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Correlation Analysis of Ligand Interactions with Cycloamyloses

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Correlation equations have been developed for the hydrolysis of phenyl acetates by cycloamyloses. These quantitative structure–activity relationships (QSAR) for the enzyme-like activity of the polysaccharides are then compared with QSAR for synthetic "mini-enzymes" which have been studied in several laboratories. The physicochemical parameters determining activity of these "pseudo-enzymes" are similar to those determining activity with real enzymes.

INTRODUCTION

The cycloamyloses are a series of macrocyclic glucose polymers produced by *Bacillus macerans* amylase acting on starch. The glucose units are attached by $\alpha(1, 4)$ linkages and are stable to alkali but undergo acid hydrolysis. Since the early work of Villiers (*1*) it has been recognized that the cycloamyloses form complexes with various ligands. Many studies have been made of their complexing and catalytic activity (*2*).

X-Ray studies have shown that the cycloamyloses are doughnut-shaped compounds with the glucose units arranged in more or less undistorted chair formations. This results in the interior of the macrolide being lined with oxygen atoms. It is assumed that this structure prevails in solution (*2*). Studies have been made *via* X-ray crystallography showing various ligands bound into the center of the doughnut (*3*).

The objective of the present report is to show that the interactions of various ligands with cycloamyloses can be formulated in QSAR and that some comparisons can be made between these and QSAR for enzymes.

METHOD

The physical constants for our correlation analysis have been taken, except where noted, from our recent compilation (*4*). The values of σ for ortho substituents were taken from Charton (*5*). Our general approach to the formulation of QSAR has been discussed (*6*, *7*). There is one example of a 3,5-disubstituted phenyl acetate and we have taken its MR value to be $3\text{-}CH_3 + 5\text{-}CH_2$; that is, in all other cases we have not parametrized 5-H. In the case of log $1/K_D$ for the 3-chloro congener for Eqs. [1]–[5], we have used the average of three values given by Van Etten *et al.* (*8*), none of which were determined under conditions exactly equivalent to the other congeners.

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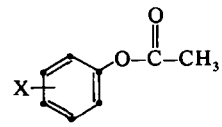
RESULTS

Case I. Hydrolysis of X-Phenyl Acetates by Cyclohexaamylose

We have formulated the following QSAR for the cyclohexaamylose catalysis and for the uncatalyzed hydrolysis of compounds of type I from the data (*2*, *8*) in Tables 1 and

TABLE 1

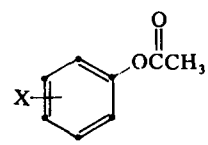
PHYSICOCHEMICAL PARAMETERS USED FOR THE FORMULATION OF EQS. [1]–[5] IN THE STUDY OF MAXIMAL RATE ACCELERATIONS AND DISSOCIATION CONSTANTS OF CYCLOHEXAAMYLOSE–PHENYL ACETATE COMPLEXES



		Log k_2/K_D		Log $1/K_D$		Log k_2							
Number	X	Obsd[a]	Calcd[b]	Obsd[a]	Calcd[c]	Obsd[a]	Calcd[d]	$MR_{3,4}$	$\pi_{3,4}$	MR_3	π_3	σ	E_{s_4}[e]
1	4-C(Me)$_3$	−0.99	−1.05	2.19	2.38	−3.17	−3.30	2.06	1.98	0.10	0.00	−0.20	−2.78
2	4-Me	−0.70	−0.59	1.96	1.81	−2.66	−2.48	0.67	0.56	0.10	0.00	−0.17	−1.24
3	2-Me	−0.42	−0.20	1.72	1.62	−2.14	−1.81	0.21	0.00	0.10	0.00	−0.13	0.00
4	H	0.00	−0.09	1.66	1.62	−1.66	−1.68	0.21	0.00	0.10	0.00	0.00	0.00
5	4-NO_2	0.31	0.33	1.92	1.88	−1.61	−1.46	0.84	−0.28	0.10	0.00	0.78	−1.02
6	3-Me	0.59	0.38	1.77	1.81	−1.18	−1.44	0.67	0.56	0.56	0.56	−0.07	0.00
7	3-Cl	0.66	0.82	1.76	1.82	−1.10	−0.99	0.71	0.71	0.60	0.71	0.37	0.00
8	3,5-(Me)$_2$	0.88	0.84	1.82	1.99	−0.94	−1.20	1.13	1.12	1.03	1.12	−0.14	0.00
9	3-C_2H_5	1.09	0.91	1.97	2.00	−0.88	−1.13	1.13	1.02	1.03	1.02	−0.07	0.00
10	3-NO_2	1.35	1.27	1.72	1.88	−0.37	−0.58	0.84	−0.28	0.74	−0.28	0.71	0.00
11	3-C(Me)$_3$	1.81	1.95	2.70	2.38	−0.89	−0.53	2.06	1.98	1.96	1.98	−0.10	0.00
12	4-Cl	—	—	1.80	1.82	—	—	0.71	0.71	0.10	0.00	0.23	−0.97

[a] From Refs. (*2*) and (*8*).
[b] From Eq. [4].
[c] From Eq. [1].
[d] From Eq. [2].
[e] From S. H. Unger and C. Hansch, *Proc. Phys. Org. Chem.* **12**, 91 (1976).

