Steric Parameters in Drug Design. Monoamine Oxidase Inhibitors and Antihistamines[1]

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Taft's E_s parameter is employed to correlate structure–activity relationships in phenoxyethylcyclopropylamine monoamine oxidase inhibitors and diphenhydramine antihistamines. New E_s values for the halogens and certain other functions have been calculated from van der Waals radii using an extension of the approach suggested by Charton.

The usefulness[3] of thermodynamically derived substituent constants for computer-based assaults on biochemical structure–activity problems continues to receive more attention. While considerable experience has accrued in the use of Hammett constants (σ, σ^-, σ^+) from homogeneous organic reactions,[4,5] the use of hydrophobic parameters (log P, π)[3,6,7] has been less thoroughly studied. Still less understood are parameters for steric effects. Taft's E_s parameter[4] and the modified form, E_s^c, suggested by Hancock, *et al.*,[8] although not extensively studied in homogeneous organic reactions, are beginning to prove of use[9] in biochemical systems quite different from that in which they were derived. How far E_s constants and other steric parameters such as Exner's molar volume values (MV) may be of use in medicinal chemical studies remains to be seen. Our initial successes[9] with E_s have prompted this further study.

E_s constants have been defined by Taft using the hydrolysis of aliphatic esters as the model reaction or the hydrolysis of *ortho*-substituted benzoic esters (E_s^o) for *ortho* substituents in aromatic systems. The two groups have been related through the methyl group of value 0.00.

Recently, Charton[10] has reexamined E_s and shown that Taft's observation that E_s parallels group radii can be expressed in quantitative terms. Charton pointed out that for a symmetrical top-type function such as CF_3, one can use either a maximum (r_v(max)) or a minimum (r_v(min)) van der Waals radius to estimate the steric action of the F atoms on neighboring atoms. The value of r_v(min) refers to the junction point of the two F atoms. In his correlations he used r_v(min). We have used an average (r_v(av)) of the two values given by Charton[10] to calculate E_s values for functions not available from Taft's work. This has been done by using the symmetrical functions in Table I for which E_s is known and r_v(av) can be calculated. From these data we have derived eq 1. In eq 1, the figures in

$$E_s = [-1.839\ (\pm 0.22)]r_v(\text{av}) + 3.484\ (\pm 0.55)$$

n	r	s
6	0.996	0.132

(1)

parentheses are the 95% confidence intervals, n is the number of data points employed, r is the correlation coefficient, and s is the standard deviation from regression. Using eq 1, the E_s values listed in Table II have been calculated.

The reason for taking r_v(av) instead of r_v(min) or r_v(max) deserves consideration. If r_v(max) is employed, we obtain a calculated value of E_s for Br of 0.345 and, if r_v(min) is used, we obtain a value of −0.33. From a study of the steric effects of Br, these values seemed too far from the standard value of 0.00 for methyl. There are many instances where Me and Br appear to have about the same steric influence; in fact, even their molar volumes[11] are quite close: Br = 26.19, methyl = 31.48. In Taft's E_s^o constants[12] (from hydrolysis of *o*-benzoates), Br and Me have the same E_s^o value of 0.00. Although Charton[10] has shown that electronic effects are involved, the net effect is that Me and Br behave in a very similar fashion. This similarity can also be seen in the ΔH of the *trans* → *gauche* conformational change[13] of liquid butane (770 ± 90 cal/mol) and liquid 1,2-dibromoethane (730 ± 50 cal/mol). Here again electronic factors are involved, but for our purposes we assume these can be neglected. We have also observed that using r_v(av) with biological data gives better correlations than r_v(max) or r_v(min) in certain examples where we believe the data to be of better than average precision. Of greatest use to us are the values of halogens in Table II which cannot be obtained by Taft's original method.

In the following two case studies, wherever possible, we have used Taft's E_s values obtained from the hydrolysis of aliphatic esters. Where such were not available, we have used the calculated values of Table II.

