and this reaction was heated on a steam bath for 6 h. It was then diluted with water and the solid precipitate was filtered. Crystallization from ethanol gave 14.2 g (77%) of **17**: mp 240–242 °C; MS, *m/e* 232 (M^+, 100); NMR (Me_2SO) δ 2.61 (s, 3 H), 3.54 (4 H), 7.29 (d, 1 H), 8.30 (d, 1 H).

9-Methyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3′,2′:4,5]-thieno[2,3-*c*]-1,2,3-triazine (18). To an ice-cold suspension of 6.6 g (0.028 mol) of **17** in 100 mL of 5% hydrochloric acid was slowly added a solution of 0.94 g (0.028 mol) of sodium nitrite in 30 mL of water. After completion of addition, the ice bath was removed and stirring continued for 45 min. It was then neutralized with saturated $NaHCO_3$ and cooled in a freezer. The solid that separated was collected, washed with water, and dried. It was dissolved in chloroform, treated with charcoal, and filtered. Upon concentration of the solution, 1.9 g (28%) of **18** was obtained: mp 216–218 °C; MS, *m/e* 243 (M^+), 200 (100); NMR ($CDCl_3$) δ 2.72 (s, 3 H), 4.31 (m, 4 H), 7.32 (d, 1 H), 8.43 (d, 1 H).

Inhibition of Histamine Release from Passively Sensitized Rat Mast Cells (RMC).[15] RMC were passively sensitized in vitro with rat antiovalbumin serum. Spontaneous histamine release (SR in the absence of antigen) and AIR (in the presence of antigen) from these passively sensitized RMC were measured after 15 min of incubation. Both the histamine released into the incubation medium and the residual histamine extracted from the RMC were measured fluorometrically with a Technicon AutoAnalyzer. Both SR and AIR are expressed as a percent of the total extractable histamine in the RMC. Net AIR was obtained by subtracting the SR from histamine released in the presence of antigen. The effect of the test compound on both SR and AIR was determined.

Test compounds were added simultaneously with antigen. The activity of the test compound is expressed as a percent inhibition of AIR or as the I_{50} value (concentration of the test compound required to inhibit AIR by 50%). Test compounds were dissolved in Me_2SO (final concentration of Me_2SO was 0.17% and did not affect AIR).

Passive Cutaneous Anaphylaxis in the Rat (PCA).[16] The effect of compounds on IgE-mediated cutaneous wheal formation in the rat was determined by a modification of the method of Watanabe and Ovary (1977).[16] Antiserum for these studies was prepared according to the following immunization protocol. Male Sprague–Dawley rats (approximately 250 g) were injected intramuscularly on days 0, 2, and 4 with 10 μg of ovalbumin and 20 mg of aluminum hydroxide (Amphojel) in 1 mL of saline. On day 0 each rat was given 10^9 *Bordatella pertussis* organisms by the intraperitoneal route. Rats were exsanguinated on day 8.

The method of passive cutaneous anaphylaxis was a follows. Naive rats were sensitized at dorsal sites by intradermal injection of the syngeneic IgE antiovalbumin antiserum (1:20 dilution). After a latency period of 48 h to allow cytophilic antibodies to bind to the cutaneous mast cells, groups of four rats were given either vehicle (1% methylcellulose, 3 mL) or graded doses of compound. Rats were challenged intravenously with antigen (4 mg of ovalbumin) in 1% Evans blue dye 10 min after oral administration of the test compound. Thirty minutes after antigen challenge, the rats were sacrificed by cervical dislocation, the dorsal skins reflected, and blued wheal areas measured. Mean values ± SD for wheal areas in control and drug-treated groups were determined and compared statistically by using the Student's *t* test.

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Registry No. **2**, 3395-04-8; **3**, 54585-47-6; **4**, 92126-72-2; **5**, 59488-61-8; **6**, 67795-42-0; **7**, 76993-15-2; **8**, 76993-12-9; **9**, 76993-13-0; **10**, 76993-14-1; **11**, 52505-52-9; **12**, 92126-73-3; **13**, 88722-19-4; **14**, 52505-53-0; **15**, 76993-16-3; **16** (R = H), 76993-29-8; **17**, 92126-74-4; **18**, 92126-75-5; **19**, 76993-18-5; **20**, 76993-17-4; **21**, 76993-19-6; **22**, 76993-20-9; **23**, 76993-22-1; **24**, 76993-23-2; **25**, 76993-24-3; **26**, 92184-38-8; **27**, 76993-25-4; **28**, 76993-26-5; **29**, 76993-29-8; **30**, 76993-27-6; **31**, 52505-51-8; **32**, 59488-62-9.