2. The parameter K_D is the molar dissociation constant for complex formation between cycloamylose and substrate (phenyl acetate). We have employed the reciprocal of K_D



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$$\log 1/K_D = 0.41(\pm 0.17)\,MR_{3,4} + 1.53(\pm 0.19) \qquad [1]$$

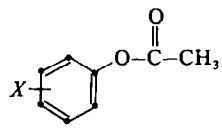
$$n = 12; \quad r = 0.858; \quad s = 0.154$$

$$\log k_2 = 0.67(\pm 0.41)\,MR_3 + 0.96(\pm 0.61)\,\sigma + 0.51(\pm 0.27)\,E_{s_4} - 1.75(\pm 0.39) \qquad [2]$$

$$n = 11; \quad r = 0.960; \quad s = 0.283$$

TABLE 2

PARAMETERS USED IN THE FORMULATION OF EQ. [6] FOR THE UNCATALYZED HYDROLYSIS RATE OF PHENYL ACETATES AT pH 10.60



Number	X	Log k_{uncat} Obsd[a]	Log k_{uncat} Calcd[b]	$\Delta \log k_{uncat}$	σ^-
1	2-Me	−3.42	−3.22	0.20	−0.13
2	3,4,5-$(Me)_3$	−3.37	−3.34	0.03	−0.29
3	3-$C(Me)_3$	−3.31	−3.19	0.12	−0.10
4	3-C_2H_5	−3.26	−3.17	0.09	−0.07
5	3,5-$(Me)_2$	−3.24	−3.22	0.02	−0.14
6	4-$C(Me)_3$	−3.22	−3.22	0.00	−0.13
7	4-Me	−3.18	−3.23	0.05	−0.15
8	3-Me	−3.16	−3.17	0.01	−0.07
9	4-MeO	−3.13	−3.22	0.09	−0.14
10	H	−3.09	−3.11	0.02	0.00
11	4-Cl	−2.82	−2.90	0.08	0.27
12	4-Br	−2.78	−2.89	0.11	0.28
13	3-Cl	−2.72	−2.82	0.10	0.37
14	3-NO_2	−2.33	−2.55	0.22	0.71
15	4-CN	−2.32	−2.32	0.00	1.00
16	2-NO_2	−2.27	−2.10	0.16	1.27
17	4-NO_2	−2.16	−2.10	0.05	1.27

[a] From Ref. (*8*).
[b] From Eq. [6].

$$\log k_2 = 0.64(\pm 0.37)\,MR_{3,4} + 0.93(\pm 0.58)\,\sigma + 0.93(\pm 0.25)\,E_{S_4} - 1.78(\pm 0.38) \quad [3]$$

$$n = 11; \quad r = 0.964; \quad s = 0.270$$

$$\log k_2/K_D = 1.15(\pm 0.25)\,MR_3 + 0.90(\pm 0.36)\,\sigma + 0.28(\pm 0.16)\,E_{S_4} - 0.21\ (\pm 0.23)$$

$$n = 11; \quad r = 0.987; \quad s = 0.168 \quad [4]$$

$$\log k_2/K_D = 1.07(\pm 0.25)\,MR_{3,4} + 0.84(\pm 0.38)\,\sigma + 0.98(\pm 0.17)E_{S_4} - 0.23(\pm 0.26) \quad [5]$$

$$n = 11; \quad r = 0.985; \quad s = 0.180$$

$$\log k_{uncat} = 0.79(\pm 0.11)\,\sigma^- - 3.11(\pm 0.06) \quad [6]$$

$$n = 17; \quad r = 0.968; \quad s = 0.111$$

(M^{-1}) since we are interested in the binding process. The rate constant for the hydrolysis step is k_2 (sec^{-1}) and k_{uncat} (sec^{-1}) is the pseudo-first-order rate constant for the uncatalyzed hydrolysis of phenylacetates, while k_{obsd} (sec^{-1}) is for the overall catalyzed hydrolysis.

We have omitted two congeners in the formulation of Eqs. [1]–[6], 3-COOH and 4-COOH; both of these substituents would be completely ionized under the experimental conditions of a high pH. Wepster and his colleagues (*9*, *10*) have summarized evidence for the fact that ionized substituents cannot normally be included with neutral substituents in correlation equations.

