ortho Effect in Alkaline Hydrolysis of *ortho*-Substituted Benzoylcholine Esters

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Abstract □ Second-order rate constants were determined for the alkaline hydrolysis of *ortho*-substituted benzoylcholine esters in 0.1 *M* aqueous NaCl at pH 9.0. Activation parameters for the reactions were calculated from the slopes of Arrhenius plots, with all plots being linear in the range of 25–45°. One derivative, *o*-methylbenzoylcholine, exhibited an abrupt change in the Arrhenius slope above 50°. Over the same extended temperature range, benzoylcholine and *o*-chlorobenzoylcholine gave no such change. An average isergonic temperature, β = 267°K., was calculated for the series from three independent correlations involving activation ($\Delta H^{\ddagger}$ *versus* $\Delta S^{\ddagger}$), rate (log k_1 *versus* log k_2), and linear free energy (ρ_T *versus* $1/T$) data. The parameters $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ each correlate independently with σ_I, the Taft inductive constant, indicating that the hydrolysis reactions are "well behaved." By comparison, thermodynamic parameters for acetic acid ionizations (*i.e.*, a model process defining σ_I) are not well behaved: ΔH° *versus* ΔS° is nonlinear, but ΔG°, ΔH°_{int}, and ΔS° correlate independently with σ_I. Rho–sigma correlations and an isergonic relationship for the benzoylcholine reactions are interpreted on the basis of the Hammett and Hepler analyses, respectively. Entropy values for this congeneric series are discussed in relation to the positive charge on the quaternary nitrogen and its effect in potentially neutralizing the substituent charge.

Keyphrases □ *Ortho* effect—alkaline hydrolysis of *ortho*-substituted benzoylcholine esters, second-order rate constants, thermodynamic parameters correlated with Taft inductive constant, relation of entropy values to nitrogen charge □ Benzoylcholine esters, *ortho*-substituted—*ortho* effect in alkaline hydrolysis, second-order rate constants, thermodynamic parameters correlated with Taft inductive constant, rho–sigma correlations □ Thermodynamic parameters—alkaline hydrolysis of benzoylcholine esters, *ortho* effect, correlated with Taft inductive constant □ Hydrolysis, alkaline—effect of *ortho*-substitution, benzoylcholine esters, thermodynamic parameters calculated

The substituent effects for a number of aliphatic (1–3) and aromatic (4, 5) reaction series have been successfully correlated using the Taft inductive constant, σ_I, originally defined according to Eq. 1 (3):

$$\sigma_I = 0.45\sigma^* = 0.45\,[\log(k/k_{CH_3})_A - \log(k/k_{CH_3})_B] \quad \text{(Eq. 1)}$$

where σ^*, the Taft polar constant, was obtained as the difference in the logarithms of the relative hydrolysis or esterification constants for acetate esters in alkaline (B) and acidic (A) solutions; k and k_{CH_3} refer to rate constants for substituted and nonsubstituted acetate esters, respectively. This definition (Eq. 1) is an inconvenient one and, more recently, it has been shown that σ_I may be expressed as a linear function of acetic acid ionizations in terms of Eq. 2 (6, 7):

$$\sigma_I = 0.276\log(K/K_{CH_3}) \quad \text{(Eq. 2)}$$

where K and K_{CH_3} are the ionization constants determined in aqueous solution for substituted and nonsubstituted acetic acid, respectively.

The electronic mechanism represented by σ_I is not entirely clear, but some evidence suggests that such a substituent effect is transmitted by a direct field effect instead of by the classical inductive mechanism (*i.e.*, induction through the chain) (8, 9). Based upon the field effect interpretation, correlations with σ_I have been used to explain the nature of the *ortho* effect in the hydrolysis of benzoate esters. For example, the effects of *ortho*-substituents in the alkaline hydrolysis of methyl and ethyl benzoates (4) and benzoylcholine iodides (5) produce rate changes which are highly correlated with σ_I. Resonance (σ_R) and steric (r_s) effects are unimportant for these reactions, and apparently only field effects (σ_I) are involved.

While the proportionality of substituent effects between acetic acid ionizations (*i.e.*, the model process defining σ_I) and *ortho*-substituted benzoate hydrolysis reactions is explicable in terms of free energy or internal potential energy changes (7), a question remains regarding the similarities or differences that exist between the respective solvation mechanisms of these reactions. A consideration of the mechanistic differences alone would suggest a dissimilarity in the solute–solvent interactions for the two processes and, hence, a difference in their observed enthalpy–entropy relationships. In this regard, previous analyses of substituent effects on enthalpy and entropy changes have greatly augmented the usual mechanistic information obtained from rho–sigma correlations; Leffler and Grunwald (10) listed a number of organic reaction series which exhibit linear enthalpy–entropy profiles in accordance with Eq. 3, the isergonic relationship[1]:

$$\delta_R\Delta H^{\ddagger} = \beta_i\delta_R\Delta S^{\ddagger} \quad \text{(Eq. 3)}$$

where δ_R = the Leffler–Grunwald operator for substituent effects (*i.e.*, the change incurred upon a reaction as a result of substituent variation); the superscript, ‡, refers to activation data; and the slope, β_i, is the isergonic temperature. For most isergonic reactions, β_i is positive in sign ($\simeq$100°–1300°K.), and a compensatory effect is imposed upon the expression for the free energy of activation (Eq. 4):

$$\delta_R\Delta G^{\ddagger} = (\beta_i - T)\delta_R\Delta S^{\ddagger} = (1 - T/\beta_i)\delta_R\Delta H^{\ddagger} \quad \text{(Eq. 4)}$$

From Eq. 4 it is seen that β_i may be defined operationally as the temperature at which a particular interaction mechanism vanishes from $\delta_R\Delta G^{\ddagger}$. Thus, when isergonic reactions are conducted near $\beta_i = T$, more useful mechanistic information is potentially available from $\delta_R\Delta S^{\ddagger}$ and $\delta_R\Delta H^{\ddagger}$ than from the simplified free energy term.

