Linear Free Energy Relationships in the Enzymatic Hydrolysis of Substituted Benzoylcholine Esters[1]

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$K_{m(app)}$ and $V_{m(app)}$ values have been determined for the hydrolysis of ortho-, meta-, and para-substituted benzoylcholine esters by Type IV cholinesterase in 0.1 M aq NaCl at a constant pH of 7.40 and 37°. Substituent effects in the 3 series were accounted for by electronic contributions with no important contributions being observed for hydrophobic or steric effects. Regression analysis on $K_{m(app)}$ and $V_{m(app)}$ showed the following dependencies on electronic parameters for the three series; ortho (F, P_E), para (σ_p, P_E), meta (σ_m). A comparison of cholinesterase catalysis *vs.* OH^- catalysis for the ortho and para series shows the distinguishing feature for the enzyme reaction to be the requirement of an electronic term for electronic polarizability (P_E). Rationale for the use of P_E in the correlations is discussed and interpreted as an intermolecular effect between substrate and enzyme. A further comparison of the two catalytic processes for the intermolecular substituent effect (σ_m and σ_p) on the reaction center reveals surprisingly similar values for the reaction constants involved. In contrast, a comparison of the two catalysts toward sensitivity to field effects (σ_I and F) in the ortho series shows substituent field contributions to be considerably less in the cholinesterase-catalyzed reactions. Results of the linear free energy relationships are consistent with a nucleophilic mechanism for enzymatic hydrolysis and suggest that either acylation or deacylation is rate limiting.

Various physicochemical approaches have been used to investigate the correlations existing between cholinesterase activity and the chemical structure of inhibitors and substrates. Thus far, the greatest success has been achieved in the area of cholinesterase–inhibitor interactions. Significant results have been reported for correlations of enzymatic inhibitor constants (K_i, I_{50}, k_i) with free energy related indices of structure such as the partition coefficient (P),[3,4] substituent constants (π, σ, σ^*, E_s),[4,5] infrared stretching frequency,[6] and, more recently, with electronic indices of structure such as charge (Q^T) and electrophilic superdelocalizability (S^E).[7]

In contrast, a review of the literature for cholinesterase–substrate interactions shows that fewer correlations have been reported and that the application of linear free energy relationships in this area has been much less successful.[8-10] In one isolated instance an analysis of the hydrolysis of p-NO_2 phenylalkyl esters by serum cholinesterase[11] has yielded significant correlations based on steric substituent effects.

We have approached the latter area in the current study by (a) determining the $K_{m(app)}$ and $V_{m(app)}$ constants[12] for series of ortho-, meta-, and para-substituted benzoylcholine esters hydrolyzed by Type IV cholinesterase and (b) performing a substituent effect analysis on these experimental constants. Although previous investigations of the benzoylcholine series have been conducted for ortho,[9] meta, and para[10] substituents, reliable values of $K_{m(app)}$ and $V_{m(app)}$ were not systematically reported. It was of further interest to compare the substituent effects observed for cholinesterase catalysis with those obtained for OH^- catalysis under identical reaction conditions (*i.e.*, 0.1 M aq NaCl, pH 7.4, 37°) using the same series of esters. We have shown that the linear free energy relationships pertaining to the alkaline hydrolysis of ortho-, meta-, and para-substituted benzoylcholine esters are given by eq 1 and 2,[13a,b] where k_2 = second-order rate constant; σ_I = the inductive constant of Taft;[14] and σ = the Hammett constant compiled by Jaffe.[15] The relationships embodied in these equations provide useful reference models with which to compare the correlations obtained for the cholinesterase-catalyzed reactions.

Experimental Section

Materials.—Synthesis of the ester substrates and treatment of the solns for the rate measurements has been reported.[13a]

Type IV horse serum cholinesterase was purchased from Sigma Chemical Co. (lot 45B-0200-1, dialyzed salt free and lyophilized). Activity of the enzyme was reported as 3.75 μM units/mg with 1 μM unit of enzyme hydrolyzing 1 μM of ACh/min at pH 8.0 and 37°. The lyophilized enzyme was stored desiccated below 0°. Solns of known enzyme activity were conveniently prepd by weighing exact amts of enzyme (2–5 mg) on a Model M-10 Cahn Electrobalance and dissolving the enzyme in an appropriate vol of 0.1 M aq NaCl (10–50 ml). The prepd enzyme solns were stored in the refrigerator or in an ice bath during rate measurements. No loss of enzyme activity could be detected during the periods of storage. All solns of enzyme, NaOH, NaCl, and ester were prepd with glass-distd H_2O. A loss of enzyme activity resulted from the use of regular distd or deionized H_2O and from

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(3) J. G. Beasley, S. T. Christian, W. R. Smithfield, and L. L. Williford, *J. Med. Chem.*, **10**, 1003 (1967).

