mp 226–233°. Recrystalization of the crystals from absolute EtOH raised the melting point to 235–238°; $[\alpha]^{28}D = +118°$ (*c* 1.015; l, 1 dm; MeOH). *Anal.* ($C_{26}H_{31}NO_4$): C, H, N.

***d*-1,2,3,4,5,6-Hexahydro-3,11β-dimethyl-6-phenyl-2,6-methano-3-benzazocin-8-ol Hydrochloride.**—The mandelate salt (mp 235–238°) was treated with NH_4OH and Et_2O to liberate the free base. The Et_2O extract was washed (H_2O), dried (Na_2SO_4), and concentrated to a smaller volume to yield colorless prisms: mp 197–198°; $[\alpha]^{28}D = +124°$ (*c* 150; l, 1 dm; MeOH). The free base was converted into its hydrochloride by treatment with alcoholic HCl in EtOH giving colorless needles: mp 310–312° dec; $[\alpha]^{28}D = +104°$ (*c* 1.26; l, 1 dm; MeOH). *Anal.* ($C_{20}H_{24}ClNO$): C, H, Cl, N.

Cyclization of *l*-1,2-Dimethyl-2-(4-hydroxybenzyl)-4-phenyl-1,2,5,6-tetrahydropyridine. l-VII (13 g) was refluxed in 300 ml of 48% HBr for 48 hr. The reaction mixture was cooled in an ice bath, neutralized with concentrated NH_4OH in ice–water, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed (H_2O), dried (Na_2SO_4), and evaporated *in vacuo* yielding a residue which was crystallized from aqueous *i*-PrOH to obtain 11.7 g (90%) of white crystals: mp 196–198°; $[\alpha]^{25}D = +120°$ (*c* 1.46; l, 1 dm; MeOH). The melting point was not depressed upon admixture of the compound with *d*-VIII obtained from the *d*-mandelate salt.

***dl*-1,2,3,4,5,6-Hexahydro-8-methoxy-3-methyl-6-phenyl-2,6-methano-3-benzazocine Hydrochloride Hydrate.**—To a suspension of *dl*-1,2,3,4,5,6-hexahydro-3-methyl-6-phenyl-2,6-methano-3-benzazocin-8-ol[1] (I, 5.0 g) in a 1:1 MeOH–$CHCl_3$ mixture (50 ml) was added freshly prepared 100 ml of CH_2N_2 ethereal solution (250 ml of solution from 20 g of nitrosomethylurea). The mixture was stirred at room temperature for 6 hr to obtain a clear solution, which was then evaporated *in vacuo* to an oil. The residue was treated with Et_2O (500 ml) and 1 *N* HCl (500 ml). The acidic layer was made alkaline (NH_4OH) and extracted with Et_2O (2 × 200 ml). The ethereal extract was washed (H_2O), dried (Na_2SO_4), and evaporated to leave a light yellow oil (5.1 g, 97%). The oil was dissolved in 0.5 *N* HCl (40 ml) with heating. The solution deposited fine prisms on cooling: 4.98 g (80%); mp 204–207° [dried at 80° (0.4 mm) for 6 hr]. *Anal.* ($C_{20}H_{24}ClNO \cdot H_2O$): C, H, Cl, N. The free base was prepared from the hydrochloride hydrate and used for the nmr study (see text).

***l*-8-(*p*-Bromobenzoxy)-1,2,3,4,5,6-hexahydro-3,11β-dimethyl-6-phenyl-2,6-methano-3-benzazocine.**—A mixture of *l*-VIII (14.67 g) 4-bromobenzoyl chloride (12.10 g, Aldrich Chem., Milwaukee, Wis.) diisopropylethylamine (14.30 g Aldrich Chem., Milwaukee, Wis.) in 300 ml of C_6H_6 was refluxed on a steam bath for 2 hr, then, brought to dryness *in vacuo* to leave a residue. The residue was treated with $CHCl_3$ and aqueous $NaHCO_3$ solution. The $CHCl_3$ layer was separated, washed (H_2O), dried (Na_2SO_4), and evaporated to dryness to leave a white crystalline solid. The solid was crystallized from *i*-PrOH to obtain 22.0 g of colorless needles: 92%; mp 161–162°; $[\alpha]^{25}D = -65°$ [*c* 0.89; l, 1 dm; $CHCl_3$–MeOH (1:1)]. *Anal.* ($C_{27}H_{26}BrNO_2$): C, H, Br, N. The compound was used for the X-ray crystallographic study (see ref 8).

Acknowledgments.—We wish to thank Dr. J. Schulze and associates of the Analytical Research Department for the microanalytical and spectral results and the rate of quaternization study, Dr. I. Fratta of the Toxicology Department for the toxicology results, and Dr. R. Hill and Mrs. M. L. Graeme of the Pharmacology Department for the pharmacological results. We are indebted to Mr. E. McMahon for his contribution in the synthesis of these compounds.