[1] The term isergonic was suggested [see R. L. Schowen, *J. Pharm. Sci.*, 56, 931(1967)] as a general term to include both isokinetic and isoequilibrium phenomena. In keeping with this suggestion, this term was adopted in the present report.

Table I—Second-Order Rate Constants for the Alkaline Hydrolysis of *ortho*-Substituted Benzoylcholine Iodides as a Function of Temperature

ortho-Substituent	k_2, M^{-1} sec.$^{-1}$[a] 25°	30°	35°	40°	45°	50°	55°	57°	60°
Hydrogen	1.22	1.65	2.05	2.87	3.66	4.77	6.50	6.61	7.69
Methyl	—	0.620	0.658	0.838	0.959	1.04	1.49	1.88	2.42
Methoxy	—	0.848	1.09	1.32	1.83	—	—	—	—
Iodo	1.66	2.16	2.82	3.64	—	—	—	—	—
Bromo	1.96	2.50	3.61	4.73	—	—	—	—	—
Chloro	2.27	2.88	4.08	5.66	—	8.82	10.4	12.4	14.7
Fluoro	2.96	3.73	5.43	7.42	—	—	—	—	—
Nitro	3.36	4.50	7.08	9.44	—	—	—	—	—

[a] Reported as an average of duplicate runs. With the exception of the methyl derivative, the mean deviation for these averages was less than 1%, excluding occasional erratic runs. The mean deviation for the methyl derivative was usually less than 3%.

In an effort to elucidate more clearly the mechanism of the *ortho* effect in the alkaline hydrolysis of *ortho*-substituted benzoylcholine esters, the activation parameters for this congeneric series were determined and the results were analyzed for the existence of an isergonic relationship. Both the Leffler–Grunwald (10) and Exner (11, 12) approaches for establishing $\Delta H^{\ddagger}$ *versus* $\Delta S^{\ddagger}$ profiles were considered to provide three independent estimates of β_i, the isergonic temperature. It was also of primary interest to compare the results of the present study with those of Hepler's (13) analysis for acetic acid ionizations to determine the analogies that may or may not exist between the two series with respect to isergonic relationships and rho–sigma dependencies.

EXPERIMENTAL

Materials—The synthesis of the ester substrates was reported previously (5). An aqueous solution of each ester was prepared just prior to investigation and stored under refrigeration during a 4-day study. All solutions were 0.1 *M* in reagent grade sodium chloride and, except for the methyl derivative, contained an ester concentration of 5×10^{-4} *M*. *o*-Methylbenzoylcholine was used at a concentration of 2.44×10^{-4} *M*. Purified nitrogen was used to provide a carbon dioxide-free environment in the reaction chamber, with the gas being passed through an aqueous solution before entering the chamber. The normalities of the sodium hydroxide titrant solutions were determined by titration with standard potassium acid phthalate solutions. A titrant normality of 0.0243 *N* was used for the methyl derivative, and one of 0.050 *N* was used for all other esters.

Kinetic Assays—Assays for the hydroxide-ion-catalyzed reactions were carried out at a constant pH of 9.00 using a pH-stat assembly (Radiometer): automatic titrator (TTT-1c), titrigraph recorder (SBR-2), syringe buret unit (SBU-1), and manual temperature compensator (PHA 924). The titration unit for this assembly consisted of a 50-ml. water-jacketed reaction chamber and an electrode head fitted with a general-purpose glass electrode (Type G 202c), reference calomel electrode (K 401), mechanical stirrer, inlet tube for nitrogen, and a titrant delivery tube for slow titrations. Solutions were maintained at desired temperatures (±0.02°) using a bath and circulator[2].

Kinetic assays were conducted in the following way. Prior to daily runs, the electrode assembly was equilibrated at the desired temperature and the pH meter was standardized with pH 9.18 buffer[3]. The 45-ml. reaction solutions were routinely flushed with nitrogen to the neutral point for the given temperature and then allowed to reach the equilibrium temperature. Nitrogen was subsequently directed across the surface of the solution during the kinetic run. Reactions were started by adding sodium hydroxide titrant to a pH of 9.00 with the automatic titrator. Base additions were continued automatically to maintain a constant pH of 9.00 during the ester hydrolysis. Depending upon the particular ester and temperature used, the length of the kinetic runs varied from 4 to 352 min., with the average percent hydrolysis for all esters being 27.9 ± 3.3%.

Since benzoic acid is virtually totally ionized at pH 9.00, the *x*–*y* recorder plot of titrant added during any kinetic run also provides a record of the acid released from the ester: $d[OH^-]/dt = d[BA]/dt = -d[RCOOR]/dt$. Data points were taken from the exponential *x*–*y* recorder tracings in calculating the pseudo-first-order rate constants. Accurate, readable plots were obtained by using appropriate chart speeds at the various temperatures.