The positive coefficient of $MR_{3,4}$ (molar refractivity of 3- and 4-substituents) in Eq. [1] suggests that dispersion forces of substituents in both the meta and para positions aid in binding ligands to the cyclocamylose. The correlation is not a good one in terms of r although it is very significant statistically [$F(1, 10) = 33.4$; $F(1, 10\alpha.001) = 21.0$]. Also, the variation in log $1/K_D$ is poor. Most of the correlation depends on the large role for the 3-*t*-butyl congener.

Equation [2] for the hydrolysis step is quite different from Eq. [1]. Most significant is the necessity for factoring $MR_{3,4}$ into MR_3 and E_{s_4}. As in Eq. [1], the positive coefficient with MR_3 indicates binding via dispersion forces or the production of a favorable conformational change in the macromolecule or both. MR is essentially a corrected molar volume term. The positive coefficient of E_{s_4}, however, now indicates a deleterious effect of bulky groups in the 4-position. Recall that the larger the group, the more negative its E_s constant. Bulky groups in the para position may displace the ligand so that it is not well placed for nucleophilic attack by one of the cycloamylose OH groups; or, such groups might distort the macromolecule in an unfavorable fashion. Van Etten *et al.* (*8*) suggest the possibility of wrong-way binding.

The positive σ term is what one would expect for the promotion of hydrolysis. It is of interest to compare the σ term of Eq. [2] with that of Eq. [6]. The magnitude of the two terms is close; however, σ^- gives a better correlation than σ in Eq. [6]. As expected, this defines a role for through resonance in the uncatalyzed hydrolysis. That such an effect is missing in the catalyzed hydrolysis suggests that the phenoxy group may be twisted so that lone-pair electrons on the oxygen atom cannot assist in the attainment of the transition state. This conclusion rests only on the goodness of fit of the 4-NO_2 substituent in the catalyzed data set and hence has only tenuous significance at the moment. It does merit further study.

We have viewed log k_2 in Eq. [3] in another way. We have included MR_4 in $MR_{3,4}$; the result is essentially the same except that the coefficient with E_{s_4} has increased so that this increase in contribution by $MR_{3,4}$ is cancelled by the E_{s_4} term. It must be noted that MR_4 and E_{s_4} are almost perfectly collinear ($r^2 = 0.96$). This same effect can be seen by comparing Eqs. [4] and [5]. These two equations are almost identical except for the larger coefficient of E_s in Eq. [4] which offsets the contribution of MR_4 to $MR_{3,4}$. Para substituents do aid binding *via* dispersion forces as Eq. [1] brings out; however, the hydrolysis is hindered by para substituents.

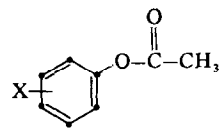
There is one example of an ortho substituent in the data set of Table 1 (2-CH_3). We have not assigned an MR value to this group. Our assumption, which appears justified by the results, is that this substituent does not contact the cycloamylose molecule.

Case II. Comparison of Catalytic Activity of Cyclohexaamylose with Cycloheptaamylose

A larger data set is available from the studies of Van Etten *et al.* (*8*) for the overall pseudo-first-order rate of hydrolysis (k_{obsd}) for phenyl acetates (**I**) with cyclohexa-

TABLE 3

PHYSICOCHEMICAL PARAMETERS USED FOR THE FORMULATION OF EQS. [7] AND [8] IN THE STUDY OF HYDROLYSIS RATES OF PHENYL ACETATES pH 10.60 IN THE PRESENCE OF CYCLOAMYLOSES



Number	X	Cyclohexaamylose $\log k_{obsd}$		Cycloheptaamylose $\log k_{obsd}$		MR_3	σ	E_{s4}[d]
		Obsd[a]	Calcd[b]	Obsd[a]	Calcd[c]			
1	4-C(Me)$_3$	−2.99	−3.22	−2.87	−2.51	0.10	−0.20	−2.78
2	4-Meo	−2.76	−2.60	−2.52	−2.60	0.10	−0.27	−0.55
3	4-Me	−2.73	−2.72	−2.35	−2.47	0.10	−0.17	−1.24
4	2-Me	−2.53	−2.29	−2.57	−2.42	0.10	−0.13	0.00
5	4-Br	−2.46	−2.29	−1.91	−1.96	0.10	0.23	−1.16
6	4-Cl	−2.34	−2.23	−1.81	−1.96	0.10	0.23	−0.97
7	H	−2.11	−2.16	−2.21	−2.25	0.10	0.00	0.00
8	3,4,5-(Me)$_3$	−2.04	−1.98	−1.50	−1.83	1.03	−0.31	−1.24
9	4-CN	−1.92	−1.67	−1.44	−1.40	0.10	0.66	−0.51
10	4-NO$_2$	−1.75	−1.70	−1.33	−1.25	0.10	0.78	−1.02
11	3-Me	−1.57	−1.79	−1.94	−1.93	0.56	−0.07	0.00
12	3,5-(Me)$_2$	−1.30	−1.43	−1.58	−1.61	1.03	−0.14	0.00
13	2-NO$_2$	−1.27	−0.93	—	—	0.10	1.24	0.00
14	3-C(Me)$_3$	−0.95	−0.50	−0.92	−0.72	1.96	−0.10	0.00
15	3-Cl	−0.67	−1.32	−1.46	−1.33	0.60	0.37	0.00
16	3-NO$_2$	−0.32	−0.86	−0.60	−0.77	0.74	0.71	0.00