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Effects of Volume and Surface Property in Hydrolysis by Acetylcholinesterase. The Trimethyl Site

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β-Substituted ethyl acetates, $XCH_2CH_2OCOCH_3$, have been prepared, and their hydrolysis by acetylcholinesterase has been studied. Log of enzymic reactivity, normalized for intrinsic reactivity in hydrolysis by hydroxide, log $(k_{cat}/K_m)_n$, rises linearly with increasing refraction volume, MR (or R_D^{25}), for substrates with β-X = H, Cl, Br, CH_3CH_2, $(CH_3)_2CH$, $(CH_3)_2S^+$, $(CH_3)_3N^+$, and $(CH_3)_3C$. Larger substituents may be accommodated, $(CH_3)_3Si$ and $(CH_3CH_2)_3N^+$, with no further increase in rate. Substrates with β-substituents CH_3S, $CH_3S(O)$, $(CH_3)_3N^+(OH)$, and $CH_3S(O_2)$ are less reactive than consistent with the relation with MR by factors of 5–40, indicating that hydrophobic surface and desolvation of the substrate–enzyme interface may be necessary for maximum reactivity correlated with MR. Values of log $(k_{cat}/K_m)_n$ for substrates with β-substituents X = CH_3S, Cl, Br, CH_3CH_2, $(CH_3)_2CH$, $(CH_3)_3C$, and $(CH_3)_3Si$ rise linearly with increasing hydrophobicity, π, but reactivity of substrates with X = $(CH_3)_3N^+$ and $(CH_3)_2S^+$ are more reactive than consistent with a relation to π by factors of 300 and 40 and with X = $CH_3S(O_2)$, $CH_3S(O)$, and $(CH_3)_2N^+(OH)$, by factors of 7–100. Reactivity appears related to (i) volume of the β-substituent and its fit in its subsite, which is *trimethyl* rather than *anionic*, and (ii) the hydrophobicity of its surface.

Although the part of the active site of acetylcholinesterase at which the trimethylammonium group of acetylcholine, $(CH_3)_3N^+CH_2CH_2OCOCH_3$, binds has generally been considered and depicted as *anionic*,[1-3] our recent studies indicate that it may not contain a specific negative charge and may be better considered *trimethyl*, as complementary to the trimethyl-substituted character of the β-substituent rather than to its positive charge.[4] This was proposed when the enzymic reactivity of a series of β-substituted ethyl acetates, $XCH_2CH_2OCOCH_3$, with cat-

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Table I. Hydrolysis of $XCH_2CH_2OCOCH_3$ by Acetylcholinesterase, pH 7.8, 0.18 M NaCl, 25 °C: Molar Refractivity, Hydrophobicity, and Kinetic Constants

	β-substituent									
no.	X	MR, cm^3	π	concn, mM	10^9[E], M	$10^{-2}k_{cat}$, s^{-1}	K_m, mM	$10^{-4}(k_{cat}/K_m)$, $M^{-1}\ s^{-1}$	k_{HO^-}, $M^{-1}\ s^{-1}$	$10^{-4}(k_{cat}/K_m)_n$, $M^{-1}\ s^{-1}$
I	$(CH_3)_2S^+$	16.4	−0.50	0.2–1.2	0.6	77	0.33	2300	3.1	2100
IA	$(CH_3)_3N^+$	17.2	−1.24	0.1–0.6	0.1	160	0.33	4800	2.8[a]	
II	$(CH_3)_3Si$	25.0	+2.59	0.1–3.0	0.4	57	3.5	160	0.06	7600
IIA	$(CH_3)_3C$	19.6	+1.98	0.7–5.3	0.2	66	5.3	125	0.07	5000
III	$(CH_3)_2N^+(OH)$	15.0	−3.0	1.5–9.0	0.3	45	18	25	0.82	86
IIIA	$(CH_3)_2CH$	15.0	+1.53	1.0–5.0	1.1	34	3.6	93	0.11	2400
IV	$CH_3S(O_2)$	13.9	−1.9	6–21	0.6	11	6.2	18	1.4[b]	36
IVA	$CH_3S(O)$	14.1	−1.85	7–22	0.5	38	16	24	0.45	150
IVB	O_2N	6.7	−0.85	7–22	1.1			(5)		
V	CH_3S	13.3	+0.45	3–21	0.6	35	15	23	0.28	230
VA	CH_3CH_2	10.3	+1.02	10–17	0.2	36	13	26	0.11[a]	660
VI	Cl	5.93	+0.39	4–40	0.2	34	14	25	0.31[a]	220
VIA	Br	8.80	+0.60	1–12	0.1	45	7.5	59	0.42[a]	400
VII	H	1.03	0	60–170	0.3	29	230.	1.3	0.11[a]	32