Monoamine Oxidase Inhibition.—In a very interesting application of the extrathermodynamic approach to a biochemical structure–activity problem, Fuller, *et al.*, correlated the inhibition of two types of monoamine oxidases by N-(phenoxyethyl)cyclopropylamines.[14] From an inspection of the data (Table III) it was apparent to Fuller, *et al.*, that the same substituent in the *meta* and *para* positions showed rather

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TABLE I

CALCULATED AND OBSERVED AVERAGE RADII

Function	r_V(av)	Obsd E_s (Taft)	Calcd E_s (eq 1)	$\lvert\Delta E_s\rvert$
H	1.200	1.24	1.276	0.04
CH_3	1.972	0.00	−0.144	0.14
CF_3	2.425	−1.16	−0.977	0.18
CCl_3	2.994	−2.06	−2.024	0.04
CBr_3	3.215	−2.43	−2.430	0.00
$C(CH_3)_3$	2.792	−1.54	−1.652	0.11

TABLE II

E_s VALUES OBTAINED USING EQUATION 1

Function	E_s	van der Waals radii
CH_3O	0.69	1.52[a]
F	0.78	1.47
Cl	0.27	1.75
Br	0.08	1.85
I	−0.16	1.98
NO_2	−1.28	2.59[b]
NO_2	0.23[e]	1.77[c]
C_6H_5	−2.58[b]	3.30[d]
C_6H_5	0.23[e]	1.77[c]

[a] Calculated using oxygen radius only. [b] Function coplanar to reaction center. The value of 2.59 is taken from the work of Charton.[10] [c] Half-thickness of C_6H_6 used.[10] [d] Estimated from Bondi values: A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964). [e] Function perpendicular to reaction center.

large differences in activity. They attributed this detrimental influence of *meta* substitution on inhibitory activity to be due to steric effects. They chose to compensate for this by assigning an arbitrary steric parameter, γ, three different values: 1.3 for a single *meta* substituent, 1.0 for a *meta* and *para* substituent, and 2.0 for 3,5 substitution. With these assumptions we have formulated eq 2 from their data. Equation 3

$$pI_{50} = [0.923\ (\pm 0.27)]\gamma + [1.585\ (\pm 0.52)]\sigma + [0.285\ (\pm 0.29)]\pi + 5.924\ (\pm 0.32)$$

$$n \quad r \quad s$$
$$18 \quad 0.940 \quad 0.342 \quad (2)$$

is comparable to eq 2 in every way except that E_s has been used instead of γ. The correlation with eq 3 is

$$pI_{50} = [0.702\ (\pm 0.20)]E_s + [1.640\ (\pm 0.50)]\sigma + [0.198\ (\pm 0.27)]\pi + 4.153\ (\pm 0.42)$$

$$n \quad r \quad s$$
$$18 \quad 0.945 \quad 0.330 \quad (3)$$

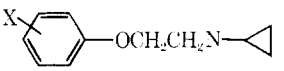
very slightly better. It is of course a satisfaction that the theoretically derived E_s constants give as good a correlation as the three strictly empirical γ values chosen for the purpose of making a good fit. This correlation also supports Charton's idea that E_s values can be based on van der Waals radii. Three of the 18 derivatives (3-Me-4-Cl, 3,5-Me_2, 3,4,5-Me_3) are poorly fit; leaving these aside, the high correlation of eq 4 is obtained. Using γ in eq 4 instead of E_s, we

$$pI_{50} = [0.766\ (\pm 0.15)]E_s + [1.752\ (\pm 0.40)]\sigma + [0.180\ (\pm 0.18)]\pi + 3.996\ (\pm 0.30)$$

$$n \quad r \quad s$$
$$15 \quad 0.976 \quad 0.203 \quad (4)$$

obtain a poorer equation having $r = 0.966$ and $s = 0.243$. While the π term in eq 3 is not significant at

TABLE III



X	E_s	π^a	σ^b	−Log (1/C) Obsd[c]	−Log (1/C) Calcd[d]	Δ log (1/C)
Inhibitors of Monoamine Oxidase (Rat Liver)						
4-Br	2.48	1.02	0.23	6.66	6.173	0.17
3,4-Cl_2	1.51	1.46	0.60	6.30	6.486	0.19
3-NO_2	0.23[e]	0.11	0.71	5.76	5.501	0.26
3-CF_3	0.08	1.07	0.42	4.98	5.110	0.13
4-Me	2.48	0.52	−0.17	5.69	5.718	0.03
3,5-Cl_2	0.54	1.52	0.74	5.68	6.063	0.38
3-Cl-4-Me	1.51	1.28	0.20	5.75	5.794	0.04
3-Br	1.32	0.94	0.39	5.64	5.905	0.27
3-Me-4-Cl	1.24	1.21	0.16	6.06	5.525	0.54
3-Cl	1.51	0.76	0.37	5.82	5.970	0.15
4-OCH_3	2.48	−0.04	−0.27	5.46	5.443	0.02
3,4-Me_2	1.24	1.03	−0.24	4.71	4.834	0.12
3,5-Me_2	0.00	1.02	−0.14	4.85	4.126	0.72
3-Me	1.24	0.51	−0.07	4.78	5.010	0.23
4-Cl-3,5-Me_2	0.00	1.72	0.09	4.70	4.642	0.06
3,4,5-Me_3	0.00	1.54	−0.31	3.54	3.950	0.41
4-N=NC_6H_5	2.48	1.71	0.64	7.56	7.282	0.28
4-NH_2	2.48	−1.63	−0.66	4.40	4.488	0.09
MAO Inhibition of Human Liver Mitochondria						
4-N=NC_6H_5	2.48	1.71	0.64	8.83	8.473	0.36
4-Me	2.48	0.52	−0.17	6.67	7.117	0.45
3,4-Cl_2	1.51	1.46	0.60	7.55	7.330	0.22
4-OCH_3	2.48	−0.04	−0.27	7.07	6.786	0.28
3-CF_3	0.08	1.07	0.42	5.32	5.506	0.19
3-Cl	1.51	0.76	0.37	6.35	6.801	0.45
3,5-Cl_2	0.54	1.52	0.74	6.20	6.518	0.32
3-NO_2	0.23[e]	0.11	0.71	5.83	5.595	0.24
3,5-Me_2	0.00	1.02	−0.14	5.10	4.794	0.31