RESULTS AND DISCUSSION

Kinetic Constants—Pseudo-first-order rate constants, k_{obs}, were obtained from the slopes of plots of log $[ester]_{remaining}$ *versus* time. Over the hydrolysis range investigated, all plots were linear with correlation coefficients of not less than 0.998. Figure 1, for the hydrolysis of *o*-bromobenzoylcholine at four temperatures, is typical of the data.

The k_{obs} for the hydrolysis of *m*-nitrobenzoylcholine at 37° was previously (5) shown to be a linear function of $[OH^-]$ in the pH region of 7.4–8.6. A zero intercept for the plot further indicated that any contribution from k_0, the water hydrolysis constant, was insignificant. In an identical manner for the present data, k_{obs} at 37° for *o*-bromobenzoylcholine is linear in $[OH^-]$ from pH 7.4–9.0, producing an apparent zero intercept and a correlation coefficient of 0.999. Values of k_2, the second-order rate constant, were therefore calculated for each derivative from the equation: $k_2 = k_{obs}/[OH^-]$. The average values from duplicate runs for k_2 at various temperatures are listed in Table I.

Activation Parameters—Arrhenius plots of the data given in Table I are illustrated by Fig. 2. The activation parameters calculated from these data are given in Table II and are based on a least-squares fit of the equation: $\log k = [-E_a/2.303R][1/T] + A$, where

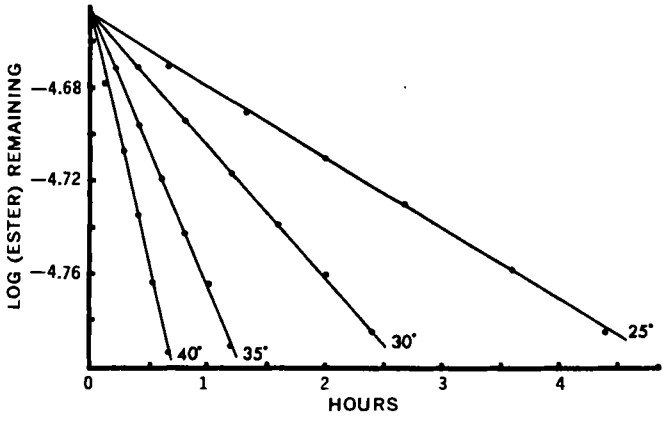

Figure 1—*Pseudo-first-order plots as a function of temperature for the alkaline hydrolysis of o-bromobenzoylcholine iodide in 0.1 M aqueous NaCl at pH 9.0.*

[2] Forma-Temp. Jr.
[3] Fisher Scientific.

Table II—Activation Parameters for the Alkaline Hydrolysis of *ortho*-Substituted Benzoylcholine Iodides[a]

ortho-Substituent	E_a, kcal./mole	$\Delta H^{\ddagger}$[b], kcal./mole	log A, M^{-1} sec.$^{-1}$	$\Delta S^{\ddagger}$[c], cal./mole/deg.
Hydrogen	10.3	9.67	7.63	−25.8
Methyl[d]	4.86	4.25	3.29	−45.7
	(18.3)	(17.7)	(12.4)	(−3.76)
Methoxy	9.60	8.98	6.84	−29.3
Iodo	9.72	9.11	7.35	−27.0
Bromo	11.2	10.6	8.48	−21.8
Chloro	11.4	10.8	8.71	−20.7
Fluoro	11.6	11.0	8.96	−19.6
Nitro	12.8	12.2	9.92	−15.2

[a] The average standard error associated with E_a and $\Delta H^{\ddagger}$ = 0.616 kcal., with log A = 0.438 unit and with $\Delta S^{\ddagger}$ = 2.00 cal. [b] $\Delta H^{\ddagger}$, the enthalpy of activation, was calculated from the relation $\Delta H^{\ddagger} = E_a - RT$, where T = 308°K. (A. A. Frost and R. G. Pearson, "Kinetics and Mechanisms," 2nd ed., Wiley, New York, N. Y., chap. 5). [c] $\Delta S^{\ddagger}$, the entropy of activation, was calculated from the relation $k_2 = (RT/Nh) \exp(\Delta S^{\ddagger}/R) \exp(-\Delta H^{\ddagger}/RT)$, where h = Planck's number and N = Avogadro's number (reference of *Footnote b*). [d] Values in parentheses were obtained using the rate constants at 50, 55, 57, and 60°.

E_a = Arrhenius activation energy, A = frequency factor, T = °K., R = 1.987 cal./deg./mole, and k_2 = second-order rate constant. The correlation coefficients for these fits were not less than 0.994, except for the methyl derivative in the temperature region 25–50° for which the value was 0.958.

A comparison of the activation enthalpies (Table II) with corresponding rate constants (Table I) reveals an unexpected trend between these parameters. Thus, within the congeneric series, rate constants are seen to parallel increases in enthalpy values. For *o*-methylbenzoylcholine, the most slowly hydrolyzed ester, the value of $\Delta H^{\ddagger}$ is 4.25 kcal./mole; for *o*-nitrobenzoylcholine, the most rapidly hydrolyzed ester, the value of $\Delta H^{\ddagger}$ is 12.2 kcal./mole. These results are seen more clearly from the tabulation of substituent effects on the free energy, enthalpy, and entropy of activation given in Table III. With benzoylcholine taken as the standard, the resulting difference in the activation parameters show that, in each case except for the *I* derivative, the $-T(\delta_R \Delta S^{\ddagger})$ term contributes more strongly to the free energy of activation than does the $\delta_R \Delta H^{\ddagger}$ term.