[a] Data from ref 4. [b] See Methods section.

ionic, uncharged polar, and nonpolar substituents was satisfactorily accounted for by two properties of the β-substituents: (i) their effects on the intrinsic reactivity as measured by hydrolysis by hydroxide and (ii) apparent molal volumes, $\bar{V}°_{25}$. This is indicated in eq 1, where log

$$\log (k_{2(n)}/K_s) = a\bar{V}°_{25} + C \quad (1)$$

$(k_{2(n)}/K_s)$ is the enzymic reactivity normalized for intrinsic hydrolytic reactivity. The effect of cationic charge in X is to increase intrinsic reactivity by a factor 25–40 as compared with analogous alkyl subsituents and correspondingly less as compared with uncharged polar substituents; substantial effects of the postulated[5] specific anionic site in attracting and orienting cationic substrates are not required.[4]

Successive methylation at N- or C-centered β-substituents was known to increase enzymic reactivity,[6,7] and the effect was correlated[8] with hydrophobicity, π.[9] Effects of uncharged polar β-substituents were related[10–12] to values of π and polar substituent constants, σ* (eq 2).[8,13]

$$\log (k_{cat}/K_m) = C + \rho^*\sigma^* + \phi\pi \quad (2)$$

However, cationic β-substituents, which are particularly relevant, impart high water solubility and low values of Π, and reactivity of such substrates may not be correlated with that of uncharged esters via eq 2, while eq 1 appeared applicable.[4] The enzyme has isoelectric point at ca. pH 5[14,15] and thus excess negative charge at pH 7–8, and evidence for Coulombic interaction effects have been found. However, cationic reversible inhibitors structurally related to acetylcholine bind better than their uncharged analogues by small factors, corresponding to ~1 kcal/mol of binding energy, much less than would be caused by interaction of $(CH_3)_3N^+$ with a "contact" anionic O^- that is implied by a specific anionic site.[16] Also, ionic strength effects on binding and on hydrolysis have been interpreted in terms of anionic charges on the enzyme surface, peripheral to the active site.[17]

Each of the inhibitors that was studied,[16] structurally related to acetylcholine, whether neutral or cationic, showed essentially the same binding constant when retarding hydrolysis of acetylcholine and its uncharged analogue, 3,3-dimethylbutyl acetate. This indicated that the β-trimethylammonio and β-*tert*-butyl groups of the two substrates and of the related inhibitors bound at the same subsite.[16] The substrate study[4] had indicated that this subsite is uncharged *trimethyl*, and this would then be more specifically explored with uncharged reagents. Indeed, arylaziridinium reagents, intended to alkylate the "anionic" site and prevent substrate access, inhibited completely hydrolysis only of cationic but not of neutral esters.[18,19] We took this to indicate not that there are separate anionic and neutral subsites but that cationic agents act at peripheral anionic groups, thus increasing positive charge and repelling cationic substrates and inhibitors; they modify the active site domain but allow uncharged substrates to bind at the one *trimethyl* site and react. This view was borne out in studies with 1-bromopinacolone, $(CH_3)_3CCOCH_2Br$, which inhibited AcChE irreversibly with the same rate toward hydrolysis of a variety of both cationic and neutral substrates, apparently via binding at the *trimethyl* site.[20] Further studies are being carried out on this process.

It was of interest to examine the size and surface character of the *trimethyl* site and the range of validity of eq 1. We have studied the enzymic reactivity of new substrates with large β-substituents of varying size, charge of core atom, and character of peripheral atom surface and reexamined some of the previously studied substrates.[4] The work is described below.