[a] From the phenoxyacetic acid system. [b] From Jaffe's compilation: H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953). [c] See ref 14. [d] Calculated using eq 3 for inhibitors of MAO (rat liver) and eq 6 for MAO inhibition of human liver mitochondria. [e] The value of 0.23 for NO_2 is obtained from eq 1 by using the van der Waals radius for the thickness of the nitro group; see Table II.

0.9 level (F test), it is significant at this level in eq 4. A closer study of the three poorly fit drugs might yield quite useful structure–activity information.

Fuller, *et al.*, also studied the inhibition of human monoamine oxidase. Equations 5 and 6 arise from

$$pI_{50} = [1.305\ (\pm 0.71)]\gamma + [0.830\ (\pm 1.60)]\sigma + [0.754\ (\pm 1.03)]\pi + 6.888\ (\pm 1.05)$$

$$n \quad r \quad s$$
$$9 \quad 0.915 \quad 0.591 \quad (5)$$

$$pI_{50} = [1.030\ (\pm 0.39)]E_s + [1.089\ (\pm 1.2)]\sigma + [0.398\ (\pm 0.76)]\pi + 4.541\ (\pm 0.88)$$

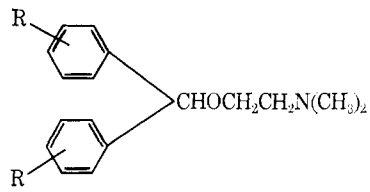
$$9 \quad 0.955 \quad 0.435 \quad (6)$$

their data in Table II. Again, E_s gives a better correlation than γ. The use of molar volume instead of E_s or γ gave poorer correlations.

It is important to consider what the high dependence on E_s means. There are two broad possibilities. The *meta* substituents may be involved in an intra- or an intermolecular steric repulsion. It is difficult to see how substituents in the *meta* position could interact strongly with the side chain; therefore, it seems most likely that the *meta* substituents in some way hinder binding of the N-phenoxyethylcyclopropylamines by the enzymes. The only *meta* substituent under consideration which is not of the symmetrical top class is NO_2. Since this function is best fit by the E_s constant derived from its thickness, it would indicate steric effects are due to a kind of fit to a surface rather than engulfment of the substituent by enzyme. It is ex-

citing to think that experimental E_s values or those directly calculated from van der Waals radii may be of some general use for enzymic interactions.

Antihistamines.—A second example in which we wish to consider the use of steric parameters is that of antihistamine activity. While an enormous amount of work has been carried out in the search for effective antihistamines, a very small amount of data are available on sets of congeners in which molecular modification was conducted in a systematic fashion amenable to substituent constant analysis. Two exceptional studies are those of Harms and Nauta[15] and Ensor, *et al.*[16] The former was an *in vitro* study and the latter an *in vivo* analysis. It is these two studies on aryl-substituted diphenhydramines of structure I with which we shall be concerned. Several different mechanisms



I

have been proposed to explain the influence of substituents of the phenyl rings on the biological activity in the di- and mephenhydramine series. Harms and Nauta suggested that in the case of the *ortho* derivatives, intramolecular interaction of the *ortho* substituents with the flexible side chain occurs, preventing a curling up of the molecule. Ariëns[17] has pointed out that electronic effects, especially hyperconjugation, are important. Other authors have also discussed steric effects of *ortho* substituents[18] and electronic effects.[19] We have analyzed the problem using regression analysis and substituent constants with the objective of disentangling steric, electronic, and hydrophobic influences of the ring substituents.

