi\) value of their acyl residue. From Eq. 3, we can obtain the interesting parameter \(\pi_0\) by setting the derivative \(\langle d \log 1/C / (d\pi) \rangle\) equal to zero.

The optimal \(\pi\) value \((\pi_0)\) which represents the ideal hydrophobic character of acyl residue was calculated to be 2.543.

2) Effect of Substituted Groups in Benzohydroxamic Acid on Urease Inhibition

\(a)\) Effect of \(meta\)-Substituents—Using substituent constants, regression analysis was made on the effect of substituents at \(meta\)-position of benzohydroxamic acid on urease inhibition. The substituent constants and the \(pI_{50}\) values which were used in this analysis are shown in Table II. From these data, we derived, via the method of least squares, Eq. 4—8.

\[
\begin{align*}
\log 1/C &= pI_{50} = -0.199\pi + 6.449 \\
\log 1/C &= pI_{50} = 0.35\pi + 6.153 \\
\log 1/C &= pI_{50} = -0.194\pi + 0.102\pi + 6.422 \\
\log 1/C &= pI_{50} = -0.134\pi^2 + 0.26\pi + 6.305 \\
\log 1/C &= pI_{50} = -0.136\pi^2 + 0.275\pi + 0.160\pi + 6.266
\end{align*}
\]

\[n \quad r \quad s\]

In this analysis, the Hammett's substituent constant, \(\sigma\), was used as an electronic parameter. Comparing Eq. 4 with Eq. 5, it is clear that the more important character of the substituent in affecting the inhibitory power on urease was found to be its hydrophobic character. Besides, the role of \(\pi\) and \(\sigma\) seems not to be simply linear and additive since Eq. 7 gave better correlation than Eq. 6. An F test indicates that the \(\pi^2\) term in Eq. 7 is justified at better than 0.975 confidence level \((F_{1.8}=11.2, F_{1.6.0.05}=10.0)\). Therefore, the \(pI_{50}\) values of \(meta\)-substituted benzohydroxamic acids seem to vary parabolically with \(\pi\) values of substituents. The addition of the \(\sigma\) term to Eq. 7 did not result in an improved correlation.

### Table II. Effect of Substituent of \(meta\)-Substituted Benzohydroxamic Acid on Urease Inhibition

<table>
<thead>
<tr>
<th>Substituent (R)</th>
<th>(\pi^a)</th>
<th>(\sigma^b)</th>
<th>(pI_{50}) Obsd.</th>
<th>(pI_{50}) Calcd.</th>
<th>(\Delta pI_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 -H</td>
<td>0</td>
<td>0</td>
<td>6.43</td>
<td>6.27</td>
<td>-0.21</td>
</tr>
<tr>
<td>2 -Cl</td>
<td>0.76±0.02</td>
<td>0.373</td>
<td>6.37</td>
<td>6.43</td>
<td>0.06</td>
</tr>
<tr>
<td>3 -NO(_2\)</td>
<td>0.11±0.10</td>
<td>0.710</td>
<td>6.43</td>
<td>6.33</td>
<td>-0.10</td>
</tr>
<tr>
<td>4 -CH(_3)</td>
<td>0.31±0.01</td>
<td>0.069</td>
<td>6.20</td>
<td>6.40</td>
<td>0.20</td>
</tr>
<tr>
<td>5 -OCH(_3)</td>
<td>0.12±0.01</td>
<td>0.115</td>
<td>6.27</td>
<td>6.33</td>
<td>0.06</td>
</tr>
<tr>
<td>6 -OC(_3)H(_5)</td>
<td>1.62a</td>
<td>0.1b</td>
<td>6.44</td>
<td>6.37</td>
<td>-0.07</td>
</tr>
<tr>
<td>7 -OC(_4)H(_5)</td>
<td>2.62c</td>
<td>0.1c</td>
<td>6.15</td>
<td>6.07</td>
<td>-0.08</td>
</tr>
<tr>
<td>8 -OC(_5)H(_7)</td>
<td>3.62d</td>
<td>0.1d</td>
<td>5.44</td>
<td>5.49</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(a)\) \(\pi\) values were obtained from the phenoxynoic acid system.  
\(b)\) \(\sigma\) values were taken from the report of Brown.  
\(c)\) calculated from Eq. 7.  
\(d)\) Other \(\pi\) values were calculated according to the Hammett's method.  
\(e)\) \(\sigma\) values have not been determined for alkoy groups above amiloxyn, but it is assumed that these would not differ significantly. Then, these values were taken from value for amiloxyn.
An F test also indicated that the $\sigma$ term in Eq. 8 was not significant. From this result, it is likely that the effect of electronic character of substituents do not play a significant role.