(4) W. P. Purcell, J. G. Beasley, R. P. Quintana, and J. A. Singer, *ibid.*, **9**, 297 (1966).

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(6) W. B. Neely, *Mol. Pharmacol.*, **1**, 137 (1965).

(7) A. Cammarata and R. L. Stein, *J. Med. Chem.*, **11**, 829 (1968).

(8) L. A. Mounter, *Biochim. Biophys. Acta*, **27**, 219 (1958).

(9) J. Thomas and J. R. Stoker, *J. Pharm. Pharmacol.*, **13**, 129 (1961).

(10) W. E. Ormerod, *Biochem. J.*, **54**, 701 (1953).

(11) C. Hansch, E. W. Deutsch, and R. N. Smith, *J. Amer. Chem. Soc.*, **87**, 2738 (1965).

(12) $K_{m(app)}$, the Michaelis constant, is defined operationally as the substrate concentration at half the maximum enzyme velocity, $V_{m(app)}$. The subscript, app, is used to indicate that these constants are based on a two-intermediate model as opposed to the classical single-intermediate scheme. A kinetic definition of the constants is given in the Appendix.

(13) (a) J. J. Zimmerman and J. E. Goyan, *J. Med. Chem.*, **13**, 492 (1970); (b) n = number of derivatives; r = the correlation coefficient; s = the standard error of estimate; F' = the F ratio; and p = significance level determined on the basis of the F test.

(14) R. W. Taft, Jr., *J. Phys. Chem.*, **64**, 1806 (1960).

(15) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).

	n	r	s	F'	p	
	ortho substituents					
$\log k_{2-x} = 2.088\sigma_I - 0.358$	6	0.982	0.109	136	0.001	(1)
	meta and para substituents					
$\log k_{2-x} = 1.540\sigma + 0.346$	9	0.999	0.026	3600	0.001	(2)
	ortho substituents					
$\log K_{m(app)-x} = 0.608F - 0.079P_E - 4.814$	6	0.942	0.135	15.1	0.050	(3)
$\log V_{m(app)-x} = 0.794F - 0.051P_E - 8.695$	6	0.984	0.076	56.3	0.005	(4)
	para substituents					
$\log K_{m(app)-x} = 1.531\sigma_p - 0.088P_E - 4.628$	5	0.996	0.057	169	0.010	(5)
$\log V_{m(app)-x} = 1.538\sigma_p - 0.075P_E - 8.537$	5	0.991	0.089	72.3	0.025	(6)
	meta substituents					
$\log K_{m(app)-x} = 1.319\sigma_m - 4.853$	5	0.998	0.030	748	<0.001	(7)
$\log V_{m(app)-x} = 0.838\sigma_m - 8.843$	5	0.845	0.193	7.50	0.100	(8)
	miscellaneous					
$F = 1.563\sigma_I + 0.008$	6	0.980	0.086	97.4	0.001	(9)
$P_E = -2.871E_{s(r_{v,ave})} + 5.506$	5	0.901	1.529	12.9	0.050	(10)
$\log k_2 = 0.415P_E - 0.162F - 0.790$	4	0.999	0.160	13688	0.010	(11)
	meta and para substitutes					
$\log K_{m(app)-x} = 1.313\sigma - 0.049P_E - 4.816$	9	0.980	0.105	85.6	<0.001	(13)

the contact of rubber stoppers with any solns used in the hydrolysis studies.

Kinetic Assays.—Enzymatic rate measurements were carried out at pH 7.4 and 37° using the semiautomatic pH-Stat and prep procedure described previously.[13a] The instrument was only slightly modified for the enzymatic studies by replacing the Ag–AgCl reference electrode with a Beckman frit-junction calomel electrode (39071).

The reaction mixt were 0.1 M in NaCl and contd substrate ranging between 2.8×10^{-6} and 6.3×10^{-4} M. Reactions were initiated by adding 1 ml of cold enzyme soln (usually corresponding to 0.06 mg of Type IV cholinesterase) to 49 ml of substrate soln at pH 7.4 and 37°. The enzyme activity was titrated for a period of 5–6 min with NaOH titrant soln ranging between 2.47×10^{-3} and 9.87×10^{-3} N. Values of initial velocities, v_0, were obtd by taking the slope of the $x - y$ recorder tracings during the 100 to 200-sec intervals for each initial ester concn, S_0, and then correcting these velocities for alk hydrolysis.