From the data in Table IV on the *in vitro* activity (guinea pig ileum) of diphenhydramine derivatives we have derived eq 7–15. In eq 7–9 are compared the

$$\log BR = [0.440\ (\pm 0.09)]E_s^{o,m} - 2.204\ (\pm 0.31)$$
$$n \quad r \quad s$$
$$30 \quad 0.886 \quad 0.307 \qquad (7)$$

$$\log BR = [-0.433\ (\pm 0.25)]\pi - 0.142\ (\pm 0.43)$$
$$30 \quad 0.550 \quad 0.555 \qquad (8)$$

$$\log BR = [2.814\ (\pm 1.4)]\sigma - 0.223\ (\pm 0.33)$$
$$30 \quad 0.629 \quad 0.519 \qquad (9)$$

$$\log BR = [0.492\ (\pm 0.14)]E_s^{o,m} - [0.585\ (\pm 1.23)]\sigma - 2.445\ (\pm 0.64)$$
$$30 \quad 0.895 \quad 0.303 \qquad (10)$$

$$\log BR = [0.474\ (\pm 0.12)]E_s^{o,m} + [0.079\ (\pm 0.20)]\pi - 2.429\ (\pm 0.64)$$
$$30 \quad 0.889 \quad 0.301 \qquad (11)$$

$$\log BR = [0.102\ (\pm 0.19)]\pi^2 - [0.828\ (\pm 0.76)]\pi + 0.152\ (\pm 0.68)$$
$$30 \quad 0.578 \quad 0.552 \qquad (12)$$

$$\log BR = [0.370\ (\pm 0.11)]E_s^{o,m} - [0.222\ (\pm 0.20)]E_s^{p} - 1.770\ (\pm 0.49)$$
$$30 \quad 0.905 \quad 0.288 \qquad (13)$$

$$\log BR = [0.326\ (\pm 0.10)]E_s^{o,m} - 0.264(E_s^{p})^2 - 0.173E_s^{p} - 1.325\ (\pm 0.55)$$
$$30 \quad 0.928 \quad 0.257 \qquad (14)$$
$$\text{ideal } E_s^{p} = -0.33(-1.7 - 0.02)$$

$$\log BR = [0.326\ (\pm 0.09)]E_s^{o,m} - 0.346(E_s^{p})^2 - 0.189E_s^{p} + [0.563\ (\pm 0.43)]E_s^{p'} - 1.878\ (\pm 0.65)$$
$$30 \quad 0.945 \quad 0.231 \qquad (15)$$
$$\text{ideal } E_s^{p} = -0.27(-0.80 - 0.03)$$

single variables $E_s^{o,m}$, π, and σ. The variable $E_s^{o,m}$ refers to the sum of the E_s values for *ortho* and *meta* substituents. The *para* position is ignored. $E_s^{o,m}$ also refers only to substituents on the most highly substituted ring. Substituents on the other ring are ignored. The above restrictions were introduced into the analysis after a perusal of the data and some preliminary calculations. By far the best of the single variable equations is that of eq 7, employing the steric parameter. The positive coefficient with E_s indicates that the larger the substituent, the lower the biological response. A most important point is that the steric effects from the *ortho* and *meta* positions are so similar that they can be treated together in one term. This strongly argues against an intramolecular action and suggests an intermolecular effect of these groups. The selection of substituents employed in this study does not allow us to make as clean a separation between the roles of π and E_s as one would like. However, it is quite clear that activity does not parallel π nearly so well as it parallels $E_s^{o,m}$. Moreover, the coefficient with π is negative. This negative dependence can be interpreted in either of two ways. The first and most likely is that a steric effect is implied. For the set of substituents in hand, the size of the substituent very roughly sets its hydrophobic character. Hence it would seem that π is telling us the same story that E_s relates. Another interpretation of the negative coefficient is that for the set of drugs under consideration, only compounds with superoptimal lipophilic character are in the set. In other words, the set falls on the "linear" portion (having a negative slope) of the normally expected parabola connecting log BR and π. Equation 12 indicates that this is quite unlikely. In the normal parabolic relationship between log BR and π, one expects and finds a negative coefficient with the exponential term. A positive coefficient is meaningless since it implies that as π approaches + or − infinity, so does biological response.