[a] From Ref. (*8*).
[b] From Eq. [7].
[c] From Eq. [8].
[d] From S. H. Unger and C. Hansch, *Prog. Phys. Org. Chem.* **12**, 91 (1976).

amylose and cycloheptaamylose. We have formulated Eqs. [7] and [8] for comparison of these two "mini-enzymes" from the data in Table 3.

Cyclohexaamylose.

$$\log k_{obsd} = 0.95(\pm 0.40)MR_3 + 0.99(\pm 0.46)\,\sigma + 0.31(\pm 0.27)\,E_{s_4} - 2.26(\pm 0.37) \quad [7]$$

$$n = 16; \quad r = 0.925; \quad s = 0.335$$

Cycloheptaamylose.

$$\log k_{obsd} = 0.89(\pm 0.20)\,MR_3 + 1.29(\pm 0.29)\,\sigma - 2.34(\pm 0.14) \quad [8]$$

$$n = 15; \quad r = 0.963; \quad s = 0.183$$

Equation [7] is very similar to Eq. [4], although the correlation is not as sharp. All of the terms in Eqs. [7] and [8] are justified by the stepwise F test at $\alpha = 0.025$ or higher.

A most striking difference between Eqs. [7] and [8] is that the latter does not contain a term in E_{s_4}. The addition of such a term does not reduce the variance in $\log k_{obsd}$; this

means that para substituents have no effect on hydrolysis through dispersion forces or steric interactions. The obvious conclusion is that these substituents do not contact the macromolecule. Their only effect on hydrolysis is *via* electron withdrawal.

The intercepts of Eqs. [7] and [8] are essentially identical, showing that cyclohexa- and cycloheptaamylose show the same catalytic activity in the hydrolysis of the phenyl acetate; however, the cycloheptaamylose, with a larger cavity, is able to handle para-substituted analogs more effectively.

As in Eqs. [1]–[6], we have not assigned an MR value to 2-substituents. Since the examples are reasonably well fit without MR, we assume that they do not contact the "enzyme". It would be worth studying a better selection of 2-substituents to more firmly establish this point.

Case III. Inhibition of m-Nitrophenyl Acetate Hydrolysis of Cyclohexaamylose by $RCOO^-$

We have formulated Eq. [9] from the data in Table 4 for the study of competitive inhibition by $RCOO^-$ of the hydrolysis of *m*-nitrophenyl acetate by Van Etten *et al.* (*8*).

$$\log 1/K_i = 0.92(\pm 0.22)\,\mathrm{MR} - 1.79\,(\pm 0.60)\log(\beta \cdot 10^{\mathrm{MR}} + 1) - 0.82(\pm 0.57) \quad [9]$$

$$n = 12; \quad r = 0.962; \quad s = 0.225; \quad \text{optimum MR} = 3.96; \quad \log \beta = -3.90$$

The acids in this equation are a mixed lot, consisting of small aliphatic compounds as well as very large inflexible aromatic compounds. Even though we are unable to parameterize steric effects for such a mixture, Eq. [9] is a reasonably good correlation,

TABLE 4

PARAMETERS USED IN THE FORMULATION OF EQ. [9] FOR THE STUDY OF COMPETITIVE INHIBITION BY R-COO⁻ OF THE HYDROLYSIS OF *m*-NITROPHENYL ACETATE

Number	*R*	Log $1/K_i$		Log $1/K_i$	MR
		Obsd[a]	Calcd[b]		
1	$MeCH_2$–	0.24	0.13	0.11	1.03
2	$(Me)_2CH$–	0.66	0.56	0.10	1.50
3	$(Me)_3C$–	0.70	0.97	0.27	1.96
4	C_6H_5–	1.09	1.48	0.39	2.54
5	4-C_6H_5CO–C_6H_4–	1.40	1.39	0.01	5.47
6	C_6H_{11}–[c]	1.72	1.59	0.13	2.67
7	4-C_6H_5–C_6H_4–	1.77	1.78	0.01	4.97
8	3-Cl–C_6H_4–	1.92	1.88	0.04	3.04
9	$C_{10}H_{15}$–[d]	2.15	2.22	0.07	4.06
10	3-Cl–C_6H_4–CH=CH–	2.15	2.21	0.06	4.13
11	4-Cl–C_6H_4–	2.22	1.88	0.34	3.04
12	4-Cl–C_6H_4–CH=CH–	2.29	2.21	0.08	4.13

[a] From Ref. (*8*).
[b] From Eq. [9].
[c] Cyclohexanecarboxylate.
[d] Adamantanecarboxylate.

accounting for 92% of the variance in $\log 1/K_I$. This equation shows that inhibitory power of the acids increases linearly up to MR = 4.0 and then falls off linearly.