The detn of $K_{m(app)}$ and $V_{m(app)}$ was complicated by a distinct upward curvature in the $1/v_0$ *vs.* $1/S_0$ plots at high substrate concns (substrate inhibition region). It was necessary therefore to experimentally determine for each substrate the concn region which produced a linear reciprocal plot.

Results and Discussions

Kinetic Constants.—Values for $K_{m(app)}$ and $V_{m(app)}$ of each of the derivatives were obtained graphically using (a) the Lineweaver–Burk plot,[16a] $1/v_0$ *vs.* $1/S_0$, and (b) the plot, S_0/v_0 *vs.* S_0, of the linear equation given by Hanes.[16b] The values of the enzyme constants derived from these 2 methods were either identical or differed by less than ± 1% of the averaged values reported in Table I.[17] Figure 1 is a Lineweaver–Burk plot for the parent compound and the *p*-nitro derivative and is typical of the plots used in evaluating our data.

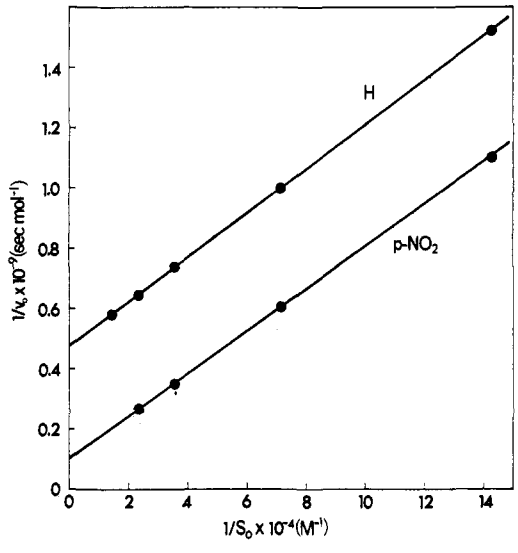

Figure 1.—Lineweaver–Burk plots for the hydrolysis of benzoylcholine iodide and *p*-NO_2 benzoylcholine iodide by Type IV cholinesterase in 0.1 M aq NaCl at pH 7.4 and 37°.

Linear Free Energy Relationships.—Analysis of the substituent effects of $K_{m(app)}$ and $V_{m(app)}$ were conducted individually for the ortho, meta, and para series.

(16) (a) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934); (b) C. S. Hanes, *Biochem. J.*, **26**, 1406 (1932).

(17) A more rigorous approach to the determination of the enzyme constants involves the use of a weighted least-squares method [G. N. Wilkinson, *Biochem. J.*, **80**, 324 (1961)]. Weighting of the data points, particularly for the $1/v_0$ *vs.* $1/S_0$ plot, corrects for the increased variance in the experimental data which is normally observed at low substrate concentrations. The need for this approach, however, is less obvious for the S_0/v_0 *vs.* S_0 plot for which the weighting factors remain more constant across the concentration range. As applied to our data, the rigorous weighting technique offers no important advantage since (a) lower substrate concentrations used in the enzyme runs were not much less than $K_{m,app}$, (b) very close agreement existed between values obtained by the two graphical methods, and (c) the experimental data excellently fit the linear plots.

TABLE I

KINETIC CONSTANTS FOR THE ENZYMATIC HYDROLYSIS OF ORTHO-, META-, AND PARA-SUBSTITUTED BENZOYLCHOLINE IODIDES BY TYPE IV CHOLINESTERASE

Substituent	$K_{m(app)}$, $M \times 10^5$	$V_{m(app)}$,[a] moles $sec^{-1} \times 10^9$
H	1.53	2.11
o-Cl	1.01	3.77
m-Cl	4.46	4.43
p-Cl	1.32	2.60
o-F	2.95	5.88
m-F	3.67	2.45
p-F	2.30	2.69
o-CH_3	0.501	0.769
m-CH_3	1.08	0.784
p-CH_3	0.377	0.483
o-NO_2	2.12	5.14
m-NO_2	12.3	4.59
p-NO_2	6.91	9.90
o-OCH_3	0.750	2.00
o-Br	0.584	2.39

[a] Maximum velocity, $V_{m(app)}$, expressed as the moles of substrate converted per sec in a 50-ml aq reaction vol (0.1 *M* in NaCl) by 0.06 mg of Type IV cholinesterase at pH 7.4 and 37°. $V_{m(app)}$ is corrected for alk hydrolysis.