The linear combinations of E_s with π or σ (eq 10 and 11) do not result in improved correlations. This again downgrades roles for electronic and hydrophobic effects of substituents. In eq 13 and 14 we have given special consideration to mono-*para* substituents. That is, E_s^{p} applies only to the mono-*para* substituent. Equation 13 is only a slight improvement over eq 7; however, it is interesting to note the negative sign of

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TABLE IV

Substituents	$\Sigma E_s^{p,m}$ [a]	E_s^p [b]	$E_s^{p'}$	$\Sigma\pi$ [d]	$\Sigma\sigma'$	Log BR Obsd [f]	Log BR Calcd [g]	$\lvert\Delta$ log BR$\rvert$
In Vitro Inhibition of Guinea Pig Ileum by Diphenylhydramines								
Unsubstituted	4.96	1.24	1.24	0.00	0.00	0.00	−0.33	0.33
4-Me	4.96	0.00	1.24	0.52	−0.17	0.58	0.44	0.15
4-Cl [h]	4.96	0.27	1.24	0.70	0.23	0.39	0.36	0.03
4-Et	4.96	−0.07	1.24	0.97	−0.15	0.42	0.45	0.03
4-*i*-Pr	4.96	−0.47	1.24	1.40	−0.15	0.30	0.45	0.15
4-*t*-Bu	4.96	−1.54	1.24	1.68	−0.20	−0.06	−0.09	0.03
2-Me	3.72	1.24	1.24	0.52	−0.17	−0.68	−0.73	0.05
3-Me	3.72	1.24	1.24	0.52	−0.07	−0.68	−0.73	0.05
2,3-Me_2	2.48	1.24	1.24	1.04	−0.24	−1.48	−1.14	0.34
2,6-Me_2	2.48	1.24	1.24	1.04	−0.34	−1.26	−1.14	0.12
2,2′-Me_2	3.72	1.24	1.24	1.04	−0.17	−1.00	−0.73	0.27
2,4′-Me_2	3.72	1.24	1.24	1.04	−0.17	−0.26	−0.73	0.47
3,3′-Me_2	3.72	1.24	1.24	1.04	−0.07	−0.56	−0.73	0.17
3,5-Me_2	2.48	1.24	1.24	1.04	−0.14	−1.48	−1.14	0.34
4,4′-Me_2	4.96	0.00	0.00	1.04	−0.17	−0.26	−0.26	0.00
2-Et	3.65	1.24	1.24	1.22	−0.15	−0.86	−0.76	0.10
2-Pr	3.36	1.24	1.24	1.43	−0.13	−0.88	−0.85	0.03
2-*i*-Pr	3.25	1.24	1.24	1.30	−0.15	−0.94	−0.89	0.05
2,2′,6-Me_3	2.48	1.24	1.24	1.56	−0.34	−1.24	−1.14	0.10
2,4′,6-Me_3	2.48	1.24	1.24	1.56	−0.34	−0.94	−1.14	0.20
2,3,5,6-Me_4	0.00	1.24	1.24	2.08	−0.48	−1.92	−1.95	0.03
2,6,2′,6′-Me_4	2.48	1.24	1.24	2.08	−0.34	−1.14	−1.14	0.00
3,5,3′,5′-Me_4	2.48	1.24	1.24	2.08	−0.14	−1.44	−1.14	0.30
2-Bu	3.33	1.24	1.24	1.90	−0.16	−1.02	−0.86	0.16
2-*i*-Bu	2.79	1.24	1.24	1.82	−0.15	−0.80	−1.04	0.18
2-*t*-Bu	2.18 [i]	1.24	1.24	1.68	−0.20	−1.22	−1.24	0.02
2-Amyl	3.32	1.24	1.24	2.40	−0.16	−0.85	−0.86	0.01
2-*t*-Amyl	2.18	1.24	1.24	2.18	−0.20	−1.00	−1.24	0.24
2,2′,4,4′,6,6′-Me_6	2.48	1.24	1.24	3.12	−0.51	−1.51	−1.14	0.37
2,2′,3,3′,5,5′,6,6′-Me_8	0.00	1.24	1.24	4.16	−0.48	−1.51	−1.95	0.44
In Vivo Inhibition by Diphenylhydramines of Guinea Pig Histamine Response								
Unsubstituted	4.96	1.24	1.24			0.00	0.08	0.08
4-F	4.96	0.78	1.24			0.50	0.19	0.31
4-Cl	4.96	0.27	1.24			0.05	0.26	0.21
4-Br	4.96	0.08	1.24			0.40	0.27	0.13
4-I	4.96	−0.16	1.24			0.63	0.27	0.36
4-Me	4.96	0.00	1.24			0.32	0.27	0.05
4-Et	4.96	−0.07	1.24			0.34	0.27	0.07
4-Pr	4.96	−0.36	1.24			0.06	0.25	0.19
4-*i*-Pr	4.96	−0.47	1.24			−0.04	0.24	0.28
4-OMe	4.96	0.69	1.24			0.04	0.21	0.17
4-C_6H_5	4.96	−2.58	1.24			−0.50	−0.53	0.03
2-Cl	3.99	1.24	1.24			−0.55	−0.60	0.05
3-Cl	3.99	1.24	1.24			−0.95	−0.60	0.35
2,4′-Cl_2	3.99	1.24	1.24			−0.61	−0.60	0.01
3,4-Cl_2	3.99	1.24	1.24			−0.61	−0.60	0.01
4,4′-Cl_2	4.96	0.27	0.27			0.00	−0.20	0.20
2-Me	3.72	1.24	1.24			−0.68	−0.78	0.10
3-Me	3.72	1.24	1.24			−0.50	−0.78	0.28
2,2′-Me_2	3.72	1.24	1.24			−0.56	−0.78	0.22
2,3′-Me_2	3.72	1.24	1.24			−0.96	−0.78	0.18
2,4′-Me_2	3.72	1.24	1.24			−0.96	−0.78	0.18
4,4′-Me_2	4.96	0.00	0.00			−0.48	−0.32	0.16