Two mathematical techniques are in current use for dealing with this type of activity. In many instances the parabolic model (*11*) (for Eq. [9] this would be: $a\mathrm{MR} - b[\mathrm{MR}]^2$) gives a good fit of the data. A bilinear model has recently been advanced by Kubinyi (*12*) which, in certain cases such as the present, gives a better fit of the data than the parabolic model. Even when the two models give equivalent quality fits, the bilinear model has the advantage that the slope of the positive MR term can be compared with simple linear QSAR. This is a distinct advantage in our present study.

In fact, the most interesting aspect of Eq. [9] is the coefficient of 0.83 with the MR term. This is in good agreement with the corresponding coefficients in Eqs. [4], [7], and [8]. We find an overall self-consistency for the role of dispersion forces as modeled by MR for the binding of ligands to the cycloamyloses.

Case IV. Decarboxylation of X-C_6H_4CH(CN)COOH by Cycloheptaamylose

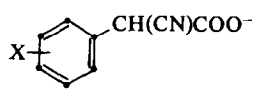
Straub and Bender (*13*) studied the cycloheptaamylose catalysis of the decarboxylation of a set of α-cyano phenylacetic acid anions. We have derived Eqs. [10]–[13] from their data in Table 5.

$$\log 1/K_D = 0.76(\pm 0.36)\,\mathrm{MR}_4 + 1.30(\pm 0.18) \qquad [10]$$

$$n = 8; \quad r = 0.905; \quad s = 0.131$$

TABLE 5

PARAMETERS USED IN THE FORMULATION OF EQS. [10]–[13] FOR THE CYCLOHEPTAAMYLOSE-CATALYZED DECARBOXYLATION OF PHENYLCYANOACETIC ACID ANIONS



		Log k_2/K_D		Log $1/K_D$		Log k_2		Log k_{uncat}			
Number	*X*	Obsd[a]	Calcd[b]	Obsd[a]	Calcd[c]	Obsd[a]	Calcd[d]	Obsd[a]	Calcd[e]	σ	MR_4
1	4-Meo	−1.25	−1.03	1.75	1.90	−3.01	−2.84	−4.21	−3.98	−0.27	0.79
2	2-Me	−1.18	−1.20	1.17	1.38	−2.35	−2.54	−3.43	−3.72	−0.13	0.10
3	4-Me	−1.02	−0.96	1.80	1.73	−2.82	−2.63	−3.92	−3.80	−0.17	0.56
4	3-Me	−0.96	−1.06	1.43	1.38	−2.38	−2.42	−3.58	−3.61	−0.07	0.10
5	H	−0.82	−0.90	1.40	1.38	−2.22	−2.27	−3.49	−3.48	0.00	0.10
6	4-Cl	0.10	0.00	1.75	1.76	−1.65	−1.79	−3.02	−3.04	0.23	0.60
7	4-Br	0.37	0.21	2.07	1.97	−1.70	−1.79	−2.92	−3.04	0.23	0.89
8	2-Cl	0.51	0.69	1.52	1.38	−1.01	−0.85	−2.31	−2.20	0.68	0.10

[a] From Ref. (*13*).
[b] From Eq. [12].
[c] From Eq. [10].
[d] From Eq. [11].
[e] From Eq. [13].

$$\log k_2 = 2.09(\pm 0.50)\sigma - 2.27(\pm 0.15) \quad [11]$$

$$n = 8; \quad r = 0.973; \quad s = 0.165$$

$$\log k_2/K_D = 2.33(\pm 0.53)\,\sigma + 0.72(\pm 0.48)\,MR_4 - 0.97(\pm 0.25) \quad [12]$$

$$n = 8; \quad r = 0.982; \quad s = 0.165$$

$$\log k_{uncat} = 1.88(\pm 0.52)\,\sigma - 3.48(\pm 0.15) \quad [13]$$

$$n = 8; \quad r = 0.963; \quad s = 0.173$$

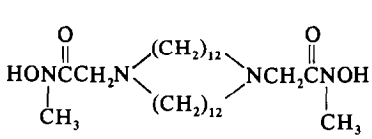
In the analysis of these data it was discovered that using MR only for 4-substituents gave better results than summing MR for 3- and 4-substituents. Since the single example of a 3-substituent is well fit by Eqs. [10] and [12], we assume that 3-substituents do not contact the cycloamylose. As usual, we have made this same assumption for 2-substituents.