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

JAMES J. ZIMMERMAN[2] AND JERE E. GOYAN

University of California, School of Pharmacy, San Francisco, California 94122

Received December 15, 1969

Rate constants have been determined for the alkaline hydrolysis of a series of *ortho*, *meta*, and *para* substituted benzoylcholine esters in 0.1 *M* aqueous NaCl at a constant pH of 7.4 and 37°. Substituent effects in the *meta* and *para* positions closely obey the Hammett equation and produce a ρ value of +1.540. The effects of substituents in the *ortho* position are accounted for by either a linear combination of σ_o^* and E_s^0 or by σ_I alone. A derivation is given to show that σ_I should be a linear function of σ_o^* and E_s^0. Interpretation of the substituent effects in the *ortho* position is most rationally based on σ_I in view of the incorrect assumptions made in defining σ_o^* and E_s^0. Substituent effects based on σ_I produce a ρ value of +2.088.

Substituent effect analysis has been successfully applied to an impressive number and variety of organic reactions, as documented by the compilations of Jaffé[3] and others.[4–6a] The success of these efforts in elucidating organic reaction mechanisms has been largely dependent on the comparisons made between the reaction rates of a new congeneric series of compounds and the substituent constants determined for an appropriate model process. The value of the reaction constant obtained from such a comparison provides a sensitive index of the susceptibility of the reaction center to the substituent effect and thus provides a means of comparing different reactions.

An examination of the reaction series for which ρ values have been determined reveals that relatively few series of biological substrates have been included in these analyses. In view of the current interest in utilizing physicochemical methods to explain drug activity, it would appear that the investigation of the purely chemical reactivity of congeneric series should be a fundamental part of many drug studies. Such an approach would provide a ρ value for the chemical reaction under the identical conditions of temperature and dielectric constant used in the biological assay and under

(1) Abstracted in part from the Doctoral Dissertation of J. J. Zimmerman, University of California, San Francisco, Calif. (1969).

(2) (a) To whom inquiries should be addressed: Temple University, School of Pharmacy, Philadelphia, Pa. 19140; (b) Trainee, National Institutes of Health Training Grant 5-T1-GM-726-03(1964–1968). American Foundation for Pharmaceutical Education Fellow (1966–1968).

(3) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).

(4) L. M. Stock and H. C. Brown, *Advan. Phys. Org. Chem.*, **1**, 35 (1963).

(5) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley & Sons, Inc., New York, N. Y., 1963, Chapter 7.

(6) (a) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley & Sons, Inc., New York, N. Y., 1963, Chapter 13; (b) *ibid.*, p 607; (c) *ibid.*, p 648; (d) *ibid.*, p 591; (e) *ibid.*, p 598; (f) *ibid.*, p 587; (g) *ibid.*, p 599; (h) *ibid.*, p 643.

less complex reaction conditions than generally encountered in the biological system.

It was of interest in the present study to direct our efforts towards the synthesis, hydrolysis, and substituent effect analysis of a congeneric series of esters which are also potential substrates of the cholinesterase enzymes. For this purpose, a series of *ortho*, *meta*, and *para* substituted benzoylcholine esters was chosen.

It was also of interest to use the results obtained to inquire further into the recent controversy regarding σ_o^* and E_s^0, the Taft substituent constants for *ortho*-substitutued benzoates.[6a] Using multiple regression analysis, Charton[7a-c] has obtained convincing evidence that σ_o^* and E_s^0 are not measures of the intrinsic polar and steric effects, respectively, of *ortho* substituents. Since these constants could be used potentially to correlate the rate data of the present series of *ortho* derivatives as well as other biological substrates, it is apparent that they require further investigation. The results suggested a theoretical relationship between the Taft and Charton approaches not demonstrated previously.

Experimental Section

Materials.—Esters used in this study were synthesized from commercially available starting materials. The preparation of benzoylcholine is a typical procedure. 2-Dimethylaminoethanol (7.0 ml, 0.07 mol) was added dropwise to a cold stirred solution of BzCl (8.2 ml, 0.07 mol) in 100 ml of anhydrous C_6H_6, and the reaction mixture was heated under reflux for 2 hr. The precipitated HCl salt was recovered from the C_6H_6 by adding a total of 50 ml of H_2O and separating the two layers. A solution of the free amine was obtained by adding 10% NaOH dropwise to the aq layer and rapidly extracting into Et_2O. After separating, drying, and cooling the ether layer, cold MeI (4.5 ml, 0.07 mol) was added slowly with stirring, and the mixture was heated under reflux for 2 hr. The Et_2O was evaporated, and the solid residue of benzoylcholine was crystallized in abs EtOH to afford an average of 11.4 g of product (48.9% yield).

Quantities (5 g) of all esters, except the *p*-NO_2 derivative, were recrystallized from abs EtOH (100–350 ml) and dried over P_2O_5 for 8–12 hr under vacuum. The average yield for this step was 3.80 g (76%). *p*-Nitrobenzoylcholine was recrystallized from 600 ml of 85% EtOH. The dried products were stored over $CaCl_2$ at 0°. The melting points (uncorrected) which were taken on a Fisher–Johns melting point apparatus are listed in Table I together with available literature values.

Stock solutions of esters, ranging between 2×10^{-3} and 2×10^{-2} *M*, were prepared immediately before use and stored in an ice bath. Both the stock ester solutions and reaction medium were 0.1 *M* in reagent grade NaCl. Purified N_2 was used to provide a CO_2-free environment in the reaction chamber, and the gas was passed through an aqueous solution before entering the chamber. The normalities of the NaOH titrant solutions were determined by titration with standard potassium acid phthalate solutions and were found to range between 2.19×10^{-3} and 1.03×10^{-2} *N*.