[a] Sum of E_s values of *ortho* and *meta* substituents on the highest substituted ring. [b] E_s value for mono-*para* substituents. [c] E_s value for the second *para* substituent in the case of *p,p′* substitution. [d] Sum of π values for all substituents on both rings. [e] Sum of σ values on the highest substituted ring. [f] Biological activities are given on a relative scale with diphenhydramine as the standard. [g] Calculated using eq 15 for the first set (*in vitro*) and using eq 18 for the second set (*in vivo*). [h] Compound tested by H. Arnold, N. Brock, E. Kuehas, and D. Lorenz, *Arzneimittel-Forsch.*, **4**, 189 (1954). [i] Approximated by E_s value for *t*-butyl.

the E_s^p term, indicating a different role for these substituents. Equation 14 is a more significant improvement over eq 7 (compare standard deviations), *i.e.*, $F_{2,26} = 4.60$. This indicates an optimum E_s^p value of about −0.3. While eq 13 and 14 are not large improvements over eq 7, they are statistically significant and they do provide information of value in making over derivatives for testing. In eq 15 a term is added for the negative effect of a second *para* substituent in the 4,4′-substituted derivatives. Since only one of these is involved in this set of data, eq 15 has little meaning when taken alone. It only becomes of interest when it is compared with the *in vivo* data below. While the above explicit and implicit postulates have

restricted the number of degrees of freedom in this correlation, the rather large number of data points gives us some confidence that the results are not fortuitous. A better selection of derivatives could shed more light on the more complex eq 13–15.

It was surprising to find that hydrophobic effects do not play a more important role in the structure–activity relationship of the diphenhydramines. A variety of attempts to find such a role for substituents were unsuccessful. Even adding $\Sigma\pi^2 + \Sigma\pi$ terms to eq 7 where π represented all substituents on both rings did not significantly improve eq 7. It seems unlikely that hydrophobic effects of substituents are completely unimportant. The correlation between π and E_s may well disguise their presence. The basic conclusions from the above analysis are (1) substituents in the *ortho* and *meta* positions of the more highly substituted ring have parallel deactivating effects, (2) mono-*para* substitution has an activating effect up to an optimum size and then a deactivating effect, (3) substituents in the *ortho* and *meta* positions of the less substituted ring have little effect, and (4) a second *para* substituent appears, on the basis of very limited evidence, to have a deactivating effect.

The second set of data in Table IV is a smaller set for *in vivo* work with guinea pigs. From these data we have derived eq 16–18. The over-all result with the *in vivo* data was so much like that for the *in vitro*

$$\log \mathrm{BR} = [0.711\ (\pm 0.23)]E_s^{o,m} - 3.431\ (\pm 1.1)$$
$$n \quad r \quad s$$
$$22 \quad 0.817 \quad 0.293 \quad (16)$$

$$\log \mathrm{BR} = [0.677\ (\pm 0.41)]E_s^{o,m} - 0.092(E_s^p)^2 - 0.026E_s^p - 3.192\ (\pm 2.0)$$
$$22 \quad 0.865 \quad 0.269 \quad (17)$$
$$\text{ideal } E_s = 0.14$$

$$\log \mathrm{BR} = [0.697\ (\pm 0.34)]E_s^{o,m} - 0.121(E_s^p)^2 - 0.002E_s^p + [0.475\ (\pm 0.33)]E_s^{p'} - 3.781\ (\pm 1.8)$$
$$22 \quad 0.914 \quad 0.223 \quad (18)$$
$$\text{ideal } E_s = 0.0(-0.6 - 6.1)$$