Over an extended temperature range of 30–60°, nonlinearity was observed in the Arrhenius plot for the *o*-methyl derivative. An attempt to fit these data nonlinearly using the equation $\log k_2 = a(1/T) + b(\log T) + c$ produced only statistically nonsignificant results. As seen in Fig. 3, however, the log k_2 values for *o*-methylbenzoylcholine may be represented as two straight-line segments when plotted against $1/T$. Such a result suggests an abrupt change in the mechanism or rate-limiting step for the reaction in the vicinity of 50°. By contrast, the Arrhenius plots for the parent compound

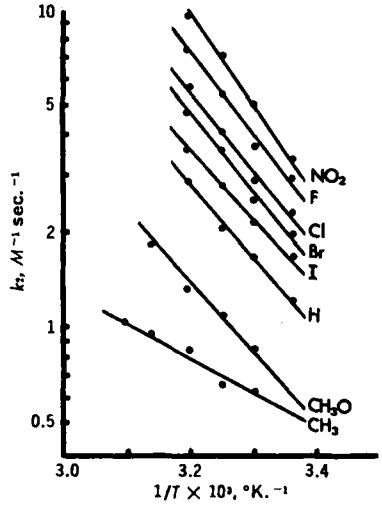

Figure 2—*Arrhenius plots for the alkaline hydrolysis of* ortho-*substituted benzoylcholine iodides in 0.1 M aqueous NaCl at pH 9.0. Kinetic constants are from Table I.*

Table III—Substituent Effects on Activation Parameters for *ortho*-Substituted Benzoylcholine Iodides[a]

ortho-Substituent	$\delta_R \Delta G^{\ddagger} = \delta_R \Delta H^{\ddagger} - T(\delta_R \Delta S^{\ddagger})$[b]		
	$\delta_R \Delta G^{\ddagger}$	$\delta_Z \Delta H^{\ddagger}$	$-T(\delta_R \Delta S^{\ddagger})$
Hydrogen	0	0	0
Methyl	0.70	−5.43	6.13
Methoxy	0.389	−0.691	1.08
Iodo	−0.196	−0.567	0.371
Bromo	−0.351	0.889	−1.24
Chloro	−0.44	1.13	−1.55
Fluoro	−0.60	1.31	−1.91
Nitro	−0.76	2.51	−3.27

[a] Units in kcal./mole; 35°C. [b] δ_R is the Leffler–Grunwald (10) operator for substituent effects (see text).

and the *o*-chloro derivative exhibit a strictly linear trend over the same temperature range.

Isergonic Relationships and Rho–Sigma Dependencies—Hepler (16) recently showed that Eq. 3 may be derived by an exact thermodynamic analysis of the Hammett equation (Eq. 5) when $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are temperature independent (*i.e.*, $\delta_R \Delta C_p^{\ddagger} = 0$):

$$\delta_R \Delta G^{\ddagger} = -2.303 RT \rho \sigma \qquad \text{(Eq. 5)}$$

Other important relationships which resulted from his analysis are given by Eqs. 6–8:

$$\delta_R \Delta H^{\ddagger} = 2.303 R \beta_i \rho_\infty \sigma \qquad \text{(Eq. 6)}$$

$$\delta_R \Delta S^{\ddagger} = 2.303 R \rho_\infty \sigma \qquad \text{(Eq. 7)}$$

$$\rho_T = \rho_\infty (1 - \beta_i/T) \qquad \text{(Eq. 8)}$$

where β_i and ρ_∞ are temperature-independent constants of integration. The enthalpy and entropy of activation are derived as linear functions of the sigma constant; ρ_T, the reaction constant for a linear free energy relationship at any given temperature, is shown to be inversely proportional to the absolute temperature. While these relationships are written in terms of σ, they would also apply to other σ constants such as σ_I.

Experimentally, β_i may be obtained as the slope of Eq. 3 or as the slope-intercept ratio from Eq. 8. Exner (11, 12), however, criticized the use of Eq. 3 on the basis of computational and experimental arguments. As an alternative, he proposed still another method of calculating β_i according to Eq. 9:

$$\log k_1 = \lambda \log k_2 + b \qquad \text{(Eq. 9)}$$

where k_1 and k_2 are rate constants at two temperatures with $T_1 > T_2$. By this method, β_i is obtained indirectly from the slope, λ, of a log k_1 *versus* log k_2 plot with the aid of Eq. 10 (14):

$$\beta_i = T_1 T_2 (T_1\lambda - T_1)/(T_1\lambda - T_2) \qquad \text{(Eq. 10)}$$

Nevertheless, it should be cautioned that β_i values calculated from

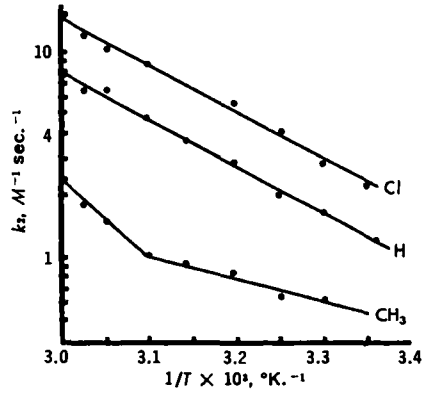

Figure 3—*Arrhenius plots over an extended temperature range for the alkaline hydrolysis of benzoylcholine iodide and* o-*chloro- and* o-*methylbenzoylcholine iodides in 0.1 M aqueous NaCl at pH 9.0. Kinetic constants are from Table I.*