The usual regression techniques were employed using substituent parameters for electronic[14,15,18-21] steric,[22,23] and hydrophobic[24] effects. A least-squares fit of the logarithms of the enzyme constants to appropriate sets of substituent constants produced the series of "best" equations (eq 3–8),[25a] where F = the substituent constant for field effects given by Swain and Lupton;[18] P_E = electronic polarizability;[19] σ_m and σ_p are the Hammett constants for meta- and para-substituted benzene derivatives, respectively.[15] The coefficients for the substituent constants in eq 3–7 are significant at the 0.050 level or better. These results indicate that electronic substituent effects govern the interaction of the present series of benzoylcholine esters with Type IV cholinesterase. Analogous sets of equations could not be obtained using substituent contributions represented by steric and hydrophobic parameters.

Ortho and Para Substituents.—The total substituent effect in both the ortho and para series (eq 3–6) is partitioned into separate contributions from a linear free energy term(F for ortho series; σ_p for para-series) and a term for group contributions from van der Waals interactions (P_E). The former of these accounts for the well-known intramolecular effect of substituents on the reaction center[15] while the latter potentially expresses a requirement for an intermolecular substituent effect on the catalytic process. In the ortho series the intramolecular effect is due solely to a field component (F). Apparently, the close proximity of groups ortho to the ester carbonyl inhibits resonance effects of the substituents. In the para series the intramolecular effect is both field and resonance in origin[25b] as seen by the use of σ_p in the correlations.

The nature of the intramolecular electronic effects in the ortho and para positions under conditions of enzymatic catalysis is equivalent to that experienced for OH^- catalysis of the same series of ester under identical reaction conditions (excluding catalyst). For the latter case it is seen from eq 1 and 2 that the substituent effect on k_2 is accounted for by σ_I in the ortho series and by σ_p in the para series. The difference in choice between σ_I and F in eq 1 and 3-4, respectively, is probably not of important consequence. While σ_I and F are based on different model reactions both are good measures of field effects and, as expected, bear a close relationship to one another. For the six substituents of the ortho series, eq 9 was obtained. The use of F in correlating the cholinesterase-catalyzed hydrolysis reactions provided statistically more significant results with both $K_{m(app)}$ and $V_{m(app)}$ than did σ_I. Thus σ_I and F produce equally acceptable correlations ($p = 0.005$) for log $V_{m(app)}$, but when σ_I replaces F in eq 3 the resulting correlation for log $K_{m(app)}$ is of lesser significance ($p = 0.100$).

From the positive sign of the coefficients for F and σ_p in eq 3–6 it is clear that electron-withdrawing substituents facilitate enzyme catalysis of the esters. It is interesting and perhaps significant that the intramolecular para substituent effect in eq 5 and 6 for the cholinesterase-catalyzed reactions ($\rho = 1.531$ and 1.538, respectively) is nearly identical with that produced in eq 1 for OH^- catalysis ($\rho = 1.540$). This similarity would suggest that the sensitivity of the attacking nucleophile to para-substituent effects in cholinesterase catalysis is the same as that for the attacking OH^- in alk hydrolysis. A similar comparison of coefficients for the ortho substituents requires substituting the value of F defined by eq 9 into eq 3 and 4. The resulting coefficients are approximately half the value of ρ in eq 2 indicating a considerable decrease in the sensitivity to the field effect of ortho substituents under conditions of enzymatic catalysis.

The distinguishing feature of the substituent effects in the ortho and para positions for cholinesterase catalysis compared with OH^- catalysis is the term, P_E, in eq 3–6. Correlation of the data using this parameter, however, is problematic from both theoretical and mechanistic points of view.

Theoretically, the treatment of the total substituent effect as a linear combination of separate model processes can be justified from a consideration of extrathermodynamic relations.[26] Inherent in this treatment is the requirement that each term be representative

(18) C. G. Swain and E. C. Lupton, Jr., *J. Amer. Chem. Soc.*, **90**, 4328 (1968).

(19) Y. K. Syrkin and M. E. Dyatkina, "Structure of Molecules and the Chemical Bond," Dover Publications, Inc., New York, N. Y., 1964.

(20) R. W. Taft, Jr., and I. C. Lewis, *J. Amer. Chem. Soc.*, **80**, 2346 (1958).

(21) J. D. Roberts and W. T. Moreland, Jr., *ibid.*, **75**, 2167 (1953).