data that we show only the three most pertinent equations. While the correlations are not so good with eq 16–18 (note especially the confidence intervals), the results confirm the assumptions made in treating the larger *in vitro* set of data. Conclusions (1–4 above) arrived at with the *in vitro* data are supported by eq 16–18. The ideal E_s value calculated from eq 17 and 18 is slightly greater than that obtained from eq 14 and 15. The tightest confidence limits are set on this ideal value of E_s in eq 15 and 18. These results indicate a Me or Br are of about optimum size. While the coefficients and intercepts of eq 16–18 are somewhat different from the corresponding *in vitro* equations, the confidence intervals are rather large. The closeness of the two results suggested combining all data in one set of equations (19–21). It is most ex-

$$\log \mathrm{BR} = [0.463\ (\pm 0.07)]E_s^{o,m} - 2.293\ (\pm 0.28)$$
$$n \quad r \quad s$$
$$52 \quad 0.880 \quad 0.311 \quad (19)$$

$$\log \mathrm{BR} = [0.355\ (\pm 0.08)]E_s^{o,m} - 0.179(E_s^p)^2 - 0.157E_s^p - 1.551\ (\pm 0.43)$$
$$52 \quad 0.919 \quad 0.262 \quad (20)$$
$$\text{ideal } E_s = -0.44(-0.9 - (-0.1))$$

$$\log \mathrm{BR} = [0.358\ (\pm 0.07)]E_s^{o,m} - 0.216(E_s^p)^2 - 0.189E_s^p + [0.482\ (\pm 0.26)]E_s^p - 2.059\ (\pm 0.47)$$
$$52 \quad 0.939 \quad 0.232 \quad (21)$$
$$\text{ideal } E_s = -0.44(-0.7 - (-0.2))$$

citing that eq 19–21 give reasonable correlations for a large number of complex derivatives. Each of the two sets are made up of quite different molecules tested in two different ways in two different laboratories. The *in vitro* work in this instance could have been used to predict the *in vivo* results. Probably even a better fit of the two sets could be obtained if metabolic effects in the one instance could be removed. These results should encourage those interested in the mathematical treatment of drug activity that all is not lost because of inaccuracies in biological data.

The conclusion that E_s for unsymmetrical groups can be used for what appear to be intermolecular effects deserves comment. If these unsymmetrical substituents were being completely immersed in a macromolecule, it is highly unlikely that an adjacent-effect parameter would give a satisfactory correlation. It would seem that these unsymmetrical functions must orient themselves so as to cause a minimum of interaction with a macromolecular surface. For example, one could picture substituent perturbation of a "charge-transfer" complex by these unsymmetrical substituents paralleling their effects on simple homogeneous reactions.

Before attempting to analyze the results in more detail, consideration must be given to the geometry of the rather complex diphenhydramine structure. Harms and Nauta[15] pointed out the importance of the relation of the dimethylamino-containing side chain to the aromatic rings. In another respect, the folding of complex organic compounds in aqueous solution has been of great concern to us.[6] For example, it was found[6] that in studying partition coefficients of molecules having the general structure $C_6H_5CH_2CH_2CH_2X$, folding of the side chain onto the ring appeared to occur in aqueous solution in all instances where X was anything but H. It would appear that whenever the chain is flexible enough, a dipolar function on the side chain can interact with the π electrons of an aromatic ring to promote folding. The folding of course is also promoted by hydrophobic bonding. Under physiological conditions the basic dimethylamino group of the diphenhydramines would be protonated. It would seem from the evidence at hand[6] that the positive charge on N plus the hydrophobic interactions could bind the side chain to one of the phenyl rings. Some evidence for such an interaction can be seen in the partition coefficient data shown in Table V. Data for compounds **1–5** come from the work of Elderfrawi and O'Brien.[20] Compound **6** is from ref 21. All log P values are for the OctOH–H_2O system. Subtracting π for the hydrocarbon units attached to the quaternary N gives us the reference standard of π for ^+N. As

(20) M. E. Elderfrawi and R. D. O'Brien, *J. Exptl. Biol.*, **46**, 1 (1967).
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TABLE V