Table IV—Estimates of β_i from a Fit of Experimental Data for *ortho*-Substituted Benzoylcholine Iodides[a]

Regression Equation	n	r	F	β_i (°K.)	Equation Number
$\Delta H^{\ddagger} = 258.1\Delta S^{\ddagger} + 16.18$	8	0.997	1131	258	11
$\log k_1 = 1.204 \log k_2 + 0.1875$	8	0.999	318	281	12
$\rho_T = -2904(1/T) + 0.1106$[b]	4	0.988	84	263	13
				β_i (average) = 267	

[a] n = number of data points used in the least-squares fit; r = correlation coefficient; and F = F-ratio. The β values were obtained as discussed in text. [b] The ρ_T values were obtained as the slopes of log k_2 *versus* σ_I correlation for data at 25, 30, 35, and 40° according to the equation $\log k_2^{\ddagger} = \rho\sigma_I + \log k_2^{CH_3}$, where $k_2^{\ddagger}$ = second-order rate constants given in Table I for substituents at a given temperature, $k_2^{CH_3}$ = calculated second-order rate constant for the methyl derivative, and σ_I = Taft's inductive constant (1–3). The regression analyses for data at the four temperatures produced correlation coefficients of 0.954, 0.959, 0.971, and 0.973, respectively.

Eq. 10 are highly dependent upon λ and may be in considerable error when scatter exists in the log k_1 *versus* log k_2 profiles.

***ortho*-Substituted Benzoylcholine Iodides**—By using the rate constants and activation parameters obtained for the hydrolysis of *ortho*-substituted benzoylcholine iodides, Eqs. 3 and 9 were plotted in Figs. 4 and 5, respectively. The Leffler–Grunwald and Exner methods are observed to produce similar values for the isergonic temperature, *i.e.*, β_i = 258°K. (Fig. 4) and β_i = 281°K. (Fig. 5). The dotted line in Fig. 4 is a transformation of the solid line from the Exner plot. There is an obvious close visual correspondence in the slopes for the two lines, but there is a slight vertical displacement because of the use of an average temperature value in the transformation equation converting rate constants to activation parameters[4]. The third estimate of β_i = 263°K. derived from Eq. 8

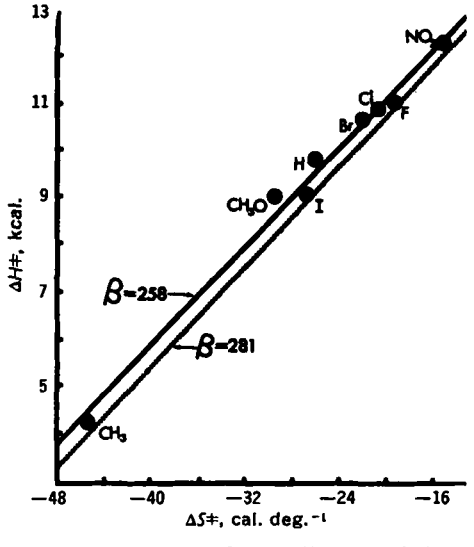

Figure 4—*Isergonic relationship for the alkaline hydrolysis of* ortho-*substituted benzoylcholine iodides in 0.1* M *aqueous NaCl at pH 9.0. Activation parameters are from Table II. Dotted line is a transformation of the solid line from Fig. 5.*

[4] The rate data of Fig. 5 are converted into the activation data of Fig. 4 using the equations (17): $\Delta H^{\ddagger} = [2.303RT_1T_2/(T_1 - T_2)](\log k_1 - \log k_2) - R(T)_{ave}$ and $\Delta S^{\ddagger} = [2.303RT_1/(T_1 - T_2)][\log k_1 - (T_2/T_1)\log k_2] - 2.303R \log[ek(T)_{ave}/h]$. When the solid line in Fig. 5 is drawn onto an E *versus* log A plot, the resulting lines coincide almost exactly since then a transformation involving only discrete T values is involved. The conversion of rate data to Arrhenius parameters is accomplished with the following expressions (11): $E = [2.303RT_1T_2/(T_1 - T_2)](\log k_1 - \log k_2)$ and $\log A = [T_1/(T_1 - T_2)][\log k_1 - (T_2/T_1)\log k_2]$.