Then, Eq. 7 was used to calculate the theoretical $pI_{50}$ values in Table II.

b) Effect of para-Substituents

Regression analysis for para-derivatives of benzohydroxamic acid was employed in a similar way to that for meta-derivatives. From the data of nine para-substituted benzohydroxamic acids, we derived Eq. 9—13 and obtained the results as shown in Table III.

<table>
<thead>
<tr>
<th>Substituent (R)</th>
<th>$\pi^a$</th>
<th>$\sigma^b$</th>
<th>$pI_{50}$</th>
<th>$\Delta pI_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 -H</td>
<td>0</td>
<td>0</td>
<td>6.43</td>
<td>-0.02</td>
</tr>
<tr>
<td>2 -Cl</td>
<td>0.70±0.03</td>
<td>0.227</td>
<td>6.52</td>
<td>-0.26</td>
</tr>
<tr>
<td>3 -NO$_2$</td>
<td>0.24±0.03</td>
<td>0.778</td>
<td>6.43</td>
<td>-0.06</td>
</tr>
<tr>
<td>4 -CH$_3$</td>
<td>0.52±0.05</td>
<td>-0.170</td>
<td>5.96</td>
<td>0.35</td>
</tr>
<tr>
<td>5 -OH</td>
<td>-0.61±0.01</td>
<td>-0.37</td>
<td>6.37</td>
<td>0.10</td>
</tr>
<tr>
<td>6 -OCH$_3$</td>
<td>-0.04±0.01</td>
<td>-0.268</td>
<td>6.64</td>
<td>0.22</td>
</tr>
<tr>
<td>7 -OC$_6$H$_5$</td>
<td>1.46</td>
<td>-0.32</td>
<td>5.75</td>
<td>0.24</td>
</tr>
<tr>
<td>8 -OC$_6$H$_4$</td>
<td>2.46</td>
<td>-0.34</td>
<td>5.64</td>
<td>-0.18</td>
</tr>
<tr>
<td>9 -OC$_6$H$_5$</td>
<td>3.46</td>
<td>-0.34</td>
<td>4.68</td>
<td>0.73</td>
</tr>
</tbody>
</table>

These results also suggest that the hydrophobic character of the substituent is of the primary importance, as well as in the case of meta-derivatives. In the case of para-derivatives Eq. 9 showed rather good fits. The addition of a $\pi^2$ term to Eq. 9 did not improve the correlation markedly, since an F test indicates that the $\pi^2$ term in Eq. 12 is only justified at 0.75 level of significance ($F_{1,6}=2.86, F_{1,6,0.75}=1.6$). Although the $\pi^2$ term is much less significant than that in the case of meta-derivatives statistically, it should have a physicochemical meaning as will be discussed later.

On the other hand, it is apparent by comparison of the correlation coefficients and the variance in these Eq. 9—13 that the role of the $\sigma$ term is not so important. Besides, an F test indicated that the $\sigma$ term in Eq. 13 was not significant as well as in the case of meta-derivatives. These results show that similar situation in meta-derivatives exists to that in para-derivatives.

3) Effect of Acyl Residues of Araliphaic Hydroxamic Acids on Urease Inhibition

The effect of substitution of a phenyl group at $\alpha,\beta$, or $\gamma$ position of aliphatic hydroxamic acid was studied on the correlation between physicochemical properties and inhibitory power on urease activity. The $\pi$ value of acyl residue of these aliphatic derivatives were calculated according to the Hansch’s method. It can be assumed that the relationship between the
π value of acyl residue and the $pI_{50}$ value of araliphatic hydroxamic acids is in accordance with that between the π value and the $pI_{50}$ value of $n$-aliphatic hydroxamic acids. Based on this assumption, therefore, the π values which were obtained for acyl residue of araliphatic hydroxamic acids were able to replace π in Eq. 3 which had previously been derived in the case of $n$-aliphatic hydroxamic acid. The results obtained are shown in Table IV.