Kinetic Assays.—Assays for the OH^- catalyzed reactions were carried out at a constant pH of 7.40 using a semiautomatic pH-Stat. Since virtually all of the BzOH produced during the reaction is fully ionized at pH 7.40, a record of the NaOH titrant added during any kinetic run to maintain a constant pH is also a record of the benzoic acid liberated from the ester: $d[OH^-]/dt = d[BA]/dt = -d[RCOOR]/dt$ at a constant pH.

The following components were used in assembling the pH-Stat: (a) a Beckman Research pH meter equipped with a Beckman type E-2 glass electrode (39004) (for stability reasons a Ag–AgCl reference electrode[8] was constructed and used in measuring the relatively slow alkaline hydrolysis rates at high meter sensitivities); (b) a Honeywell *x–y* recorder(Model s-153x33vv-x-120) of which the *x*-axis input voltage was supplied by an SI-100 Integrator (Self Organizing Systems Inc.) connected to a constant voltage source and of which the *y*-axis was supplied by a voltage source constructed for the base-delivering micropipet; (c) a reaction chamber assembly consisting of a 50 ml, water-jacketed Pyrex glass reaction chamber fitted with a Lucite top [The Lucite top was constructed to protect the reaction solution from atmospheric CO_2 and to serve as a mounting unit for all the necessary solution probes (electrodes, gas inlet, titrant inlet, etc.). Solutions were magnetically stirred and maintained at 37° (±0.06°) with a P. M. Tampson circulation thermostat]; (d) a semiautomatic titration assembly constructed by attaching a 10-turn Beckman Helipot to the delivery knob of a 1-ml Manostat micropipet. The Helipot was driven by a 1.35-V Hg battery connected to the *y*-axis input of the *x–y* recorder. In this way any addition of titrant was automatically recorded as a function of time.

Kinetic assays were conducted in the following way. Prior to daily runs the electrode assembly was equilibrated at 37° and the pH meter standardized with Beckman pH 6.84 buffer. The 50-ml reaction solutions with 2×10^{-4} to 1.2×10^{-2} *M* ester in 0.1 *M* NaCl were routinely flushed with N_2 for 10 min to the neutral point (pH 6.811 at 37°) and then allowed to reach 37°. N_2 was subsequently directed across the surface of the solution during the kinetic run. The reactions were initiated by manually adding NaOH titrant from the micropipet to a pH of 7.4. Base additions were then continued manually at frequent intervals for 15–30 min to maintain a pH of 7.40 during the course of ester hydrolysis. The initial velocity, v_0, for each individual hydrolysis measurement was obtained from the slope of the linear portion of the *x–y* recorder plot. Slopes which were accurately readable were achieved by using an appropriate combination of chart speed, ester concentration, and NaOH concentration.

Table I
Melting Points of Substituted Benzoylcholine Iodides

Substituent	Mp, °C	Mp (lit.), °C
H	240.5–241	243[a]
o-CH_3	153–153.5	157[b]
m-CH_3	175–175.5[c]	110–112[a]
p-CH_3	199–199.5	197–198[a]
o-Cl	170–170.5	166–168,[a] 174[b]
m-Cl	194.5–195	192–195[a]
p-Cl	215.5–216	
o-F	211–212	
m-F	209.5–210	206–209[a]
p-F	178.5–179	173–175[a]
o-NO_2	154.5–155	155[b]
m-NO_2	195.4–195 dec	183–186[a]
p-NO_2	238–239 dec	
o-OCH_3	159.5–160	162[b]
o-Br	142.5–143	142[b]

[a] See W. E. Ormerod, *Biochem. J.*, **54**, 701 (1953). [b] See J. Thomas and J. R. Stoker, *J. Pharm. Pharmacol.*, **13**, 129 (1961). [c] Both the acyl chloride and the ester used in this study were analyzed by ir and gave the results expected of a *meta*-substituted benzoyl derivative. Furthermore, this derivative exhibits the normal inductive effect on the rate of alkaline hydrolysis based on the correlation of log k_2 with σ.

Results and Discussion

Kinetic Constants.—Pseudo-first-order rate constants, k_{obsd}, were obtained from the slopes of plots of v_0, the initial rate, *vs.* $[RCOOR]_0$, the initial ester concentration in accordance with the equation, $v_0 = k_{obsd} \cdot [RCOOR]_0$. At least four different values of $[RCOOR]_0$, extending over an approximately fourfold concentration range, were used in determining the k_{obsd} for each ester. All plots were linear yielding correlation coefficients of not less than 0.998.

At slightly alkaline pH values, k_{obsd} can be expressed as $k_{obsd} = k_0 + k_2[OH^-]$ where k_0 is the water hydrolysis

(7) (a) M. Charton, *J. Amer. Chem. Soc.*, **91**, 615 (1969); (b) *ibid.*, 619; (c) *ibid.*, 624.

(8) J. J. Lingane, "Electroanalytical Chemistry," Interscience Publishers, Inc., New York, N. Y. (1953), p 263.