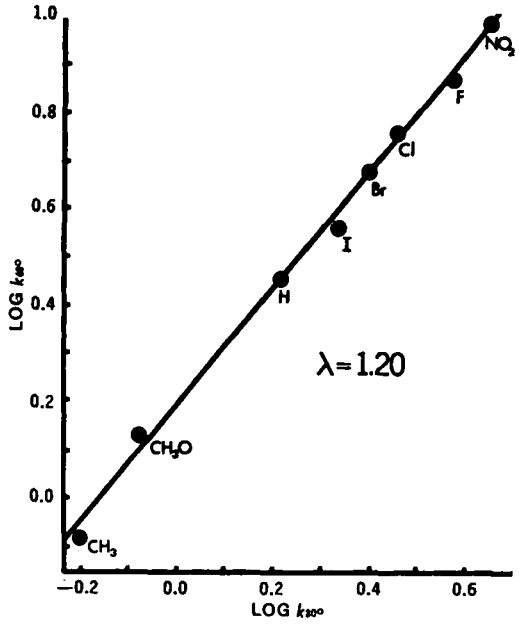

Figure 5—*Relationship between the logarithms of the second-order rate constants at 30 and 40° for the alkaline hydrolysis of* ortho-*substituted benzoylcholine iodides in 0.1* M *aqueous NaCl at pH 9.0. Kinetic constants are from Table I.*

is intermediate to the values of 258 and 281°K., resulting in an average for the three of β_i = 267°K. Correlations (Eqs. 11–13) for the fit of the experimental data to the theoretical equations are given in Table IV.

The relationships of the activation parameters for the benzoylcholine series to σ_I are given by Eqs. 14–16 in Table V. Each parameter is excellently correlated by σ_I. These results, together with those for Eqs. 11 and 13, indicate that the present congeneric series fits the "well-behaved" category of rho–sigma correlations (10).

Table V—Correlations of Activation Parameters for Acetic Acid and Benzoylcholine Series[a,b]

	n	r	s	F	Equation Number
***ortho*-Substituted Benzoylcholine Iodides**[c]					
$\Delta G^{\ddagger} = -2.312\sigma_I + 0.2626$	7	0.971	0.137	84	14
$\Delta H^{\ddagger} = 11.43\sigma_I + 4.641$	7	0.981	0.543	131	15
$\Delta S^{\ddagger} = 44.60\sigma_I - 44.77$	7	0.991	1.440	285	16
α-Substituted Acetic Acids[d]					
$\Delta H^{\circ} = 9.504\Delta S^{\circ} - 0.9597$	14	0.063	0.610	0.05	17
$\Delta H^{\circ} = 0.7186\sigma_I - 0.6207$	14	0.308	0.582	1.25	18
$\Delta G^{\circ} = -5.146\sigma_I + 6.441$	14	0.965	0.366	162	19
$\Delta H^{\circ}_{int} = -4.889\sigma_I + 6.004$	14	0.966	0.342	168	20
$\Delta S^{\circ} = 14.89\sigma_I - 23.66$	14	0.960	1.137	141	21
Enthalpy and Entropy Comparisons between Series[e]					
$\Delta H^{\circ}_{int} = -0.4184\Delta H^{\ddagger} + 8.013$	6	0.985	0.208	130	22
$\Delta S^{\circ} = 0.246\Delta S^{\ddagger} - 12.07$	6	0.987	0.434	152	23

[a] n = number of data points used in the least-squares fit; r = correlation coefficient; s = standard error of estimate; F = F-ratio (F = 106 at the 0.0005 level for 1,4 degrees of freedom, and F = 63.6 at the same level for 1,5 degrees of freedom). [b] The superscripts, ° and ‡, refer to acetic acid and benzoylcholine series, respectively. [c] Fluoro, chloro, bromo, iodo, methyl, methoxy, and nitro (hydrogen consistently produced lower correlations and was not included in these analyses) (5); 35°. [d] Fluoro, chloro, bromo, iodo, methoxy, ethyl, propyl, isopropyl, butyl, isobutyl, cyano, carboxymethyl, and hydrogen (data for nitro were not available for analysis). The experimental data for methoxyacetic acid at 25° were taken from E. J. King, *J. Amer. Chem. Soc.*, 82, 3575(1960). All other values for acetic acids (25°) were taken from *Reference 15*. The H°_{int} values were calculated from the relation: $\Delta H^{\circ}_{obs.} - 280\ \Delta S^{\circ}_{obs.}$. [e] Fluoro, chloro, bromo, iodo, methyl, and methoxy.

α-Substituted Acetic Acids—An analysis of the thermodynamic constants of α-substituted acetic acids is presented in Table V by Eqs. 17–21. Analogous to the benzoylcholine series, $\Delta G°$ and $\Delta S°$ for the acetic acid series both correlate independently with σ_I. The $\Delta H°$ *versus* $\Delta S°$ relationship, however, is highly scattered ($r = 0.063, n = 14$), and $\Delta H°$ is not a linear function of σ_I. Furthermore, $\delta_R\Delta C_p° \neq 0$ for these reactions (15), and it may be shown under such conditions that β_i is no longer a constant but is temperature dependent (16). While the observed enthalpy changes for acetic acid ionizations are not well behaved, $\Delta G°$ values for the series still approximate a linear free energy model for σ_I because of the compensatory effect inherent in Eq. 4.