(22) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1963, Chapter 13.

(23) E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).

(24) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

(25) (a) The statistical calcns for the substituent effect analyses presented in this paper were performed on the IBM 360, Model 50, using the multiple regression routine of the Reactive Terminal Services (RTS) statistical package. All values of the correlation coefficients and standard errors of estimate were corrected for small sample size by the routine according to the equations: $r_{corr}^2 = 1 - (1 - r^2)(n - 1)/(n - m)$; $s_{corr}^2 = s^2(n - 1)/(n - m)$, where m = the number of independent variables (STATPK Statistical Analyses Library, EP-100-0769-8S, ITT Data Services, Paramus, N. J.). (b) Swain and Lupton (ref 18) have calcd the sensitivities to resonance and inductive effects for σ_p values as 53 and 47%, respectively. For σ_m values the sensitivities are 22 and 78%, respectively.

(26) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, Chapters 6 and 7.

of a physically real model process.[27] P_E, *per se*, does not represent such a model process, but its relationship to one is implied by the success of eq 3–6. For the present series, an intercorrelation was indeed observed to exist between P_E and linear steric $E_{s(r_{v,ave})}$ values[28] for the para substituents. While there is a relatively high correlation between these two constants, the linear relationship does not appear to be general since the inclusion of Br and OCH_3 substituents (ortho series) in the regression analysis leads to a marked decrease in the correlation coefficient ($n = 7$, $r = 0.668$). Equation 10, therefore, does not guarantee a totality of steric effects for P_E correlations but is merely sufficient to show a proportionality between P_E and some model process. Taft[22] and Charton[29] have shown a more general relationship between the linear free energy σ constant and another nonmodel process, the dipole moment.

Mechanistically, the substituent effect represented by P_E in eq 3–6 may be viewed as an intermolecular effect between substituent and enzyme. Since P_E is proportional to London dispersion energies[19] such an interaction would necessarily involve one between substituent and a polarizable group at the enzyme active site. The serine oxyanion of the esteratic site could be such a group as could a hydrophobic group adjacent to this site.[30] While direct proof of this is not available it is pertinent that Bunnett[31] has cited examples for the interaction between substituents and attacking nucleophiles in nucleophilic bimolecular reactions. A special characteristic of substituents near the reaction center was observed to affect the reaction of α-substituted methyl halides and substituted benzyl chlorides toward nucleophiles. This special characteristic was identified as polarizability. Regression analysis of the data for the reaction of sodium methoxide with α-substituted methyl bromides (appearing in Table III of ref 31) in terms of P_E and F produces eq 11[32] where the coefficients for P_E and F' are significant at the 0.010 and 0.025 levels, respectively. Equation 11 has not been presented as proof for the model proposed for cholinesterase catalysis (eq 3–6), but success of this equation does lend some credence to the hypothesis of an intermolecular substituent effect based on P_E.

The negative coefficient of P_E in eq 3–6 indicates that polarizability effects hinder the reaction. In relation to enzymatic activity this hinderance may be manifested in either of two ways.[33] First, attractive dispersion forces between substituent and enzyme may lead to an incorrect alignment of substrate at the active site decreasing the reaction. Secondly, since P_E has units of volume (ml) and increases with the size of the substituent, the importance of this term may reflect an intermolecular steric effect on the enzyme reaction. Regarding the latter interpretation, the relationship between P_E and $E_{s(r_{v,ave})}$ is not linear in general as discussed above. Further, the use of $E_{s(r_{v,ave})}$ values in place of P_E in eq 3–6 did not yield the overall success in correlating the data experienced by using the P_E. Thus the replacement of P_E by $E_{s(r_{v,ave})}$ values in eq 3–5 leads to statistically nonsignificant coefficients for $E_{s(r_{v,ave})}$ ($t = 0.44$, 1.74, and 1.81, respectively) while the same change in eq 6 produces a significant coefficient ($t = 4.32$). It would appear therefore that the intramolecular substituent effect accounted for by P_E in the hydrolysis of the ortho- and para-substituted benzoylcholine esters by Type IV cholinesterase is not enitrely due to bulk or steric effects but may also involve the hindering effect of attractive dispersion forces.