Results

Ethyl acetates, $XCH_2CH_2OCOCH_3$, with varied large β-substituents were prepared: X = $(CH_3)_3Si$, for com-

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parison with the smaller C analogue, 3,3-dimethylbutyl acetate; X = $(CH_3)_2S^+$, acetylsulfocholine, with one less methyl, and X = $(CH_3)_2N^+(O^-)$, the tertiary amine oxide with basic (O^-) and one less methyl, for comparison with acetylcholine; X = $CH_3S(O_2)$, $CH_3S(O)$, O_2N, and CH_3S, for study of effects of dipolar charge and unshared electrons in the peripheral and central atoms of the β-substituent. Rate constants for hydrolysis by hydroxide, k_{HO^-}, and conditions and results of the enzymic study are summarized in Table I. The value of k_{HO^-} for acetylsulfocholine, I, 3.1 M^{-1} s^{-1}, is similar to that found previously for acetylcholine IA, 2.8 M^{-1} s^{-1}. That for the trimethylsilyl substrate, II, 0.06 M^{-1} s^{-1}, is similar to that for the carbon analogue, IIA, 0.07 M^{-1} s^{-1}, which was redetermined in this work and found to be lower than previously reported, 0.11 M^{-1} s^{-1}. In hydrolysis of 2-(methylsulfonyl)ethyl acetate, IV, at pH 10 a value of k_{HO^-} = 2.5 M^{-1} s^{-1} was observed. However, absorption at 280–300 nm developed during the hydrolysis and is attributed to methyl vinyl sulfone,[21] formed by β-elimination, in 43% yield. Correction for this leads to k_{HO^-} = 1.4 M^{-1} s^{-1} for hydrolysis, a value consistent with σ_I = 0.62 for $CH_3S(O_2)$ compared with 0.90 for $(CH_3)_3N^+$.[22] Such absorption was not observed during the enzymic hydrolysis of compound IV at pH 7.8 and also when the hydrolysis product, 2-(methylsulfonyl)ethanol, was kept at pH 10. β-Elimination did not appear to be a problem in the study of the sulfoxy or dimethylsulfonio substrates. 2-Nitroethyl acetate, IVB, reacted very rapidly with hydroxide, and even at pH 6.5 nonenzymic generation of acid appeared slightly greater than the enzymic rate. We estimate an enzymic bimolecular rate constant one-third of that reported;[8] this substrate will not be discussed in detail.

Since values of $\bar{V}°_{25}$ are not available for the newly studied substrates, we obtained two alternate estimates of molar volume, refraction volumes, MR, and van der Waals volumes, V_w. Molar refractivity, MR, has been commonly used in pharmacological structure–reactivity correlations.[23-25] Values were taken from compilations[23,24,26] or calculated as described in the Methods section. van der Waals volumes, V_w, are used less commonly in such reactivity correlations.[27] They are calculated from van der Waals radii and bond radii,[28] and values of the ratios V_w/MR appeared similar for most of the substituents, ~2.3; lower values of this ratio for Br, CH_3S, and Cl may reflect polarizability of outershell electrons. The discussion will be based on refraction volumes, MR.

Values of π for the uncharged substituents and $(CH_3)_3N^+$ were selected from the literature.[9,23,29-31] An estimate of the effect of positive charge is included in the value for $(CH_3)_2S^+$ and of an aromatic–aliphatic difference in $CH_3S(O_2)$. The value of π for $(CH_3)_2S^+$ is 0.75 more positive than that for $(CH_3)_3N^+$, despite one less CH_3 group, just as π for CH_3S is 0.75 more positive than that for $(CH_3)_2N$. This is consistent with the larger size of S and the generally lower water solubility and hydrogen bonding of S compounds than that of analogous N and O compounds. The π values are substantially more negative for $CH_3S(O_2)$ and $CH_3S(O)$ than that for $(CH_3)_2S^+$, despite the net positive charge and water solubility of the latter. Replacement of peripheral CH_3 by hydrophilic O and unit positive charge by the X–O bond strongly decrease hydrophobicity. This effect is particularly large in the amine oxide III. The value π = −3.0 for the hydroxyl form, $(CH_3)_2N^+(OH)$, will be used. Strong hydration[32] may justify use of the hydroxyl form. The value would be even more negative for the oxide structure.

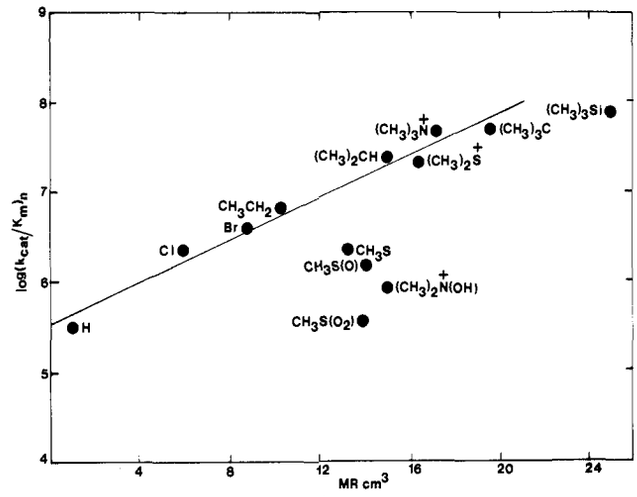

Figure 1. Normalized reactivity of $XCH_2CH_2OCOCH_3$, log $(k_{cat}/K_m)_n$ and molar refractivity, MR_X.