An explanation of the compensatory effect was offered by Hepler (13, 16) based upon an extrathermodynamic model for substituent and solvent effects. Accordingly, the observed free energy changes may be written as a sum of two interaction energies:

$$\delta_R\Delta G°_{obs} = \delta_R\Delta G°_{int} + \delta_R\Delta G°_{env} \qquad \text{(Eq. 24)}$$

where "int" signifies the internal or intrinsic effect of the substituent on the reaction center, and "env" refers to the environmental effect due to the interaction between solvent and substituent. In a similar manner, the observed enthalpy and entropy may be expressed according to Eqs. 25 and 26, respectively:

$$\delta_R\Delta H°_{obs} = \delta_R\Delta H°_{int} + \delta_R\Delta H°_{env} \qquad \text{(Eq. 25)}$$

$$\delta_R\Delta S°_{obs} = \delta_R\Delta S°_{int} + \delta_R\Delta S°_{env} \qquad \text{(Eq. 26)}$$

From evidence which suggests that substituent effects on $\Delta S°_{int}$ are negligible (*i.e.*, $\delta_R\Delta S°_{int} \simeq 0$) and that the solvation enthalpy and entropy are isergonically related (*i.e.*, $\delta_R\Delta H°_{env} = \beta_e\Delta S°_{env}$), Eq. 27 may be written:

$$\delta_R\Delta H°_{obs} = \delta_R\Delta H°_{int} + \beta_e\delta_R\Delta S°_{obs} \qquad \text{(Eq. 27)}$$

where β_e is now a parameter related to solvent and is not necessarily identical to β_i. Finally, upon combining the appropriate equations, the free energy is expressed in terms of Eq. 28:

$$\delta_R\Delta G°_{obs} = \delta_R\Delta H°_{int} + (\beta_e - T)\delta_R\Delta S°_{obs} \qquad \text{(Eq. 28)}$$

Since it is observed that $\beta_e \simeq T$ for many reactions conducted in aqueous solution, Eq. 28 reduces further to an expression (Eq. 29) that underlies the general success of the Hammett equation:

$$\delta_R\Delta G°_{obs} \simeq \delta_R\Delta H°_{int} \simeq -2.303RT\rho\sigma \qquad \text{(Eq. 29)}$$

The result of Eq. 20 (Table V) shows that a correlation between σ_I and the calculated $\delta_R\Delta H°_{int}$ values for acetic acid ionizations does indeed produce the linear fit predicted by Eq. 29. Thus, experimental measures of the free energy clearly contain contributions from intrinsic substituent effects ($\delta_R\Delta G°_{int}$), but the effects from solvent–substituent interactions are apparently not seen because of compensatory phenomenon (*i.e.*, $\delta_R\Delta G°_{env} \simeq 0$). A set of equations analogous to Eqs. 24–29 may also be written for kinetic reactions in aqueous solution.

Mechanistic Inferences—In drawing mechanistic inferences from the results for the benzoylcholine series, it is important to distinguish between the meaning of the rho–sigma correlations and the linear $\Delta H^{\ddagger} - \Delta S^{\ddagger}$ profiles. From the Hammett propositions (17), it is understood that linear rho–sigma correlations based on $\Delta G^{\ddagger}$ reflect solely internal potential energy changes of substituents. Mechanistic contributions from such analyses would then be limited normally to those for inductive and/or resonance effects exclusive of solvent effects. For the present series, therefore, Eq. 14 reflects the high dependency of $\Delta G^{\ddagger}$ on the inductive effect of the substituents. Furthermore, the negative slope for the relationship indicates that hydrolysis rates increase as the electron-withdrawing power of the substituents increases. An increasingly positive character of the carbonyl carbon facilitates attack by hydroxide ion. It is not necessary to invoke a description that bases the *ortho* effect on resonance or steric interactions.

Information from the $\Delta H^{\ddagger} - \Delta S^{\ddagger}$ profile can be interpreted in light of the Hepler analysis. The value of β_i for the *ortho*-substituted esters approximates that of β_e for solvation processes in general; thus it is likely that the results of Fig. 4 are highly dependent upon solvent–substituent interactions. Such a linear profile need not reflect the existence of only a single-interaction mechanism. Leffler and Grunwald (10) indicated that linear $\Delta H^{\ddagger} - \Delta S^{\ddagger}$ profiles are also obtained for multiple-interaction mechanisms when the β values for the interactions are identical or nearly so. In these cases, the regression lines for the different mechanisms cannot be separated, and they are superimposed upon one another on the isergonic plot. For the present series, it is likely that β_i and β_e are nearly identical since both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ have been shown to correlate independently with σ_I (Eqs. 15 and 16, respectively). The $\Delta H^{\ddagger}$ is interpreted to include both internal potential energy and solvation enthalpies, while $\Delta S^{\ddagger}$ should reflect solely the solvation entropy involved. A similar consistency between β values is not obtained for the acetic acid series. The correlation between $\Delta H°_{int}$ and $\Delta S°$ values produces an isergonic temperature of −465.8°K. when β_e = 280°K. A further comparison between the two series for a limited number of congeners shows a surprisingly close correlation between their respective enthalpy and entropy values as indicated by Eqs. 22 and 23.

While the solvation entropy does not contribute to the observed free energy changes or the ordering of the substituents within the benzoylcholine series, the $\Delta S^{\ddagger}$ values given in Table II can be utilized to gain further insight into the solvation mechanism. An unexpected trend is observed to exist between entropy and the charge on the substituent. Since it has been shown that σ_I is an increasing linear function of substituent charge (18), it would be expected that the most highly charged substituent would be the most strongly solvated, producing the greatest decrease in entropy. This is clearly not the case for the present series; the most highly charged substituent, nitro, exhibits the greatest entropic freedom (*i.e.*, the most positive entropy value); the least highly charged substituent, methyl, displays the greatest entropy requirement (*i.e.*, the most negative entropy value). The apparent inconsistency is explained potentially by considering the positive charge on the choline moiety. Thus, the close proximity of a diffuse positively charged electron cloud, such as that of a quaternary nitrogen (19), could be expected to neutralize partially the substituent charge and lead to an alteration of expected solvent effects. In such a case, the conformation of the benzoylcholine molecule would be a critical factor in altering the substituent–solvent interactions. From conformational analysis studies (19), there is evidence for acetylcholine, suggesting that minimum energy conformations do exist which would bring the quaternary nitrogen and the α-carbon of the acyl group into reasonably close proximity. A similar analysis will be necessary for the benzoylcholine molecule to verify this hypothesis.