Alternative to the linear free energy approach taken above, the correlation of the data with eq 3–6 is potentially based on an equation suggested by Cammarata[34] for the electronic component (E_{rs}) of drug–receptor interactions

$$\delta A_n = \sum_s (aq_s + bS_s^E) + C' \qquad (12)$$

where S^E is the electrophilic π superdelocalizability for a substituent atom on the substrate molecule incurring a change in the enzyme activity, δA; q is the total charge on the same atom. A summation of S^E and q over all atoms of a substituent results in parameters presumably bearing a close relationship to P_E and σ, respectively. Equation 12 has been successfully applied in accounting for the inhibitory effect of 3-hydroxyphenyltrimethylammonium derivatives on AChE activity.[7]

Meta Substituents.—The total substituent effect in the meta series (eq 7 and 8) is best correlated with the linear free energy constant, σ_m.[25b] The high correlation obtained for log $K_{m(app)}$ (eq 7) using this constant would preclude any further contribution from an intermolecular effect as shown above for the ortho and para series. The resulting linear relationship between log $K_{m(app)}$ and σ_m is illustrated in Figure 2. It appears that the substrate molecules must be oriented at the active site such that the meta substituents do not come in contact with groups which influence the catalytic activity of the enzyme.

The sign and magnitude of the reaction constant for log $K_{m(app)}$ of the meta series ($\rho = 1.319$) is close to that observed for log $K_{m(app)}$ of the para series ($\rho = 1.531$). This similarity suggests a near equivalency for the intramolecular substituent effect on $K_{m(app)}$ for meta and para substituents. Equation 5 and 7 were therefore combined into a single equation (eq 13) assuming a value of $P_{E(x)} = 0$ for meta substituents, for which the coefficients of the constants are significant

(27) The model processes employed in linear free energy relationships are predominantly those for rate-equilibrium phenomena with the free energy change for the process being the difference between the initial and final states (*i.e.*, $\Delta\bar{G}^\circ = -RT \ln k$(or K). The free energy change for the substituents in these processes is represented by $\Delta\Delta\bar{G}^\circ = -RT \ln (k_X/k_0)$ (or K_X/K_0). Physical constants such as P_E and μ are based on the potential energy (E) of single states and are obtained as experimental constants or from theoretical calcns. In this regard, however, the proportionality which frequently exists between free energy differences and energies of single states has been discussed (ref 15, 22).

(28) Charton has demonstrated a linear relationship between the van der Waals radii, $r_{v,min}$ and $r_{v,max}$, for symmetric-top substituents and Taft E_s values (ref 22) obtained from the acid-catalyzed hydrolysis and esterification of aliphatic esters and carboxylic acids (M. Charton, *J. Amer. Chem. Soc.*, **91**, 615 (1969)). Kutter and Hansch (ref 23) extended Charton's work with the use of the van der Waals radii, $r_{v,ave}$, in calc previously unavailable steric values, $E_{s(r_{v,ave})}$. These values would be expected to be equiv to those obtained for the actual model process employed by Taft.

(29) M. Charton, *J. Org. Chem.*, **30**, 552 (1965).

(30) M. I. Kabachnik, A. P. Brestkin, N. N. Godovikov, M. J. Michelson, E. V. Rozengart, and V. I. Rozengart, *Pharmacol. Rev.*, **22**, 355 (1970).

(31) J. F. Bunnett, *J. Amer. Chem. Soc.*, **79**, 5969 (1957).

(32) Substituents used in the analysis were CH_3, F, Cl, and Br; k_2 = rate coefficient for the reaction with units of 1 mole^{-1} sec^{-1}.

(33) A. Cammarata, *J. Med. Chem.*, **10**, 525 (1967).

(34) A. Cammarata, *ibid.*, **11**, 1111 (1968).

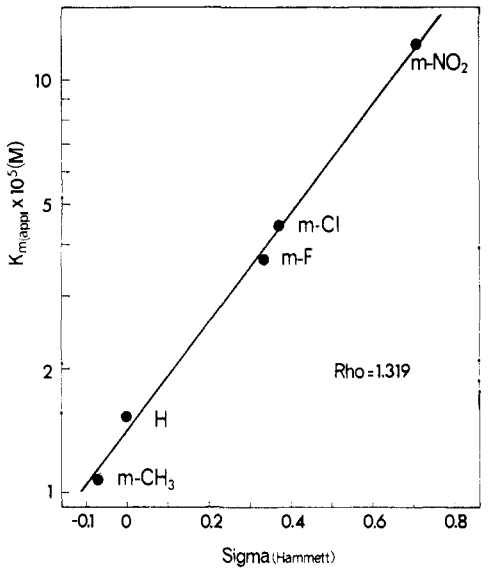

Figure 2.—Log $K_{m,app}$, for the hydrolysis of meta-substituted benzoylcholine iodides by Type IV cholinesterase in 0.1 *M* aq NaCl at pH 7.4 and 37°, plotted against σ. The regression line is a fit of eq 7.

at better than the 0.050 level. The results obtained for log $V_{m(app)}$ of the meta series (eq 8) are significant at only the 0.100 level and indicate an incomplete description of the substituent effects. Attempting to explain the data through the use of various other parameters for electronic, steric, and hydrophobic effects did not result in improved correlations.