Although the correlation with Eq. [10] is not high, it is quite significant ($F(1,6) = 27$; $F(1,6\alpha.005) = 18.6$). Adding a term in σ to Eq. [10] does not give a significant improvement in the correlation. Using π_4 in place of MR_4 gives a much poorer correlation ($r = 0.792$); hence, even though there is collinearity between MR and π ($r^2 = 0.50$), our results show, as usual, that the binding region is not typically hydrophobic. The results with Eqs. [10] and [12] show that binding for decarboxylation of the nitriles is much different from that of the hydrolysis of the phenyl acetates.

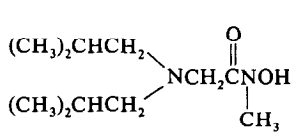
Straub and Bender noted the good correlation between log k_2 and σ (they obtained $\rho = 2.72$ and 2.44 for Eqs. [11] and [13], respectively, omitting ortho substituents) and discussed its implications for enzymic catalysis. The agreement between the coefficients of σ in Eqs. [11]–[13] is good. Equations [10] and [11] bring out the independent and quite different character of the binding ($1/K_D$) and catalytic steps in the decarboxylation reaction.

Case V. Hydrolysis of para-Nitrophenyl Carboxylates by Synthetic Macrocyclic and Acyclic Amines

Hershfield and Bender (*14*) carried out a most interesting study of the hydrolysis of 4-nitrophenyl carboxylates using compounds **II** and **III** as catalysts. We have formulated Eqs. [14]–[16] from the data in Table 6. In these equations log k_{II} and log k_{III} (M^{-1} sec^{-1}) are the apparent second-order rate constants for the appearance of *p*-nitrophenol.



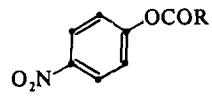
II



III

TABLE 6

PARAMETERS USED IN THE FORMULATION OF EQS. [14]–[16] FOR THE HYDROLYSIS OF PARA-NITROPHENYL CARBOXYLATES BY SYNTHETIC MACROCYCLIC AND ACYCLIC AMINES CONTAINING HYDROXAMIC ACID GROUPS



Number	R	Log k_{II}		Log k_{III}		π
		Obsd[a]	Calcd[b]	Obsd[a]	Calcd[c]	
1	Me	0.07	0.00	−0.16	−0.13	0.54
2	$MeCH_2$	0.12	0.23	−0.28	−0.27	1.08
3	$(Me)_2CH$	0.36	0.37	−0.64	−0.35	1.42
4	$Me(CH_2)_3$	0.52	0.67	−0.47	−0.54	2.16
5	$MeCH_2CH_2$	0.60	0.45	−0.38	−0.40	1.62
6	$Me(CH_2)_4$	0.80	0.89	−0.46	−0.68	2.70
7	$Me(CH_2)_6$	1.53	1.34	−0.72	−0.95	3.78
8	$Me(CH_2)_{10}$	2.18	2.23	−1.70	−1.50	5.94

[a] From Ref. (*14*).
[b] From Eq. [14].
[c] From Eq. [15].

Catalysis by **II**.

$$\log k_{II} = 0.41(\pm 0.07)\,\pi - 0.22(\pm 0.21) \qquad [14]$$

$$n = 8; \quad r = 0.985; \quad s = 0.135$$

Catalysis by **III**.

$$\log k_{III} = -0.25(\pm 0.10)\,\pi + 0.01(\pm 0.30) \qquad [15]$$

$$n = 8; \quad r = 0.926; \quad s = 0.196$$

$$\log k_{III} = 0.029(\pm 0.36)\,\pi - 0.043(\pm 0.05)\,\pi^2 - 0.30(\pm 0.46) \qquad [16]$$

$$n = 8; \quad r = 0.961; \quad s = 0.157$$

We have arbitrarily used π rather than MR in the formulation of Eqs. [14]–[16]. The parameters are almost perfectly collinear for the set of substituents under consideration; hence they both give the same quality correlations. In the case of catalysis by **II** and **III**, we believe that the hydrocarbon portions of "mini-enzymes" are promoting catalysis and that π is the appropriate parameter. Further work would be necessary to prove this assumption.

Equation [14] is an excellent correlation and very similar to other equations we have formulated for hydrolysis by similar "mini-enzymes" (see below). Catalysis by **III** is more complicated. While not a very good correlation, Eq. [15] is very significant statistically [$F(1,6) = 35.9$; $F(1,6\alpha.001) = 35.5$]. Adding a term in π^2 (Eq. [16]) does improve the correlation, but confidence limits on the π terms are so large that an

optimum π_0 cannot be calculated (*15*). Statistically, Eq. [16] is not as solid an improvement over Eq. [15] as one would like [$F(1,6) = 4.30$; $F(1,6\alpha.001) = 4.06$]; therefore, our discussion will be limited to Eq. [15] and its interesting negative coefficient with π.