Enzymic kinetic parameters for acetylsulfocholine were similar to those for acetylcholine, with k_{cat} half as great and K_m the same. A factor of 3 in reactivity, with both k_{cat} and k_m being more favorable for acetylcholine, has been reported.[33] Reactivity of the trimethylsilyl substrate II was similar to that of the carbon analogue IIA, with k_{cat} essentially the same and K_m slightly more favorable. The amine oxide III binds substantially less well than the uncharged Si and C compounds II and IIA, leading to low k_{cat}/K_m, 0.5% that of acetylcholine. Relatively high k_{HO^-} leads to low normalized reactivity. This compound has been reported not to be hydrolyzed by acetylcholinesterase.[34] The methylsulfonyl substrate IV also has low enzymic reactivity, in this case due to low k_{cat}, despite three groups attached to the core of the β-substituent and good binding, similar to that of the more reactive uncharged trimethyl substrates II and IIA. Then high k_{HO^-} leads to very low normalized reactivity. The methylsulfoxy and methylthio substrates IVA and V bind less well than the sulfone, and k_{cat} values and normalization factors are more favorable, leading to similar enzymic reactivity to that of the sulfone and higher normalized rectivity than that of the sulfone. Enzymic reactivity of IVA and V is similar to that of *n*-butyl acetate, VA, while the normalization factor of the latter is more favorable. Compounds IIA, IIIA

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and VA and ethyl acetate and its β-Br and B-Cl derivatives were reexamined for this comparison; they show higher reactivity than previously reported.[4]

Discussion

Values of log $(k_{cat}/K_m)_n$ and refraction volumes, MR, are plotted in Figure 1. A linear correlation may be observed between MR and log $(k_{cat}/K_m)_n$ of substrates with β-substituents, H, Cl, Br, CH_3CH_2, $(CH_3)_2CH$, $(CH_3)_3C$, $(CH_3)_3N^+$, and $(CH_3)_2S^+$, slope 0.117 cm^{-3}, intercept 5.53, correlation 0.97. At highest MR, 18–25 cm^3, similar normalized reactivities, $\sim 6 \times 10^7$ M^{-1} s^{-1}, are seen for the substrates with the largest β-substituents, $(CH_3)_3N^+$, $(CH_3)_3C$, and $(CH_3)_3Si$. Increase in reactivity with increase in volume[4] levels off; $(CH_3)_3Si$, significantly larger than $(CH_3)_3N^+$ is accommodated by the *trimethyl* subsite, as is the still larger $(C_2H_5)_3N^+$ substituent, 31 cm^3;[35] however, reactivity appears to approach its maximum with that of the natural substrate IA and may be nearly diffusion controlled.[36] That the normalization applied to the two large uncharged substrates II and IIA leads to essentially the same value as that of the natural substrate lends confidence to the procedure. Acetylsulfocholine, I, shows high reactivity, appropriate to its MR, slightly less reactivity than that of acetylcholine, and the same reactivity as the normalized reactivity of its uncharged analogue of similar MR, isoamyl acetate, IIIA.

The substrates with substituents containing peripheral O, β-$(CH_3)_2N^+(OH)$, β-$CH_3S(O_2)$, and β-$CH_3S(O)$, show lower reactivity than consistent with this relation to MR by factors of 20, 40, and 10 respectively. The β-CH_3S substrate also shows lower reactivity than consistent with MR by a factor of 5. Thus, while volume of the β-substituent, over a range of structures, may set an upper limit to enzymic reactivity, an additional property seems needed if this maximum reactivity is to be achieved. This may be hydrophobicity of surface, present in CH and halogen and absent in peripheral O of compounds III, IV, and IVA. The hydrophilic surface of these substituents, with MR of 14–15 cm^3, may retain substantial water in the active site associated with dipolar X–O bonds [20,32,37]. The similarly sized isopropyl and dimethylsulfonium groups of compounds IIIA and I, with hydrophobic surface and interactions, may exclude water, nearly fully utilize the site, and lead to much higher reactivity, since, although the site may accommodate 25–30 cm^3, full activity results from such groups of MR ≥ 17 cm^3, compounds I, II, and IIA. Solvation by water has been proposed previously to stabilize esters, paradoxically perhaps, even against hydrolysis.[38,39] Normal resonance stabilization of the ester group, RC(O)—OR′ ↔ $RC(O^-)=O^+R$, is enhanced by hydrogen bonding interactions with water, and desolvation must precede attack of the serine hydroxyl on the carbonyl carbon. Similarly, the catalytic esteratic groups of the enzyme may require desolvation. These processes will be furthered by hydrophobic surface interactions between substrate and enzyme and diminished by highly hydrated substrate groups. The hydrophilic surface of the sulfone does not appear to decrease its binding substantially as compared with *tert*-butyl. Effect of surface on binding is being studied with inhibitors and will be described in a later report.

