Reactions were carried out with a concentration of 0.734 *M* D-glucal triacetate in the solvents and appropriate amounts of IBD.

Product Isolation.—A mixture of 1.0 g of D-glucal triacetate, 1.21 g of IBD, and 50 ml of carbon tetrachloride was degassed by a freeze–thaw method, and nitrogen gas dried with concentrated sulfuric acid was introduced. The ampoule was sealed and placed in a thermostated bath adjusted at 30 ± 0.1°. When the mixture was irradiated by a 200-W incandescent light bulb, the reaction proceeded very quickly. After 2 min of irradiation, the ampoule was opened and the reaction mixture was washed with sodium thiosulfate and water, dried, and evaporated. The residue was fractionated by repeated preparative tlc on silica gel using benzene–ether (1:1) as the developer, and 543 mg (43.1%) of **5**, mp 63–63.5°, 81 mg (6.4%) of **2**, mp 100–101.5°, and 140 mg (11.1%) of **4**, mp 120–121.5°, were obtained. These compounds were found to be identical with the authentic samples.

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Steric Parameters in Structure–Activity Correlations. Cholinesterase Inhibitors[1]

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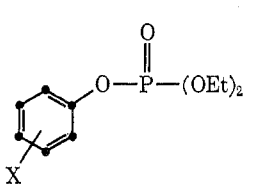
Newly formulated E_s constants based on Charton's work showing a direct relationship between the steric parameter E_s and van der Waals radii are used to help correlate the chemical structure of diethyl phenylphosphates with their cholinesterase-inhibitory potency.

We have been interested in the correlation of chemical structure and reactivity of organic compounds with enzymes and pharmacological systems.[2,3] The approach employed is that now termed extrathermodynamic[4] in which the linear combination of free energy based parameters is used to correlate structure with activity. A generally useful model for enzymic reactions[5] is shown in eq 1. In some instances higher order equations should be considered.[6] In eq 1, C represents molar concentration of organic compound causing a standard response (in the present work 50% inhibition of cholinesterase activity). Alternatively, a rate or equilibrium constant, K, may be used. The constants k_1, k_2, k_3, and k_4 are obtained by the method of least squares. The Hammett constant σ or its variations[4] may be used to represent electronic effects of substituents on log $1/C$ or K. The hydrophobic parameter,[7] π, represents the free energy of transfer of a substituent from an aqueous to an apolar phase and E_s is Taft's steric parameter.[4] The present analysis is directed toward the application of eq 1 and its simpler forms to cholinesterase inhibitors of structure I. The activity data

$$\log 1/C(K) = k_1\pi + k_2\sigma + k_3E_s + k_4 \qquad (1)$$



I

($1/C$) come from the extensive studies of Metcalf and Fukuto on the inhibition of fly head cholinesterase by phosphorous esters[8,9] and carbamates.[10]

Metcalf and Fukuto showed that the inhibitory activity of the esters (I) was strongly related to the electron-withdrawing effect of the substituents (X). In an attempt to sharpen their correlation it was found[11] that there was a very good correlation between the *para* isomers and the Hammett constant, but that the *meta* isomers gave an extremely poor correlation. Metcalf and Fukuto[12] have advanced evidence to show that the *meta* substituents appear to fit into a specific enzymic site. The geometry of the substituent could of course be crucial in such a fit. At the time of our first analysis of the *meta*-substituted phenylphosphates only a limited set of E_s values for substituents on the benzene ring were available. While the steric parameter E_s was formulated for intramolecular interactions, we have found that this parameter can be employed when intermolecular interactions may be involved.[5,11,13,14]

Recently, Charton[15] has shown that E_s can be quantitatively related to van der Waals radii for symmetrical-top-like substituents. He has calculated the maximum van der Waals radii, $r_{V\,max}$, and the minimum, $r_{V\,min}$, for groups such as methyl, trifluoromethyl, etc. We have found that using $r_{V\,av}$ (an average of $r_{V\,max}$ and $r_{V\,min}$), E_s values calculated from van der Waals radii can be placed on the same scale as Taft's values. Equation 2 can be used for this purpose. Equation 2 was established[13] by the correlation of six (n = number of data points employed in the regression) symmetrical-top substituents of known E_s values with $r_{V\,av}$ values taken from the work of Charton.[15] Using eq 2, E_s values formerly not available for the halogens, NO_2, SF_5, etc., can be calculated from $r_{V\,av}$ values. Thus we are now

	n	r	s	
$E_s = -1.839r_{V\,av} + 3.484$	6	0.996	0.132	(2)