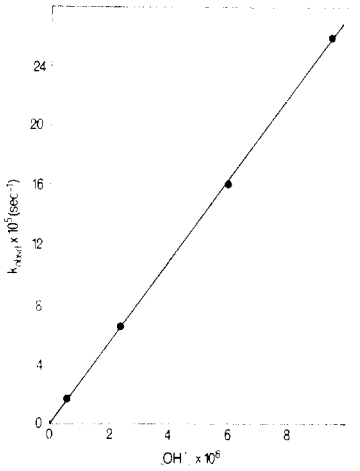


Figure 1.—Pseudo-first-order rate constants for the alkaline hydrolysis of *m*-NO_2 benzoylcholine iodide in 0.1 *M* aqueous NaCl at 37°, plotted against hydroxide ion concentration.

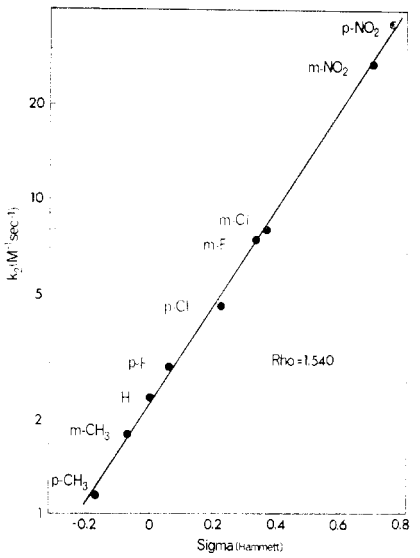


Figure 2.—Logarithm of the second-order rate constants, for the alkaline hydrolysis of *meta* and *para* substituted benzoylcholine iodides in 0.1 *M* aqueous NaCl at pH 7.4 and 37°, plotted against σ. The regression line is a fit of eq 2.

constant and k_2 is the second-order alkaline hydrolysis constant.[9] When $k_0 \ll k_2[OH^-]$, however, a plot of k_{obsd} *vs.* $[OH^-]$ will have an apparent zero intercept. Figure 1 demonstrates this relationship for the *m*-NO_2 derivative for which rate measurements were made at pH values of 7.4, 8.0, 8.4, and 8.6. The H_2O hydrolysis constant, k_0, for other charged esters has indeed been shown to be considerably less than k_2 by 9–10 orders of magnitude,[10] and therefore the results of Figure 1 are not unexpected. On the basis of the apparent zero intercept obtained for *m*-nitrobenzoylcholine, one of the more rapidly hydrolyzed esters, a zero value for k_0 was assumed for each of the esters; and k_2 values were calculated from the equation $k_2 = k_{obsd}/[OH^-]$. The results obtained from the substituent effect analyses appear to verify the correctness of this assumption since correlations with substituent constants would not be expected *a priori* for reactions in which the rate constant

(9) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin Inc., New York, N. Y., 1966, p 4.

(10) (a) M. G. Wright, *J. Chem. Soc. B*, 548 (1968); (b) B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2982 (1969).

TABLE II

KINETIC CONSTANTS FOR THE ALKALINE HYDROLYSIS OF *ortho*, *meta*, AND *para* SUBSTITUTED BENZOYLCHOLINE IODIDES

Substituent	$k_{obsd} \times 10^6$, sec^{-1}	pH[a]	k_2, M^{-1} sec^{-1}
H	1.41	7.40	2.34
o-Cl	3.61	7.40	6.02
m-Cl	4.81	7.40	8.01
p-Cl	2.75	7.40	4.58
o-F	4.50	7.40	7.50
m-F	4.45	7.40	7.42
p-F	1.77	7.40	2.94
o-CH_3	0.289	7.40	0.481
m-CH_3	1.08	7.40	1.79
p-CH_3	0.683	7.40	1.14
o-NO_2	5.88	7.40	9.79
m-NO_2	16.2	7.40	27.0
p-NO_2	21.8	7.40	36.3
o-OCH_3	0.800	7.40	1.33
o-Br	3.50	7.40	5.84
m-NO_2	65.3	8.00	27.3
m-NO_2	161	8.40	26.8
m-NO_2	259	8.60	27.3

[a] pK_w = 13.622 at 37°.

contained contributions from more than one mechanism.[11]

Values of k_{obsd} and k_2 for the alkaline hydrolysis rate measurements are given in Table II. Although the values for k_{obsd} were not determined in duplicate at pH 7.4, a measure of the reproducibility of the data is provided by comparing the k_2 values for the *meta* derivative determined at the pH values of 7.4, 8.0, 8.4, and 8.6. The standard deviation for these four estimates is 0.25 demonstrating good precision in the experimental measurements.

Linear Free Energy Relationships. *meta* and *para* Substituents.—Effects of the *meta* and *para* substituents on the second-order rate constants were investigated using the Hammett equation.[12]

$$\log k_{2-x} = \rho\sigma + \log k_{2-H} \qquad (1)$$

where x and H refer to the substituted and unsubstituted derivatives, respectively. ρ and σ have their usual meaning. Figure 2 illustrates the resulting linear relationship between $\log k_{2-x}$ and σ. The equation fitting the data was obtained by the method of least squares.[13a,14]

	n	r	r^2	s	p	
$\log k_{2-x} = 1.540\sigma + 0.346$	9	0.999	0.998	0.026	0.001	(2)

(11) K. B. Wiberg, "Physical Organic Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1964, p 403.

(12) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y. 1940, p 184.