**Table IV. Effect of Acyl Residue of Araliphatic Hydroxamic Acid on Urease Inhibition**

<table>
<thead>
<tr>
<th>Acyl Residue</th>
<th>$\pi$ $^a)$</th>
<th>$pI_{50}$ $^b)$</th>
<th>$\Delta pI_{50}$</th>
</tr>
</thead>
</table>
| 1 phenylaceto | CH$_2$ \[
\begin{array}{c}
\end{array}
\] | 2.69 | 5.92 | 6.49 | 0.57 |
| 2 β-phenylpropio | CH$_2$CH$_2$ \[
\begin{array}{c}
\end{array}
\] | 3.15 | 6.70 | 6.47 | -0.23 |
| 3 α-phenylbutyro | CH$_2$CH$_2$CH$_2$ \[
\begin{array}{c}
\end{array}
\] | 3.49 | 4.96 | 6.43 | 1.47 |
| 4 β-phenylbutyro | CH$_2$CHCH$_2$ \[
\begin{array}{c}
\end{array}
\] | 3.45 | 6.34 | 6.43 | 0.09 |
| 5 γ-phenylbutyro | CH$_2$CH$_2$CH$_2$ \[
\begin{array}{c}
\end{array}
\] | 3.68 | 6.00 | 6.40 | 0.40 |
| 6 α-phenylhexyro | CH$_2$CH$_2$CH$_2$CH$_2$ \[
\begin{array}{c}
\end{array}
\] | 4.49 | 5.05 | 6.20 | 1.15 |
| 7 β-phenylhexyro | CH$_2$CH$_2$CH$_2$CHCH$_2$ \[
\begin{array}{c}
\end{array}
\] | 4.45 | 5.92 | 6.21 | 0.29 |
| 8 1-naphthylaceto | CH$_2$ \[
\begin{array}{c}
\end{array}
\] | 3.83 | 5.07 | 6.37 | 1.30 |

$a)$ These π values were calculated according to the Hansch’s method.$^{40}$

$b)$ calculated from Eq. 3

From this results, it was found that β- or γ-substituted derivatives show good fits to Eq. 3 whereas α- substituted derivatives do not. Distinct differences in α-phenyl derivatives between the observed $pI_{50}$ value and the calculated $pI_{50}$ according to Eq. 3 were observed. These differences, as compared with those in β- or γ-substituted derivatives, were markedly large. It will be inferred from the poor fit of α-phenyl derivatives that their lower inhibitory powers are due to a steric effect of the bulky phenyl group in the α-position preventing the hydroxamic acid group from proper fit on the active site of urease molecule.

**Discussion**

1) Effect of Hydrophobic Character

From the results obtained in the series of such congeners as $n$-aliphatic hydroxamic acids, meta-substituted benzohydroxamic acids and para-substituted benzohydroxamic acids, it is
concluded that the primary effect of acyl residues in altering the inhibitory power of the corresponding parent hydroxamic acid is associated with their hydrophobic character. It has been reported that the relative activity of drugs in a series of congeners is parabolically dependent on their hydrophobic character in many biochemical and pharmacological systems.\textsuperscript{17)}