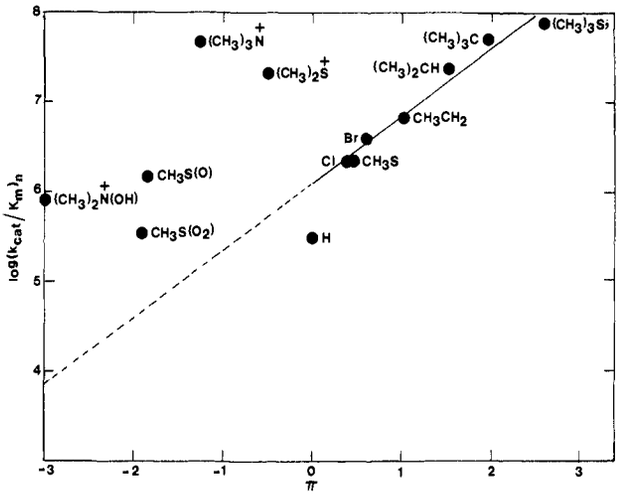

Figure 2. Normalized reactivity of $XCH_2CH_2OCOCH_3$, log $(k_{cat}/K_m)_n$ and hydrophobicity, π_X.

The somewhat low reactivity of the methylthio substrate, in relation to its MR, might not be thought due to hydrophilic surface, but comparison of π values of CH_3S, CH_3CH_2, Br, and Cl (Table I) indicates that S does contribute substantial hydrophilicity. However, surface hydrophobicity and hydrophilicity which we are proposing is to be distinguished from the property measured by π from the water–octanol distribution.[9] Values of log $(k_{cat}/K_m)_n$ and π are plotted in Figure 2. A linear relationship may be observed between log $(k_{cat}/K_m)_n$ and π for the β-substituents CH_3S, Cl, Br, CH_3CH_2, $(CH_3)_2CH$, $(CH_3)_2CH$, $(CH_3)_3C$, and $(CH_3)_3Si$, slope 0.74, intercept 6.13, correlation 0.97; reactivity of ethyl acetate itself appears slightly low. However values of II are strongly affected by water solubility per se, even of compounds with hydrophobic surface, such as the highly reactive tetraalkylammonium and trialkylsulfonium substrates IA and I. Their reactivity is greater by factors of 300 and 40, respectively, than would be consistent with the relationship of uncharged substrates to π. Similarly the amine oxide, sulfone, and sulfoxy substrates with peripheral O in the β-substituents, III, IV and IVA, have reactivity higher than the correlation with π by factors of 100, 7, and 30, respectively.

One might say that normalized reactivity is correlated with hydrophobicity, π, with other factors, i.e., net cationic charge and Coulombic interaction, providing an "antihydrophilicity factor".[40] Other factors would presumably be needed to account for enhancement by hydrophilic X–O bonds. However, the correlation of both cationic and uncharged substrates (Figure 1) makes it appear preferable to propose a primary relation of reactivity to the volume of the β-substituent and its effect on fit, restriction of substrate motion, and displacement of water in the active site and a secondary effect of surface hydrophilicity, decreasing reactivity by retention of water in the active site.

Materials and Methods

2-(Trimethylsilyl)ethyl acetate, $(CH_3)_3SiCH_2CH_2OCOCH_3$, II, was prepared by a reported preocedure[41] from 0.037 mol of

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2-(trimethyl silyl)ethanol (Fluka), 0.037 mol of pyridine, and 0.037 mol of acetyl chloride, ether–water extraction, and distillation at reduced pressure. Starting alcohol and product ester had appropriate NMR spectra.