(13) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 6th ed, The Iowa State University Press, Ames, Iowa, 1967, (a) Chapter 6; (b), Chapter 13.

(14) (a) The statistical calculations for the substituent effect analyses presented in this paper were performed on the IBM 360, Model 50, and the CDC 6400 computers using program BMDO2R. Subsequently, all values of the correlation coefficients, coefficients of determination, and standard errors of estimate obtained with this program were corrected for small sample size using the equations: $r^2_{corr} = 1 - (1 - r^2)(n - 1)/(n - k)$; $s^2_{corr} = s^2(n - 1)/(n - k)$; where k = the number of parameters estimated by the equation (F. C. Mills, "Statistical Methods," 3rd ed., Henry Holt and Co., New York, N. Y., 1955, p 626); (b) n = the number of derivatives; r = the correlation coefficient; r^2 = the coefficient of determination ("explained variance"); s = the standard error of estimate; and p = the significance level determined on the basis of the *F*-test.

In eq 2 the coefficient of σ is the reaction constant, ρ, and the constant for the equation is the calculated value of log k_2 for benzoylcholine. Values of σ used in the regression analysis were taken from the compilation of Jaffé.[3]

From the sign and magnitude of ρ in eq 2 it is evident that electron-withdrawing substituents facilitate the alkaline hydrolysis of *meta* and *para* substituted benzoylcholine derivatives (*i.e.*, electron withdrawal from the ester CO increases the ease of nucleophilic attack). This result is in agreement with many similar analyses appearing in the literature regarding substituent effects in the alkaline hydrolysis of benzoate esters[3] and is consistent with the hypothesis of a rate-limiting attack by OH^- on the ester CO.[15] The value of $\rho = 1.540$ which we observe is lower than values of ρ for benzoate esters ($\rho > 2$) obtained in less polar solvents,[3,6b] but the decrease is expected when effects attributable to dielectric constant and temperature are considered. Hammett[16] has suggested that the reaction constant is inversely related to both the dielectric constant and temperature; and in general, this view is supported by a decrease in the observed reaction constant for benzoate esters as the polarity of the solvent and the reaction temperature increase. For example, the alkaline hydrolysis of ethyl benzoates at 25° in 85% EtOH has a $\rho = 2.537$, although in 75% MeOH $\rho = 2.193$.[3] Similarly, the alkaline hydrolysis of methyl benzoates at 0° in 60% acetone has a $\rho = 2.460$, but at 50° in the same solvent $\rho = 1.980$.[3] More recently, Chapman, *et. al.*,[17] have attempted to quantitate the solvent effect on reaction rate using various solvent parameters. For the reactions of *meta* and *para* substituted benzoic acids and phenylacetic acids with diazodiphenylmethane in eight different alcohols, ρ values were satisfactorily correlated with a linear combination of $(D - 1)/(2D + 1)$ and σ^* where D is the dielectric constant and σ^* is the Taft polar constant for aliphatic substituents.[6a] These workers attributed the negative effect of a high dielectric to the decreased transmission of polar effects through the medium and to a decrease in the energy required to effect charge separation in the transition state. Both of these solvent effects can lead to a reduction in substituent effects.

The aqueous medium used in the hydrolysis studies of benzoylcholine esters would further increase the dielectric effect of solvent to decrease ρ, and therefore a value of $\rho = 1.540$ is not unreasonable. The absolute magnitude of this decrease is of the order expected on the basis of other reactions conducted in both aqueous and organic solvents. For the ionization of benzoic acids at 25°, the reaction constants in water and ethanol are 1.000 and 1.957, respectively.[3] A similar difference exists for the ionization of anilinium ions.

The potential influence of the charged choline moiety on the reaction is also of interest since the formal charge could affect the susceptibility of attack by OH^- as well as affecting the susceptibility of the reaction center to ring-substituent effects. Evidence against the former of these potential effects has recently been obtained for the hydrolysis of a series of α substituted *o*-nitrophenyl esters in aqueous solution, including both charged and uncharged α substituents.[10b] On the basis of a linear correlation between log k_0 for H_2O catalysis and log k_2 for OH^- catalysis, it was concluded that a formal charge–charge attraction or repulsion is unimportant in the nucleophilic attack by OH^- on the ester CO. It would also appear that the charged choline moiety would have a negligible effect on the transmission of ring substituent effects, although evidence for this has not been presented.

ortho **Substituents.**—Substituent effects on the hydrolysis rates of the *ortho* derivatives were analyzed initially using the equation proposed by Taft.[6c]

$$\log k_{2-x} = \rho^*\sigma_0^* + \delta E_s^0 + \log k_{2-CH_3} \quad (3)$$

where σ_0^* is the Taft polar substituent constant and E_s^0 is the Taft steric constant, both for *ortho* substituted benzoates. ρ^* and δ are the reaction constants associated with σ_0^* and E_s^0, respectively. Equation 3 implies that the total substituent effect on the rate of hydrolysis of *ortho* substituted benzoates is accounted for by a linear combination of polar and steric energy terms. In testing the applicability of this model to the present set of *ortho* derivatives, the following set of equations were obtained by stepwise regression analysis.[18]

	n	r	r^2	s	p	
$\log k_{2-x} = -0.321 E_s^0 + 0.585$	6	0	0			(4)
$\log k_{2-x} = 1.020 \sigma_0^* + 0.212$	6	0.749	0.561	0.382	0.100	(5)
$\log k_{2-x} = 1.969\sigma_0^* + 0.815 E_s^0 - 0.214$	6	0.934	0.872	0.283	0.025	(6)
$\log k_{2-x} = 1.948\sigma_0^* + 0.828 E_s^0 - 0.298$	4	0.996	0.993	0.092	0.050	(7)