DISCUSSION

Equations [1]–[9] give a good self-consistent picture of the catalytic hydrolysis of the phenyl acetates by the cycloamyloses. One of the features of these correlation equations which we find most helpful in understanding enzymic catalysis is the dependence of activity on molar refractivity (MR) of the substituents. If we replace $MR_{3,4}$ with the hydrophobic parameter (*16*) $\pi_{3,4}$ in Eq. [1], the correlation drops to $r = 0.76$. In the case of k_2 (Eq. [2]), replacing MR_3 with π_3 yields an equation with $r = 0.931$ and in the example of Eq. [4] for k_2/K_D, this replacement produces an equation with r of 0.931. MR_3 in these equations appears to be the better parameter, but the distinction is not great since the collinearity between π_3 and MR_3 is high ($r^2 = 0.80$).

Substituting π for MR in Eq. [7] gives an equation with $r = 0.852$ and such a substitution in the case of Eq. [8] gives an equation with $r = 0.886$. MR_3 and π_3 are also highly collinear ($r^2 = 0.82$) in these cases. Taken as a whole, these results indicate that MR is the superior parameter.

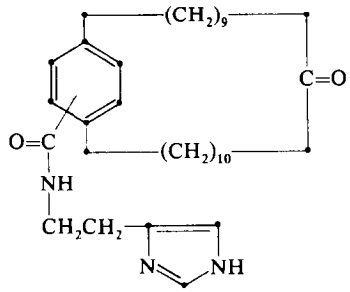
We have been concerned with defining two limiting kinds of space on enzymes with which substituents interact (*6*, *17–20*). One type of space is correlated with MR and the other with π. Evidence so far suggests that π-space is made up of apolar amino acid residues and that MR-space is made up of polar residues. The cycloamyloses are most interesting to us since we know that their doughnut-like structure is lined with polar oxygen atoms. On the basis of past experience, we would expect ligand interaction to be better correlated with MR than with π and it is gratifying to see our expectations fulfilled. Since π and MR are so collinear in the present data sets, more research is needed on this point.

Bender *et al.* have compared cycloamylose hydrolysis with well-known protolytic enzymes. We too believe that this is a valuable comparison to make. As we have noted (*18*), the so-called hydrophobic pocket in chymotrypsin is mostly lined with polar amino acid residues; hence we expect and find binding in this region (ρ_2 space in Hein–Niemann nomenclature) to be correlated with MR (*18*, *19*). In this sense, chymotrypsin and the cycloamyloses are similar.

For the competitive inhibition of phenyl acetate hydrolysis by cyclohexaamylose, Van Etten *et al.* (*8*) note a rather poor correlation ($r = 0.79$) with parachor. Using the bilinear model, we find an excellent correlation with MR in Eq. [9]; this shows that one gets increasingly better inhibitors up to the point of MR = 3.96. At this point a sharp break occurs and inhibitory power falls off as MR increases. It is significant that the role of MR in competitive inhibition (Eq. [9]) is so similar to that in catalysis (Eqs. [4], [7], [8], [10]).

A comparison of the elegant results of Bender's group on hydrolysis by "mini-enzymes" with work from other laboratories can be made *via* our correlation equations. The closest system for comparison is that of Murakami *et al.* (*21*) who studied the

hydrolysis of *p*-nitrophenyl carboxylates using the macrocyle **IV**. We have derived (*22*) Eq. [17] from their data.



IV

$$\log k = 0.45(\pm 0.09)\,\pi - 0.53(\pm 0.39) \qquad [17]$$
$$n = 11; \quad r = 0.968; \quad s = 0.260$$

Both the slopes and the intercepts of Eqs. [17] and [14] are in as close agreement as one could expect for the two different systems studied in two different laboratories. The dependence of hydrolysis on hydrophobic character is what one would expect.

The results of Bender *et al.* and Murakami *et al.* can be compared with an imaginative study by Gitler and Ochoa-Solano (*23*) who also studied the hydrolysis of para-nitrophenyl carboxylates; however, their reaction was carried out in micelles of cetyltrimethylammonium bromide. They used two catalysts, *N*-myristoyl-L-histidine and *N*-acetyl-L-histidine. The former, with its large lipophilic tail, would tend to concentrate within the micelle, while the latter polar compound would remain in the aqueous phase. Equations [18] and [19] have been derived (*24*) from the work of Gitler and Ochoa-Solano.