Kinetic Inferences.—The results of eq 3–8 can be analyzed in terms of the two-intermediate kinetic scheme demonstrated for the cholinesterase enzymes.[35] Linear free energy expressions which apply to this kinetic model may be written as follows (see Appendix)

$$\log K_{m(app)rel} = \log k_{-1,rel} - \log k_{1,rel} \quad (14)$$

$$\log K_{m(app)rel} = \log k_{2,rel} - \log k_{1,rel} \quad (15)$$

$$\log K_{m(app)rel} = \log k_{3,rel} - \log k_{1,rel} \quad (16)$$

$$\log V_{m(app)rel} = \log k_{2,rel} \quad (17)$$

$$\log V_{m(app)rel} = \log k_{3,rel} \quad (18)$$

where all constants are expressed relative to a standard (*e.g.*, $K_{m(app)rel} = K_{m(app)-x}/K_{m(app)-0}$; x refers to the substituted derivative and 0 refers to the standard for the series), and where k_{-1}, k_1, k_2, and k_3 are kinetic constants for dissociation, association, acylation, and deacylation, respectively. The values of log $k_{n,rel}$ appearing on the right-hand side of eq 14–18 are potentially linear functions of substituent constants. Equations 14–16 provide a basis for an interpretation of the correlations obtained between log $K_{m(app)}$ values and the electronic substituent parameters, while eq 17–18 offer alternatives for substituent effects on log $V_{m(app)}$.

Equation 14 is eliminated as a likely alternative from the following considerations. When eq 14 holds, positive coefficients for the linear free energy terms in log $K_{m(app)}$ correlations (eq 3, 5, and 7) indicate that electron withdrawal from the ester carbonyl facilitates dissociation of the ES-complex (*i.e.*, decreases binding). Further, since the linear free energy terms for log $V_{m(app)}$ correlations (eq 4, 6, and 8) also have positive coefficients, it would be necessary to conclude that decreased binding leads to a faster reaction. This appears unlikely and is in conflict with the "better binding:better reaction" hypothesis advanced for still another hydrolytic enzyme, α-chymotrypsin.[36] The elimination of eq 14 indicates that it would have been incorrect to assume, *a priori*, that the Michaelis constant was simply an equilibrium constant inversely taken as the affinity of the substrate for the enzyme.

Equations 15–18 are therefore the most likely alternatives on which to base the interpretation of the results. When eq 15 and 17 hold, then positive coefficients for σ_m, σ_p, and F in correlations for log $K_{m(app)}$ and log $V_{m(app)}$ (eq 3–8) indicate the electron withdrawal from the ester carbonyl in the ES-complex facilitates an attack by the serine oxyanion in the acylation step (k_2). If on the other hand eq 16 and 18 hold, then the positive coefficient for the linear free energy terms suggests that electron withdrawal from the C=O in the acyl intermediate facilitates an attack by H_2O in the deacylation step (k_3). Both of these alternatives are consistent with the nucleophic mechanism postulated for cholinesterase enzymes.[37] On the basis of the evidence presented here it is not possible to distinguish, however, between acylation (k_2) or deacylation (k_3) as the potential rate-limiting step. The similarity of the coefficients for $K_{m(app)}$ and $V_{m(app)}$ in the ortho (eq 3–4) and para (eq 5–6) series would indicate that within each of these series the correlation equations are describing the same model processes and hence the same rate-limiting step. In view of this the term, log $k_{1,rel}$, in eq 15 and 16 must make only minor contributions to log $K_{m(app)rel}$.