The coefficients of the substituent constants in eq 4–7 are the respective reaction constants, and the constant for the equations is the calculated value of log k_2 for *o*-toluoylcholine, the standard for this series. Values of the substituent constants used in this analysis were those given by Taft.[6d,e] From the statistical values for eq 4 and 5 it is clear that substituent effects in the *ortho* position cannot be accounted for solely by σ_0^* or E_s^0. The results of eq 6 and 7, on the other hand, strongly suggest the correctness of the assumption that both σ_0^* and E_s^0 are required in explaining the total substituent effect on the rate. On the basis of the F-test, ρ^* and δ are significant at the 0.025 and 0.050 levels, respectively.

The physical significance of the results obtained with eq 6 and 7 is obscured in light of recent evidence produced by Charton.[7a–c] Using multiple regression techniques Charton has shown that: (a′) the Taft E_s^0 values for *ortho* substituents are independent of van der Waals radii and are primarily a measure of resonance effects; (b′) the Taft σ_0^* constants are not a measure of inherent polar inductive effects but instead

(15) M. L. Bender, R. D. Ginger, and J. P. Unik, *J. Amer. Chem. Soc.*, **80**, 1044 (1958).

(16) L. P. Hammett, *ibid.*, **59**, 96 (1937).

(17) N. B. Chapman, J. R. Lee, and J. Shorter, *J. Chem. Soc. B*, 769 (1969).

(18) (a) *o*-Cl, *o*-Br, *o*-F, *o*-CH_3, *o*-OCH_3, and *o*-NO_2 for the $n = 6$ set; *o*-F, *o*-CH_3, *o*-OCH_3, and *o*-NO_2 for the $n = 4$ set. H was excluded from these series since the benzene ring and the carboxyl group are coplanar in benzoic acid [M. Charton and B. I. Charton, *J. Org. Chem.*, **33**, 3872 (1968)]. In most *ortho*-substituted benzoic acids the plane of the carboxyl group makes an angle greater than zero with the plane of the benzene ring. The inclusion of H in the regression analysis of the present *ortho* series consistently produced lower correlations; (b) r and r^2 for eq 6 and 7 are the multiple correlation coefficient and multiple coefficient of determination, respectively. See reference 13b for multiple regression techniques.

are a mixture of the electrical substituent effects produced in acidic and basic hydrolysis; (c′) the substituent effect in the alkaline hydrolysis of *ortho* substituted benzoates can be accounted for solely by the intrinsic general inductive effect of the substituents.

Rate data for the *ortho* substituted benzoylcholine esters was further analyzed in light of the conclusions, a′–c′, above. The stepwise correlation of log k_2 for the *ortho* derivatives with σ_I[19a,b] and σ_R[19c] results in the following equations.[20]

	n	r	r^2	s	p	
$\log k_{2-x} = 2.088\,\sigma_I - 0.358$	6	0.982	0.964	0.109	0.001	(8)
$\log k_{2-x} = 2.030\,\sigma_I - 0.369$	4	0.985	0.969	0.133	0.025	(9)
$\log k_{2-x} = 2.082\sigma_I + 0.034\,\sigma_R - 0.351$	6	0.976	0.952	0.145	0.005	(10)

The results of eq 8 and 9 indicate that σ_I adequately accounts for the total substituent effect on the rate. Addition of σ_R to the equation does not improve the correlation (F-ratio for the coefficient of $\sigma_R = 0.025$). It is noted that in this series of equations the $n = 4$ and $n = 6$ sets produce nearly identical r values in contrast to eq 6 and 7 given previously. Since Charton[19b] has shown σ_I values to be independent of steric effects, the results of eq 8 and 9 further indicate that the substituent effect on alkaline hydrolysis rates of *ortho*-substituted benzoylcholine esters is due primarily to a field effect of the substituent.

A discussion of the variable solvent effect on ρ may be introduced to potentially explain the larger ρ value of 2.088 obtained for the *ortho* series compared with the ρ value of 1.540 obtained for the *meta* and *para* series. It has been shown that the reaction constant obtained for correlation of σ_I with log K_x for the ionization of *ortho* substituted benzoic acids is independent of solvent effects.[18a] Similar but less convincing evidence has been presented for the alkaline hydrolysis of *ortho* substituted benzoates.[7c] In contrast, for the ionization of *meta* and *para* substituted benzoates the reaction constant decreases as solvent polarity increases.[18a] This negative effect of solvent, discussed previously in relation to the ρ value of the *meta* and *para* derivatives, would appear to be nonexistent in the *ortho* derivatives; and therefore a larger value of ρ would be expected.