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TABLE I

CHOLINESTERASE INHIBITION BY DIETHYL PHENYLPHOSPHATES

$$X\text{-}C_6H_5OP(OEt)_2$$

Registry no.	X	σ	E_s	—Log I_{50}— Obsd	Calcd[a]	$\lvert\Delta(-\log I_{50})\rvert$
13538-40-4	4-$C(CH_3)_3$	−0.20	1.24	4.00	3.86	0.14
5076-63-1	4-Cl	0.23	1.24	4.52	4.85	0.33
3070-13-1	4-SCH_3	0.21[b]	1.24	4.48	4.80	0.32
16498-00-3	4-COOH	0.73	1.24	6.07	5.99	0.08
6132-17-8	4-SO_2CH_3	1.05	1.24	6.60	6.72	0.12
22955-88-0	4-CHO	1.13	1.24	6.82	6.91	0.09
6132-16-7	4-CN	1.00	1.24	6.89	6.61	0.28
311-45-5	4-NO_2	1.27	1.24	7.59	7.23	0.36
2789-05-1	3-SF_5	0.61	−1.67[c]	7.12	7.33	0.21
13538-32-4	3-OCH_3	0.12	0.69	3.89	3.93	0.04
13538-33-5	3-$C(CH_3)_3$	−0.12	−1.54	6.05	5.58	0.47
4532-06-3	3-NO_2	0.71	−1.28	7.30	7.18	0.12
20611-03-4	3-$\overset{+}{N}(CH_3)_3$	0.88	−1.60	7.52	7.88	0.36

[a] Calculated using eq 8. [b] From F. G. Bordwell and P. J. Boutan, *J. Amer. Chem. Soc.*, **78**, 854 (1956). [c] Calculated using eq 2 with values of $r_{V_{max}}$ 3.03 and $r_{V_{min}}$ 2.57 calculated by M. Charton, private communication.

able to explore steric effects of the *meta* substituents of I. From the data in Table I we have derived eq 3–5,

$$-\log I_{50} = (2.685 \pm 4.4)\sigma + 5.184 \pm 2.6 \quad n = 5,\ r = 0.743,\ s = 1.159 \quad (3)$$

$$-\log I_{50} = (-1.366 \pm 1.4)E_s + 4.900 \pm 1.6 \quad n = 5,\ r = 0.911,\ s = 0.714 \quad (4)$$

$$-\log I_{50} = (-1.090 \pm 0.59)E_s + (1.576 \pm 1.4)\sigma + 4.499 \pm 0.83 \quad n = 5,\ r = 0.993,\ s = 0.248 \quad (5)$$

correlating the *meta* isomers. It is interesting to note that in the case of the *meta* isomers a much better correlation is obtained using E_s (eq 4) than using eq 3. The *meta* isomers are therefore quite different in their mode of inhibition. The linear combination of E_s and σ yields a much improved correlation (eq 5). An F test indicates eq 5 to be statistically quite a significant improvement over eq 4; $F_{1,2} = 22.9$.

The *para* isomers alone yield eq 6. If the normal

$$-\log I_{50} = (2.490 \pm 0.44)\sigma^- + 4.184 \pm 0.37 \quad n = 8,\ r = 0.985,\ s = 0.254 \quad (6)$$

Hammett σ values are used in eq 6 instead of σ^- values, an extremely poor correlation ($r = 0.479$) results.

Combining both *meta* and *para* derivatives, eq 7 and 8

$$-\log I_{50} = (-0.556 \pm 0.20)E_s + (2.452 \pm 0.54)\sigma^- + 4.818 \pm 0.41 \quad n = 13,\ r = 0.962,\ s = 0.408 \quad (7)$$

$$-\log I_{50} = (-0.966 \pm 0.36)E_s + (2.287 \pm 0.44)\sigma^- - (1.201 \pm 0.96)X + 5.519 \quad n = 13,\ r = 0.980,\ s = 0.313 \quad (8)$$

are obtained. In eq 7 and 8, σ_m and σ_m^- are presumed to have the same value. While eq 7 gives a good correlation, eq 8 gives a significantly better result ($F_{1,9} = 8.1$). In eq 8, X is a dummy parameter used to account for the basic stereoelectronic difference in inhibitory mechanism between *meta* and *para* isomers. Since *meta* isomers are given the arbitrary value of 1 and *para* isomers the value of 0, the negative coefficient with X indicates that, steric and electronic factors being equal, the *meta* isomers are less effective. In addition to the F test, this dummy parameter is justified by the fact that the coefficient with E_s in eq 8 is close to that of eq 5, while the coefficient with E_s in eq 7 is considerably different from that of eq 5.

The addition of the hydrophobic constant, π, to the above equations does not result in an improvement in correlation. This would indicate that substituents in the *meta* and *para* positions do not bind hydrophobically to the enzyme; that is, they appear not to be desolvated in the inhibitory process.

The coefficient with σ in eq 5 and 8 can be compared with the value of 1.907 obtained by Aldridge and Davison[16] for the hydrolysis of four (4-Cl, 4-NO_2, 3-NO_2, H) diethyl phenylphosphates in buffer (pH 7.6) at 37°. The value for ρ in eq 8 and that of 1.91 are well within the 95% confidence intervals. This indicates the close parallelism between ease of hydrolysis and anticholinesterase activity of the phosphate esters. The value of ρ, measured from experiments using sheep erythrocytes as a source for cholinesterase, was 4.08 ± 0.74. The activity of cholinesterase in the red cells appears to be different from the source (homogenized fly heads) used by Fukuto and Metcalf. Equation 8 confirms the hypothesis of Metcalf and Fukuto[12] that the *meta* substituent fits into an enzymic pocket. The quality of this fit would appear to determine the positioning of the phosphate ester for cleavage by the enzyme.

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