No.	Structure	Log P	$-$	$\Sigma\pi$ for hydrocarbon residues	$=$	$\pi(-\overset{+}{N}Me_3)$
1	$C_2H_5\overset{+}{N}Me_3$	−3.01		−2.5		−5.1
2	$C_4H_9\overset{+}{N}Me_3$	−2.60		−3.5		−6.1
3	$C_6H_{13}\overset{+}{N}Me_3$	−1.84		−4.5		−6.34
4	$C_8H_{17}\overset{+}{N}Me_3$	−1.07		−5.5		−6.57
5	$C_{10}H_{21}\overset{+}{N}Me_3$	−0.16		−6.5		−6.67
6	$C_6H_5(CH_2)_4\overset{+}{N}Me_3$	−2.02		−5.13		−7.15

one goes down the series from **1** to **5**, solubility in the H_2O phase increases relative to that in OctOH. The differences are small between members **2** and **5**. This is most easily explained by assuming a tendency for the larger side chain to coil up and become more compact. It is of interest to note that when C_6H_5 is introduced (**6**), we obtain a larger effect than even with the long C_{10} chain of **5**. This suggests pronounced folding for **6**, promoted by the interaction of the ring π electrons and the positive charge of the N atom. In the case of the diphenhydramines, such side-chain folding might be strongest on the least substituted ring. There is considerable support for the fact that when bulky groups are present,[22-24] van der Waals complexes[25,26] between substituted aromatic rings and interacting molecular moieties are hindered. In van der Waals complexes in aqueous biochemical systems we must consider hydrophobic forces in addition to the forces considered by Dewar[25,26] in defining van der Waals complexes. Thus one might question whether side-chain folding of the dimethylamino-containing unit onto a phenyl ring would persist after transfer of the drug from an aqueous phase into a nonaqueous medium where the restraining pressure of the water molecules would be gone. In this connection it is of interest to note that Dewar[26] has shown that 2-(1-pyrenyl)ethyl *p*-toluenesulfonate appears to be folded even in a nonaqueous solvent. Hence one must consider the possibility that the dimethylamino side chain is folded onto a phenyl ring even in or on the receptor site. If such folding in fact occurs, then one of the phenyl rings could be involved in such an intramolecular complex and would possibly not be suitable sterically for binding to the receptor site. This could account for the fact that *ortho*- and *meta*-substituent effects on one ring can be neglected.

There are so many instances in which very important drugs contain an aromatic ring to which a two- or three-carbon ω-dialkylamino side chain is affixed, that one wonders whether there is some general pharmacological significance involved in this kind of folding. Since such N atoms would be protonated under physiological conditions, such action in essence mounts a cationic N onto one side of a flat hydrophobic ring. The above hypothesis is of course tentative. Further work is in progress using partition coefficients and nmr studies to more fully understand the conformations aromatic drugs with polar side chains assume in aqueous solutions. Such knowledge about intramolecular complexing is of great importance in the current effort to more precisely define drug conformation.[27]

From our analysis in eq 19–21, one cannot come to any final conclusions about the intimate details of the required geometry for antihistamines in general. The reason for relative unimportance of substituents on one ring may be that suggested above or, since it is known that there is a difference in activity of optical antipodes, it may be that each phenyl ring has a preferred receptor site, one less sterically demanding than the other. The results of this study indicate that regression analysis employing steric constants should enable one to obtain more information about the receptor site. Also, one should be able, using more suitably designed derivatives, to more carefully assess the beneficial effects of 4 substituents up to a certain size as well as the detrimental effects of a second *para* substituent.

It must be emphasized that in the antihistamine analyses the most important equations are 7, 16, and 19. One can place considerable confidence in these simple linear equations since so many varied derivatives fit the single hypothesis. Improvement in correlation is not large in going to the higher order equations such as 13–15. Also, the number of data points used to justify the terms in E_s'' and E_s''' are fewer in number. In fact, for E_s'' only three points are available. However, the effect of substituents in this position is so large that the additional term in E_s'' is statistically significant. These higher order equations do suggest ideas for further research.

We feel that the results in this report open up a general approach to the study of intermolecular interactions between drugs and their receptor sites. These results show that the fit of drugs onto or into macromolecules is not an all-or-none situation which the "lock and key" theory often conjures up. The present results are hard to explain without assuming that the binding or partial insertion of groups of moderate size on or into a macromolecular pouch is, at least over a limited range, a continuous linear process. Since the free-energy change involved parallels so closely that for simple shielding of an ester group in a hydrolytic process (E_s), one would assume a great deal of flexibility in the macromolecular receptor site. In fact, it would appear to approach that of liquids. No doubt there are strict limits to this method of treating intermolecular steric effects. How far such a grossly simple treatment can be carried remains to be seen. At present, we find the door to a new field ajar. It appears ever more likely that, thanks to large computers, we can seriously consider n-dimensional analyses, using large numbers of *well-designed* derivatives, of the electronic, hydrophobic, and steric interactions of drugs with their receptors.

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