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GLC Determination of Meperidine in Blood Plasma

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Abstract □ A GLC technique utilizing a flame-ionization detector is described for the analysis of meperidine in blood plasma. The meperidine is extracted with benzene from plasma that has been made basic with sodium carbonate. A linear calibration curve is found in the range 0.1–1.25 mcg./ml., with the precision of the assay estimated to be ±9.9% (*RSD*). The method has been used in the determination of the half-life of meperidine in dog plasma.

Keyphrases □ Meperidine—GLC-flame-ionization analysis in dog plasma, determination of half-life □ Plasma levels, meperidine—determination of half-life in dogs, GLC-flame ionization □ GLC-flame-ionization detection—analysis, meperidine in dog plasma, determination of half-life

Until recently, the colorimetric technique of Burns *et al.* (1), with a sensitivity of 0.3 mcg./ml., was the best method available for analysis of meperidine[1]. This method, which depends upon the formation of a methyl orange–base complex, is applicable for most amines. Thus, its adaptation for analysis of meperidine raises the question of specificity. Beckett *et al.* (2) showed that, even with modifications such as buffer washes, nicotine interferes with the assay. This should be true of many other bases as well. Dal Cortivo *et al.* (3) developed a fluorometric assay for meperidine, but a lower limit again of only 0.3 mcg./ml. was established. Jenkins *et al.* (4) used a GLC method for the estimation of meperidine in a study of its placental transfer in pregnant ewes. A sensitivity of 0.025 mcg./ml. was reported; however, in our hands this sensitivity was not obtainable. Moreover, there is a limitation of only two analyses per hour and a relatively large volume of plasma is needed (3–4 ml.).

Although other papers have been published concerning GLC techniques, either complete details of the methodology were not reported (5) or no attempt was made to adapt the method for the analysis of biological fluids (6–11). Other approaches used for the determination of meperidine in biological fluids include another colorimetric approach utilizing bromthymol blue (12, 13), a UV absorption spectrophotometric technique (14), and two methods using column chromatography, to separate meperidine from contaminating material, coupled with either paper chromatography (15) or colorimetry (16) for quantitation. Most of the therapeutic serum concentrations have been estimated using the method of Burns *et al.* (1) with widely varying values (1, 17–21). Consequently, a method having greater sensitivity and greater specificity was desired. A GLC method was developed which has a sensitivity slightly better than 0.1 mcg./ml. and the required specificity.

[1] Demerol, Sterling-Winthrop Research Institute.

EXPERIMENTAL

Apparatus—A gas chromatograph[2] equipped with a flame-ionization detector was used with gas flows of 45, 60, and 600 ml./min. for nitrogen, hydrogen, and air, respectively. A column oven temperature of 180° was used; injection port and detector temperatures were maintained at 220 and 210°, respectively.

Column—A 2-mm. i.d. × 180-cm. glass U tube column was packed with 3% OV-17 on 100–120-mesh Gas-Chrom Q[3]. Before it was packed, the empty glass column was thoroughly rinsed with methanol and acetone, dried, and conditioned 1 hr. with a 5% solution of dimethyldichlorosilane[3] in toluene to silylate reactive sites. The column was again rinsed with acetone and dried.

Analytical Procedure—Two milliliters of blood plasma[4] was transferred to a 50-ml. glass-stoppered centrifuge tube made basic with 1.5 ml. 10% sodium carbonate and extracted with 15 ml. analytical reagent grade benzene by shaking for 2 min. The tubes were centrifuged for 3 min., and 12.5 ml. of the benzene phase was then transferred to a screw-top test tube. One drop of methanolic 1 *N* HCl (prepared from 12 *N* HCl) was added to the benzene phase. The benzene was evaporated at no more than 40° under a flow of nitrogen. The residue was taken up in 30 μl. of analytical reagent grade dimethylformamide and 20 μl. of hexamethyldisilazane[3], taking care to rinse the sides of the tube well. GLC determinations were made by injecting 4 μl. of the dimethylformamide solution onto the column with a 1-μl. solvent wash. Quantitation was accomplished by the measurement of peak heights.

Standards for a calibration curve were prepared by adding aliquots of an aqueous solution of meperidine hydrochloride to 50-ml. glass-stoppered centrifuge tubes containing 2 ml. of blood plasma[5] to give final plasma concentrations of 0.1–1.25 mcg./ml. The tubes were shaken to ensure mixing and then analyzed as mentioned. To determine percent recoveries, it was necessary to compare the calibration curve data with a detector response curve. This was obtained by taking aliquots of an aqueous solution of meperidine hydrochloride to give standards of 0.2–2.5 mcg. The aliquots were evaporated to dryness and taken up in 30 μl. of dimethylformamide and 20 μl. hexamethyldisilazane, and 4-μl. samples were injected onto the column.

[2] Packard model 7301.
[3] Applied Science Laboratories, State College, Pa.
[4] Citrate and fluoride have been used successfully as anticoagulants.
[5] Red Cross citrated plasma.