The success in correlating the log k_2 values for the *ortho* derivatives with σ_I or with a linear combination of σ_0^* and E_s^0 suggests a general relationship between these sets of substituent constants. From the equations of Taft and Charton it can be shown that σ_I should be a linear function of σ_0^* and E_s^0 (see Appendix). The regression equations for this relationship are given as follows.[21]

	n	r	r^2	s	p	
$\sigma_I = 0.950\sigma_0^* + 0.411\,E_s^0 + 0.064$	6	0.948	0.899	0.104	0.025	(11)
$\sigma_I = 0.904\sigma_0^* + 0.385\,E_s^0 + 0.101$	8	0.933	0.870	0.089	0.005	(12)

Equations 11 and 12 demonstrate the high correlation which exists between σ_I and a linear combination of σ_0^* and E_s^0. The coefficients of σ_0^* and E_s^0 in eq 12 are significant at the 0.005 and 0.010 levels, respectively. These results explain the apparent equivalency of the two approaches used in correlating the rate data of the *ortho* derivatives. It is further noted that the substitution of σ_I, defined by eq 11, into eq 9 results in $\log k_2 = 1.929\,\sigma_0^* + 0.834\,E_s^0 - 0.305$ which is almost identical with eq 7.

It is necessary now to comment on the physical significance of interpretations based on σ_0^* and E_s^0 *vs.* those based on σ_I. From eq 6 and 7 it could be argued that separate polar and steric effects must be invoked to explain the total substituent effect on the rate. The evidence of Charton[7a–c] and the discussion given here however indicate that such an interpretation is not warranted. Thus, E_s^0 is shown to be primarily related to resonance effects by the following equations.[21]

	n	r	r^2	s	p	
$E_s^0 = -2.439\,\sigma_R - 0.128$	8	0.973	0.948	0.143	0.001	(13)
$E_s^0 = -2.356\,\sigma_R - 0.384\sigma_I + 0.043$	8	0.981	0.963	0.132	0.001	(14)

The coefficient of σ_I in eq 14 is significant only at the 0.250 level. It is evident that effects attributed to E_s^0 are adequately accounted for by σ_R. Steric effects at a maximum contribution could only account for 5.2% of the variance in this data.

Interpretations based on σ_0^* as an intrinsic *ortho* polar effect are equally questionable. This can be seen from eq 6′ in the Appendix where σ_0^* is expressed as a term reflecting the difference between the inductive effect of the substituent in alkaline hydrolysis and the resonance effect of the substituent in acidic hydrolysis. In view of the above objections regarding σ_0^* and E_s^0, correlation of the rate data for the *ortho* substituted benzoylcholine esters is most legitimately interpreted on the basis of σ_I.

Acknowledgment.—The authors wish to thank the following individuals for their generous help in running program BMDO2R: Dr. R. M. Elashoff, Mr. P. Johnson, Mr. M. Butterworth, and Mr. F. Krikorian of the University of California Computer Center staff, San Francisco; Dr. S. F. Zimmerman, V. A. Hospital, Coatesville, Pa. We also wish to acknowledge the helpful suggestions of Dr. A. Cammarata, Temple University School of Pharmacy, Philadelphia, Pa.

Appendix

Taft[6f,g] has defined σ_0^* and E_s^0 in the following way.

$$\sigma_0^* = (1/2.48)[\log (k_x/k_{CH_3})_B - \log (k_x/k_{CH_3})_A] \quad (1')$$

$$E_s^0 = \log (k_x/k_{CH_3})_A \quad (2')$$

where the log terms refer to the relative rates of alkaline (B) and acidic (A) hydrolysis and where the constant, 2.48, can be represented by

$$2.48 = \rho_B^* - \rho_A^* \quad (3')$$

(19) (a) σ_I is the inductive constant given by Taft for aliphatic series [R. W. Taft, Jr., *J. Phys. Chem.*, **64**, 1806 (1960); M. Charton, *J. Org. Chem.*, **28**, 3121 (1963)]; (b) Charton has shown that the σ_I constants may be related through a simple linear equation to the pK_a of substituted acetic acids in water and are independent of steric effects [M. Charton, *ibid.*, **29**, 1222 (1964)]. The equation for this relationship is $\sigma_{I,x} = m(pK_{a,x}) + c$ where m and c are the least squares slope and intercept, respectively; (c) σ_R is the resonance substituent constant calculated from the relationship $\sigma_R = \sigma_p - \sigma_I$ where σ_p is the Hammett constant for *para* substituents [R. W. Taft, Jr., and I. C. Lewis, *J. Amer. Chem. Soc.*, **80**, 2346 (1958)].

(20) The $n = 4$ and $n = 6$ sets refer to the same substituents given in footnote 18a.

(21) The $n = 6$ set refers to the same substituents given in footnote 18a. *o*-OC_2H_5 and *o*-I were added to the above for the $n = 8$ set.