N-Myristoyl-L-histidine Catlysis.

$$\log k = 0.62(\pm 0.12) \log P - 0.28(\pm 0.34) \qquad [18]$$
$$n = 5; \quad r = 0.995; \quad s = 0.060$$

N-Acetyl-L-histidine Catalysis.

$$\log k = -0.31(\pm 0.19) \log P - 0.23(\pm 0.55) \qquad [19]$$
$$n = 5; \quad r = 0.948; \quad s = 0.106$$

Equation [18] is similar to Eqs. [14] and [17] except that the slope of Eq. [14] is a little higher. The difference is probably real, even though the confidence limits are rather large when compared to the differences in slope. Somewhat better desolvation may occur during partitioning into the micelle compared to partitioning into the hydrophobic pockets of **II** and **IV**.

Equation [19] shows that the more hydrophobic compounds are more slowly hydrolyzed when the catalyst is held in the aqueous phase which, of course, is expected. It is interesting that Eq. [19] is so much like Eq. [15]. The meaning of this is not clear, but the negative slope of Eq. [15] suggests that interaction of the substrate with the

hydrophobic portion of **III** hinders catalysis. A better attack by the oxime moiety on the ester linkage appears to be possible when the substrate is free in solution.

Recently, Komiyama and Bender (*25*) have demonstrated that the interaction of cycloamylose is enthalpy controlled. This suggests to us that ligand–enzyme interactions which are correlated by MR may be *predominantly* enthalpy controlled while ligand interactions correlated by π may be predominantly entropy controlled.

The elegant studies by Bender and his colleagues and the results of others can be rationalized and compared in a general way *via* QSAR. The emerging picture is that we are indeed beginning to develop synthetic "mini-enzymes" with properties similar to real enzymes. We strongly believe that correlation analysis, as described above, will facilitate our understanding of both types of catalysis.

ACKNOWLEDGMENT

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REFERENCES

1. A. Villiers, *C. R. Acad. Sci.* **112**, 536 (1891).
2. D. W. Griffiths and M. L. Bender, *Adv. Catal.* **23**, 209 (1973).
3. M. M. Harding, J. M. MacLennan, and R. M. Paton, *Nature (London)* **274**, 621 (1978).
4. C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikatiani, and E. J. Lien, *J. Med. Chem.* **16**, 1207 (1973).
5. M. Charton, *Prog. Phys. Org. Chem.* **8**, 235 (1971).
6. J. Y. Fukunaga, C. Hansch, and E. E. Steller, *J. Med. Chem.* **19**, 605 (1976).
7. C. Silipo and C. Hansch, *J. Amer. Chem. Soc.* **97**, 6849 (1975).
8. R. L. Van Etten, J. F. Sebastian, G. A. Clowes, and Myron L. Bender, *J. Amer. Chem. Soc.* **89**, 3242 (1967).
9. A. J. Hoefnagel, J. C. Monshouwer, E. C. G. Snorn, and B. M. Wepster, *J. Amer. Chem. Soc.* **95**, 5350 (1973).
10. A. J. Hoefnagel and B. M. Wepster, *J. Amer. Chem. Soc.* **95**, 5357 (1973).
11. C. Hansch and J. M. Clayton, *J. Pharm. Sci.* **62**, 1 (1973).
12. H. Kubinyi, *J. Med. Chem.* **20**, 625 (1977).
13. T. S. Straub and M. L. Bender, *J. Amer. Chem. Soc.* **94**, 8875 (1972).
14. R. Hershfield and M. L. Bender, *J. Amer. Chem. Soc.* **94**, 1376 (1972).
15. C. Hansch, A. R. Steward, S. M. Anderson, and D. L. Bentley, *J. Med. Chem.* **11**, 1 (1968).
16. A Leo, C. Hansch, and D. Elkins, *Chem. Rev.* **71**, 525 (1971).
17. M. Yoshimoto and C. Hansch, *J. Med. Chem.* **19**, 71 (1976).
18. M. Yoshimoto and C. Hansch, *J. Org. Chem.* **41**, 2269 (1976)
19. C. Hansch, C. Grieco, C. Silipo, and A. Vittoria, *J. Med. Chem.* **20**, 1420 (1977).
20. C. Hansch, R. N. Smith, A. Rockoff, D. F. Calef, P. Y. C. Jow, and J. Y. Fukunaga, *Arch. Biochem. Biophys.* **183**, 383 (1977).
21. Y. Murakami, Y. Aoyama, M. Kida, and A. Nakano, *Bull. Chem. Soc. Japan* **50**, 3365 (1977).
22. C. Hansch, *J. Org. Chem.*, **43**, 4889 (1978).
23. C. Gitler and A. Ochoa-Solano, *J. Amer. Chem. Soc.* **90**, 5004 (1968).
24. W. J. Dunn III and C. Hansch, *Chem.-Biol. Interact.* **9**, 75 (1974).
25. M. Komiyama and M. L. Bender, *J. Amer. Chem. Soc.* **100**, 4576 (1978).