However, such a parabolic correlation has not been established in the enzymic reaction system. Therefore, the result obtained in this study is very interesting to elucidate the mechanism of the urease inhibition by hydroxamic acids. We found that the steric effect of acyl residue of hydroxamic acids influences significantly their urease inhibition.\textsuperscript{9)} Recently, Gale\textsuperscript{18)} also reported that hydroxamic acids such as 3,4,5-trimethoxy benzo-, 3,4,5-trimethoxy-phenyl aceto-, and 3,5-diisopropyl salicylohydroxamic acid did not inhibit urease activity. It has been well known that urease molecule is a simple protein, of which molecular weight is 480000.\textsuperscript{19)} These facts suggest that the active area of urease is not situated at the surface, but at the internal territory of the molecule. Therefore, our present results can best be explained by assuming that the active site is located within holes or crevices in the internal territory and is surrounded with hydrophobic amino acid residues of urease molecule. This assumption suggests that a random walk process plays an important role in urease inhibition by hydroxamic acids, in which the hydroxamic acid in question makes its way from an aqueous solution outside the enzyme molecule to an active site in its internal territory. That is, hydroxamic acids which are more hydrophilic will have a low probability of reaching an active site because these may be held in an aqueous solution outside the urease molecule, whereas hydroxamic acids which are extraordinary hydrophobic will be difficult to reach the active site because these will be strongly trapped by many nonspecific hydrophobic territories. The findings that the coefficients of $\pi^2$ term and the intercepts are almost the same in equation 7 and 12 may indicate that $m$- and $p$-substituted benzo hydroxamic acids reach to the active site of urease in the same manners of random walk process. Therefore, it may be interpreted that the difference between Eq. 7 and 12 is mainly based on that of stereospecific hydrophobic bonding of acyl residue to the hydrophobic territorities near the active site between these hydroxamic acids. Namely, the findings that the coefficient of $\pi$ term is plus in Eq. 7 and minus in Eq. 12 suggest that there may exist hydrophobic group in the part corresponding to $m$-position and hydrophilic group to $p$-position when benzo hydroxamic derivative fits to the active site of urease molecule. Furthermore, a comparison of Eq. 3 with these Eq. 7 and 12 suggests that the random walk process to the active site also are not so different between aliphatic and aromatic hydroxamic acids, because the coefficient of $\pi^2$ term are almost the same in these equations.

Then, it may be concluded from these results that hydrophobic character of acyl residue of hydroxamic acids plays two kinds of role in urease inhibition; the role in the random walk process of hydroxamic acids to the active site; the other in the stereospecific hydrophobic bonding at the active site, and hence the former are almost the same, but the latter are rather different in all the congeners of hydroxamic acids.

2) Electronic Effect

As the parameter representing electronic effect, we used I values, which was calculated according to the technique of Chiang, et al.,\textsuperscript{15)} for acyl residue of $n$-aliphatic hydroxamic acids and the Hammett substituent constants $\sigma$ for substituents of meta- and para- substituted benzo hydroxamic acids. From Eq. 2, 5 and 10 obtained using these parameters, it is seen that electronic effects play only a minor role in this study of the structure-activity relationship of hydroxamic acids on urease inhibition.

In the present study of structure-activity relationship according to the Hansch's equation, we cannot suggest the contribution of electronic factor of acyl residues or substituents to the inhibitory power. However, the degree of their contribution may be remarkably affected when we use other parameters for electronic effect. Based on the results obtained in this work, further studies have now been carried on according to the other methods of study for structure-activity relationship.

3) Steric Effect

In the previous paper,\textsuperscript{9} we reported that \textit{ortho}-substituted derivatives of benzohydroxamic acid were markedly less inhibitory than \textit{meta}- and \textit{para}-substituted derivatives, and \textit{\alpha}-phenyl \textit{n}-aliphatic hydroxamic acids were much less inhibitory than the corresponding \textit{\beta}- or \textit{\gamma}-phenyl derivatives.

These observations can not be explained as due to the electronic effect such as steric interference of resonance and polarization effects, but can be interpreted as "\textit{ortho}-effect" or "\textit{\alpha}-effect" in the sense of the steric hindrance which prevents the formation of enzyme-inhibitor complex between hydroxamic acid and urease at its active site.

The present observations also support our concept\textsuperscript{9} that the formation of enzyme-inhibitor complex between urease and hydroxamic acid is sterically hindered by \textit{\alpha}-phenyl group.

Most of the variation in the structure-activity relationship appears to be associated with the relative hydrophobic character of the derivatives. However, it is obvious that steric effect influences inhibitory power of substituted benzohydroxamic acids on urease activity. Further studies have to be continued from this point of view.