2-(Methylthio)ethyl acetate, $CH_3SCH_2CH_2OCOCH_3$, V, was prepared by a reported procedure[42] from 45 g of (methylthio)-ethanol (Aldrich) and 50 mL of acetic anhydride heated under reflux in 175 mL of toluene for 1 h. The solution was washed with sodium bicarbonate and salt solutions, dried, and distilled, leading to the product, bp 80 °C (28 mm), with appropriate NMR and IR spectra.

2-(Dimethylsulfonio)methyl acetate bromide, $[(CH_3)_2S^+CH_2CH_2OCOCH_3]Br^-$, "acetylsulfocholine", I, was prepared from 2.0 g of 2-(methylthio)ethyl acetate and a large excess of methyl bromide in 10 mL of dry ether, allowed to stand for 3 weeks. The product was filtered and washed with dry ether: 0.80 g (24% yield), mp 72–74 °C, appropriate NMR spectrum. Anal. ($C_6H_{13}BrO_2S$) C, H. The compound was stored in a freezer under argon. The dimethylsulfonium iodide has been prepared as a hygroscopic oil,[42] and the chloride also appeared difficult to crystallize.[43]

2-(Methylsulfinyl)ethyl Acetate, $CH_3S(O)CH_2CH_2OCOCH_3$, IVA. The β-sulfoxy substrate was prepared by slow addition of 8.1 mL of 30% hydrogen peroxide in 20 mL of acetone to a cold stirred solution of 12.2 g of 2-(methylthio)ethyl acetate in 30 mL of acetone. The solution was allowed to stand overnight, treated with platinum black to destroy excess peroxide, filtered, and concentrated. Water was removed by azeotropic distillation of benzene, 4.3 g of starting ester was recovered, bp 38 °C (1.4 mm), and the sulfoxy product was obtained: bp 100 °C (0.25 mm), 4.3 g (49% yield), appropriate NMR and IR spectra. Anal. ($C_5H_{10}O_3S$) C, H.

2-(Methylsulfonyl)ethyl Acetate, $CH_3S(O_2)CH_2CH_2OCOCH_3$, IV. The β-sulfone substrate was prepared from 5.0 g of 2-(metylsulfonyl)ethanol (Fluka), 10 mL of acetic anhydride, and 2.4 mL of sulfuric acid, heated at 100 °C for 1 h, cooled, treated with ice and water, extracted with chloroform, dried, and distilled: bp 106 °C (0.07 mm), 3.2 g (48% yield), mp 45–48 °C, appropriate NMR and IR spectra. Anal. ($C_5H_{10}O_4S$) C, H.

2-(Dimethylamino)ethyl Acetate *N*-Oxide Hydrochloride, $[(CH_3)_2N^+(OH)CH_2CH_2OCOCH_3]Cl^-$, III. 2-(Dimethylamino)ethyl acetate,[44] bp 95 °C (80 mm), was prepared from (dimethylamino)ethanol and acetic anhydride. A solution of 3.0 g of this in 15 mL of dry benzene was added to 5.0 g of *m*-chloroperbenzoic acid in 50 mL of benzene, and the mixture was allowed to stand for 5 days, then treated with 20 mL of ether saturated with dry HCl, and concentrated in vacuum. The residue was washed with ether and crystallized from 1-butanol–hexane; the crystals were washed with ether and dried: 1.1 g (26% yield), mp 78–79 °C (lit.[34] mp 73–74.5 °C).

2-Nitroethyl acetate, $O_2NCH_2CH_2OCOCH_3$, was prepared from 5.4 g of 2-nitroethanol, 7 mL of acetic anhydride, and 1 drop of sulfuric acid at room temperature: 4.5 g (57% yield), bp 59–60 °C (1.3 mm) [lit.[45] bp 118–119 °C (30 mm)].

Other substrates were available from previous work.[4]

Acetylcholinesterase from electric eel (EC 3.1.1.7), type V-S, lyophilized powder was obtained from Sigma. Acetylcholine iodide was from Aldrich, mp 160–161 °C.

Kinetic studies were carried out as described previously,[4] with slight modification. Enzyme solution was prepared in 0.18 M NaCl instead of 0.05 M phosphate, and titrations were carried out under a slow stream of argon instead of nitrogen. Rate constants for hydrolysis of the substrates by hydroxide, k_{HO^-}, were determined in the pH stat at pH 10. Normalized enzymic reactivity, $(k_{cat}/K_m)_n$, was taken as $(k_{cat}/K_m) \times (k_{HO^-}\text{-acetylcholine}/k_{HO^-}\text{-substrate})$.