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Appendix

The following kinetic scheme has been shown to apply to reactions of cholinesterase[35,37]

$$E + S \underset{-1}{\overset{1}{\rightleftharpoons}} ES \xrightarrow{2} ES' \xrightarrow{3} E + P \quad (1')$$

where ES is the Michaelis–Menten complex and ES′ is the acyl intermediate. $K_{m(app)}$ values obtained graphically are related to the individual reaction steps by the following equation

$$K_{m(app)} = [(k_{-1} + k_2)/k_1][k_3/(k_2 + k_3)] \quad (2')$$

Expressing eq 2′ as the logarithm of the relative $K_{m(app)}$ value produces

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_3/(k_3)_0] - \log [k_1/(k_1)_0] + \log [(k_{-1} + k_2)/(k_{-1} + k_2)_0] - \log [(k_2 + k_3)/(k_2 + k_3)_0] \quad (3')$$

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where the subscript, 0, refers to the reference standard (subscript x has been omitted for convenience). Equation 2′ reduces to the following cases after applying rate-limiting assumptions.

$$K_{m(app)} = k_{-1}/k_1 \text{ when } k_3 \gg k_2 \text{ and } k_{-1} \gg k_2 \quad (4')$$

$$K_{m(app)} = k_2/k_1 \text{ when } k_3 \gg k_2 \text{ and } k_2 \gg k_{-1} \quad (5')$$

$$K_{m(app)} = k_3/k_1 \text{ when } k_2 \gg k_3 \text{ and } k_2 \gg k_{-1} \quad (6')$$

Expressing eq 4′–6′ as the logarithm of the relative $K_{m(app)}$ values results in the following equations.

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_{-1}/(k_{-1})_0] - \log [k_1/(k_1)_0] \quad (7')$$

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_2/(k_2)_0] - \log [k_1/(k_1)_0] \quad (8')$$

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_3/(k_3)_0] - \log [k_1/(k_1)_0] \quad (9')$$

An analogous treatment can be applied to the $V_{m(app)}$ values. Accordingly, the $V_{m(app)}$ values obtained graphically are written as

$$V_{m(app)} = [k_2k_3/(k_2 + k_3)]E_t \quad (10')$$

where E_t is the total enzyme concentration. Taking the logarithm of the relative $V_{m(app)}$ value yields

$$\log [V_{m(app)}/V_{m(app)0}] = \log [k_2/(k_2)_0] + \log [k_3/(k_3)_0] - \log [(k_2 + k_3)/(k_2 + k_3)_0] \quad (11')$$

After applying rate-limiting assumptions to eq 10′

$$V_{m(app)} = k_2E_t \text{ when } k_3 \gg k_2 \quad (12')$$

$$V_{m(app)} = k_3E_t \text{ when } k_2 \gg k_3 \quad (13')$$

Expressing eq 12′ and 13′ as the logarithm of the relative $V_{m(app)}$ values yields

$$\log [V_{m(app)}/V_{m(app)0}] = \log [k_2/(k_2)_0] \quad (14')$$

$$\log [V_{m(app)}/V_{m(app)0}] = \log [k_3/(k_3)_0] \quad (15')$$

Separation of the observed rate coefficients into a single relative rate term or into a sum or difference of relative rate terms provides justification for the correlation of rates with substituent parameters. For the latter case (eq 7′–9′) when both rate terms contribute significantly to the observed rate, the observed reaction constant will necessarily be a difference quantity for the two reaction steps (*i.e.*, for σ contributions to eq 7′, $\rho_{obsd} = \rho_{-1} - \rho_1$).

Notes

Structure–Activity Relationships Having a Basis in Regular Solution Theory

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Lipophilic properties of drug molecules are well known to limit the magnitude of a biological response by governing the penetrability of the molecules through tissues and by affecting "hydrophobic" interactions between drug agents and biomacromolecules. Partition coefficients have been a favorite measure of lipophilicity used in the correlation of drug effects,[1] but other measures such as polarizabilities[2] or molar attraction constants[3] have also been used. Leo, *et al.*,[4] prefer partition coefficients over other lipophilic measures on the grounds that better correlations with biological activities are obtained. In this preliminary communication we wish to point out the relationship that exists between partition coefficients and other measures of lipophilicity, notably polarizability and the molar attraction constant. Correlations of biological activity involving these indexes may thus be interpreted as reflections of the solubility properties of the compounds involved, irrespective of the context in which the correlations were originally presented.

From a consideration of the chemical potentials μ_a and μ_o for a substance in an aqueous and in an organic environment, respectively, it can readily be shown that when the reference state is taken as the pure substance the partition coefficient P is determined by the ratio of the activity coefficients γ_a and γ_o for the substance in each of the respective phases. This relationship can be given as

$$\log P = \log (S_o/S_a) = \log \gamma_a - \log \gamma_o \quad (1)$$

where S_o and S_a are the solubilities of a substance in an organic and in an aqueous phase. Equation 1 is known to relate partition coefficients with relative solubilities[5] and also forms a basis for the determination of activity coefficients from partitioning experiments.[6]

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