The linear free energy relationships applicable to the relative rate terms in eq 1′ and 2′ have been determined by Charton[7b,c] to be

$$\log (k_x/k_{CH_3})_A = \rho_A\sigma_R \quad (4')$$

$$\log (k_x/k_{CH_3})_B = \rho_B\sigma_I \quad (5')$$

Upon substituting eq 3′, 4′, and 5′ into eq 1′, the value of σ_0* becomes

$$\sigma_0^* = [1/(\rho_B^* - \rho_A^*)](\rho_B\sigma_I - \rho_A\sigma_R) \quad (6')$$

Rearrangement of eq 1′ yields

$$\log (k_x/k_{CH_3})_B = 2.48\sigma_0^* + \log (k_x/k_{CH_3})_A \quad (7')$$

Further, the linear steric energy relationship applicable to eq 2′ has been given by Taft[6h] as

$$\log (k_x/k_{CH_3})_A = \delta E_s^0 \quad (8')$$

Substituting eq 3′, 5′, and 8′ into eq 7′ yields

$$\rho_B\sigma_I = (\rho_B^* - \rho_A^*)\sigma_0^* + \delta E_s^0 \quad (9')$$

Therefore

$$\sigma_I = [(\rho_B^* - \rho_A^*)/\rho_B]\sigma_0^* + (\delta/\rho_B)E_s^0 \quad (10')$$

The Metabolism of Pyrovalerone Hydrochloride

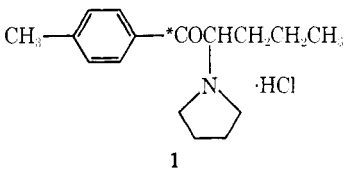
W. MICHAELIS, J. H. RUSSEL, AND O. SCHINDLER

Research Institute, Dr. A. Wander S.A., Berne, Switzerland

Received December 3, 1968

The absorption, distribution, and excretion of ^{14}C-labeled pyrovalerone hydrochloride (1,4′-methyl-2-(1-pyrrolidinyl)valerophenone hydrochloride) were investigated after both oral and intravenous administration of a single dose of 20 mg/kg and 10 mg/kg, respectively, to the mouse. After oral administration, the substance was rapidly and completely absorbed and after both intravenous and oral administration, the radioactivity was excreted rapidly in the urine. Regardless of the mode of administration, within 24 hr over 90% reappeared in the urine whereas less than 10% was detected in the feces. The radioactivity found in the body was concentrated in the liver, bile, and kidneys. The brain contained only traces of radioactivity; this consisting of unchanged pyrovalerone. An examination was also made of human, rabbit, and mouse urine after administration of single doses of 60 mg for the human, 40 mg/kg po. for the rabbit, and 10 mg/kg iv. for the mouse. The substance was excreted very rapidly by all three species and mainly as metabolite **8**. In no instance could unchanged pyrovalerone be detected.

Pyrovalerone hydrochloride (**1**)[1] is a psychostimulant synthesized by Heffe.[2] Both in pharmacological[3] and in clinical[4] experiments, it differs markedly from amphetamine.



1

An account is given of the absorption, distribution, and excretion of pyrovalerone·HCl (**1**) in the mouse after both oral and intravenous administration of a single dose of the ^{14}C-labeled substance (*C = ^{14}C). Attempts to elucidate the chemical nature of the radioactivity detected in the brain and experiments to identify pyrovalerone and its metabolites in the urine of mice, rabbits, and humans are also described.

Results and Discussion

Absorption, Distribution, and Excretion in the Mouse. —In a preliminary trial with mice housed in a closed metabolite cage, no $^{14}CO_2$ was expired during 24 hr. The results[5] presented were obtained from animals which, during the experiment, were housed in open metabolite cages.

Using the methods described in the experimental section, the excretion curves shown in Figure 1 were obtained for urine and feces. Figure 2 is a graphical presentation of the distribution of activity in the gastrointestinal tract.

After oral administration, pyrovalerone·HCl was absorbed rapidly. Thirty minutes after administration, only 29% of the dose remained in the gastrointestinal tract. Radioactivity was excreted in the urine rapidly, and, only 4 hr after administration, 70% had been excreted by this route. In all, over 90% of the administered radioactivity was excreted in the urine and 6–8% in the feces. The fractions of radioactivity in the stomach and intestine correspond with the excretion patterns (see Figure 2).

After intravenous administration, the onset of excretion of radioactivity in the urine was even more rapid. Radioactivity was detected in the urine only 5 min after injection and 15 min after administration 20% of the dose had already been excreted by this route. From Figure 1 it is evident that the pattern of excretion in the urine and feces is about the same with both methods of administration. This confirms that pyrovalerone·HCl is absorbed rapidly and completely after oral administration.

The concentrations in the most important organs further confirm the rapid absorption. After oral administration (Table I), all investigated organs except the bile attained their highest concentrations during the

(1) 4′-Methyl-2-(1-pyrrolidinyl)valerophenone hydrochloride (F-1983) (Dr. A. Wander S.A., 3001 Berne, Switzerland).

(2) W. Heffe, *Helv. Chim. Acta*, **47**, 1289 (1964).

(3) G. Stille, H. Ackermann, E. Eichenberger, and H. Lauener, *Arzneim. Forsch.*, **13**, 871 (1963).

(4) (a) H. Heimann and K. Vetter, *Schweiz. Med. Wochenschr.*, **95**, 306 (1965); (b) A. R. Holliday, R. B. Morris, and R. P. Sharpley, *Psychopharmacologia*, **6**, 192 (1964); H. Heimann and G. Lukacs, *ibid.*, **8**, 79 (1965).

(5) The concentrations of radioactivity in the organs are presented as micrograms of radioactive substance (calculated as unchanged pyrovalerone·HCl) per gram of fresh tissue. The listed fractions of radioactivity (as a percentage of the dose) and the concentrations represent the average of the single pools of both animals. The points on the excretion curve (Figure 1) for the times listed are the arithmetic averages of all estimations.