Refraction Volume. R_D[25] or MR (ref 23, p 44) is molar volume modified by an index of refraction function $(MW/d)[(n^2-1)/(n^2+2)]$; it reflects polarizability and may be related to attractive dispersion forces. Index of refraction of compounds related to the large β-substituents of this study range from 1.37 for aliphatic hydrocarbon to 1.42 for dimethylsulfone and to 1.48 for dimethyl sulfoxide.

Data for calculation of MR are found in the extensive publications of Vogel and his collaborators,[26] and results of calculations based on atomic and group additive values and on measurements have been tabulated by Hansch and Leo et al.[23,24] Values for uncharged β-substituents, listed in Table II, are taken from ref 23, except for $CH_3S(O)$ and $CH_3S(O_2)$. The listed value for $CH_3S(O)$, 13.7 cm^3,[23] similar to that for CH_3S, 13.3 cm^3,[25] is consistent with S(O) in sulfites, 8.05 cm^3,[26] and CH_3, 5.65 cm^3. However, a value of 8.65 cm^3 for S(O), derived from sulfoxides,[26] leads to 14.3 cm^3, and a value of 0.8 cm^3 for O from sulfoxide–sulfide leads to 14.1 cm^3. The latter will be used. A second O, as in sulfone, appears to decrease MR by 0.21 cm^3 in sulfate–sulfite–sulfide comparisons,[26] leading to 13.9 cm^3 for $CH_3S(O_2)$. Mention is also made of 8.7 cm^3 for O_2 in sulfone, leading to 22 cm^3 for $CH_3S(O_2)$, but this is considered very approximate,[26] and 13.9 cm^3 will be used.

Calculation of refraction volumes of charged substituents was based on data and effects of positive charge in Chapter 10 of ref 46. Values of NH_3 = 5.67 cm^3, $^+NH_4$ = 4.1 cm^3, H = 1.03 cm^3 indicate that positive charge in ammonium compounds decrease MR by 2.6 cm^3. We apply the same contraction to $(CH_3)_2S^+$. Values of 14.14, 5.65, 1.03 and 13.3 cm^3 for $(CH_3)_2N$, CH_3, H, and CH_3S, respectively,[23] and the contraction for charge lead to the values listed in Table I for $(CH_3)_3N^+$ and $(CH_3)_2S^+$. A reported[37] value of MR for trimethylamine oxide, $(CH_3)_3N^+O^-$, 22 cm^3, indicates 16.4 cm^3 for $(CH_3)_2N^+(O^-)$ and, combined with values of 5.1 cm^3 for HO, 3.75 cm^3 for H_2O[46], 15 cm^3 for $(CH_3)_2N^+(OH)$. The values for $(CH_3)_2N$, above, and the values for HO, H, and positive charge lead to 15.6 cm^3 for $(CH_3)_2N^+(O^-)$, 14.2 cm^3 for $(CH_3)_2N^+(OH)$. Values based on the amine oxide will be used.

Hydrophobicity, π. The values of π in aliphatic compounds for the substituents $CH_3S(O)$, Cl, Br, and O_2N are from ref 23 and for CH_3S, from ref 9. A value for aliphatic $CH_3S(O_2)$ is not available; the aromatic value, −1.63, is very similar to that for aromatic $CH_3S(O)$, −1.58, for which the aliphatic value is −1.85. We may estimate that the difference will be similar for $CH_3S(O_2)$, leading to π = −1.9 for aliphatic $CH_3S(O_2)$. Only values in aromatics are listed[23] for the nonpolar substituents, C_2H_5, *i*-C_3H_7, *t*-C_4H_9, and $(CH_3)_3Si$; the difference for CH_3 is small, 0.50 and 0.56 in aliphatic and aromatic compounds, and the aromatic values appear suitable.[30]

Two disparate values of π have been given for $(CH_3)_3N^+$: −5.96,[23] apparently based on 3-phenylpropyl derivatives,[31] and −1.24,[29] based on acetylcholine, which is more relevant. The values for $(CH_3)_3N^+$, −1.24, $(CH_3)_2N$, −0.30,[9] and CH_3 indicate that the cationic charge may decrease π by 1.44. If this may be applied to the sulfur compound, π = +0.45 for CH_3S leads to π = −0.5 for $(CH_3)_2S^+$. The values of π for $(CH_3)_3N^+$, CH_3, and OH lead to π = −3.0 for $(CH_3)_2N^+(OH)$.

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