Pure & Appl. Chem., Vol. 50, pp. 987-994.
Pergamon Press Ltd. 1978. Printed in Great Britain.

0033-4545/78/1001-0987 $02.00/0

STERIC EFFECTS IN QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS

Toshio Fujita

Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan

Abstract - Separating the stereospecific factor from others governing the manifestation of biological activity to estimate its relative significance is the first step in understanding the stereospecific effect of biologically active compounds. In certain cases, this can be performed by means of quantitative analysis of structure-activity relationship of congeneric series of bioactive compounds using physical-organic models and multiple regression analysis. Examinations were made for the use of the Hancock E_s^c values as the model of intra- as well as inter-molecular steric effects. A new procedure to analyze the steric effect in various physical-organic reactivities was proposed. The procedure was successfully extended to analyzing the steric effect between quaternary ammonium cationic head of acetylcholine and its analogs and their target, acetylcholinesterase. Other examples where E_s^c values are applicable in structure-activity correlations are briefly reviewed. A comparison is made between E_s^c and other steric parameters currently used in structure-activity studies.

INTRODUCTION

Stereospecificity has long been known as being very important for biologically active compounds to exhibit their activity. In particular, it has been believed to play significant roles in the interactions with their targets in vivo as postulated in the classical lock-and-key theory of enzymatic reactions (1) as well as in the receptor theory of drugs (2). However, a number of events which are governed non-stereospecifically by various physicochemical properties are usually involved in the overall chain of processes for bioactive compounds to reach and to interact with their targets. Considering the complicated transport processes including biodegradation pathways and the heterogeneous as well as topological architecture of macromolecular biotargets, one would face perplexing problems in understanding the ultimate origin of stereospecificity, such as:
1) How to separate the stereospecific factors from others governing the manifestation of the activity?
2) How significant are the stereospecific factors relative to other factors?
3) What is the stereospecifically critical step in the overall process?
4) Why is the stereospecificity required? What is the physicochemical significance (or the significance at the molecular level for both the bioactive compounds and the interacting biomacromolecules) of the stereospecificity?

Although countless efforts from various directions have to be made for complete answers to above questions, there have been considerable developments in this field of science, recently. One of these developments has been initiated by Hansch and his coworkers (3-6). Their approach to these problems is based on the quantitative analysis of structure-activity-relationships (QSAR) with the use of physicochemical models and multiple regression analysis. It assumes that physicochemical properties determining the physical-organic processes can be used as models for characterizing the behaviors of bioactive compounds in vivo. Variations in the "intensity" of a certain biological activity exhibited by a certain series of bioactive compounds can be correlated to variations in various physicochemical factors associated with their structure. The situation can be expressed as eq 1, where ΔBR is the variation in the biological response and E_1, E_2 ····· and E_n are parameters for physicochemical factors.

$$\Delta BR = f(\Delta E_1, \Delta E_2, \cdots \Delta E_n) \quad (1)$$

Most significant factors governing ΔBR are the variations in hydrophobic, electronic and steric properties of bioactive compounds. Factors for hydrogen bonding, van der Waals and charge-transfer forces and others may be needed depending on the situation. In terms of free-energy related substituent parameters for congeneric compounds having a common skeleton but varying substituents, eq 1 is transformed, for example, to eq 2 by taking the BR value of the unsubstituted compound as the reference.

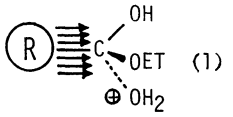
$$\log(1/C) = a\pi + \rho\sigma + \delta E_s + \text{constant} \quad (2)$$

In eq 2, C is the concentration (or dose) of congeneric members which gives a standard response such as EC_{50}, LD_{50}, etc. on a molar basis. π is the hydrophobic substituent parameter defined from oil/water (generally, 1-octanol/water) partition coefficients, P, as $\pi \equiv \log P_X - \log P_H$, where subscripts denote substituted and unsubstituted compounds (7). σ is the Hammett constant for electron withdrawing property of substituents defined from dissociation constants of benzoic acids (8). Depending on the situation, the Taft σ^* derived from reaction rate constants of aliphatic esters (9) and others can be used as the electronic parameter. E_S is the Taft steric parameter (9) in this example. In some cases, the squared terms for hydrophobic and steric parameters are required to account for the optimums for these effects. a, ρ and δ are susceptibility constants which are determined as regression coefficients by multiple least square method. The level of significance of the regression coefficients is examined by t- and/or F-tests. Some of the terms in eq 2 are not always significant indicating that some factors are not always critical in governing the variation in the activity. If this procedure is successfully applied to congeneric bioactive compounds, we can, at least, separate the stereospecific factor from others and estimate its relative significance in determining the ΔBR value.

The most widely used steric parameter is the Taft E_S constant as in eq 2, but other parameters can be used depending on the mode of steric interactions involved. Recently, we have been examining the application of the Hancock "corrected" steric E_S^C constant (10) to QSAR. In spite of the original definition, the E_S^C values are useful as intermolecular steric parameters in certain in vitro reactions between low-molecular compounds. The purpose of this paper is to report the current state of our effort in quantitatively separating the steric effect intermolecularly operating between bioactive compounds and their targets, in particular, the effect of the cationic head of acetylcholine analogs on the anionic site of acetylcholinesterase.

DEFINITION OF THE E_S^C VALUE

In reactivities of aliphatic compounds, the substituents generally exert not only electronic but also steric effects in varying degrees intramolecularly on the functional reaction center because of their conformational flexibility. However, the variation in the acid-catalyzed hydrolytic rate of carboxylic esters of the type RCOOEt is mostly subject to the steric effect of substituents R. The steric effect of R on the tetrahedral intermediate formation is far more significant than the electronic effect (1). Taft defined the parameter E_S as eq 3 where $(k_R/k_{Me})_A$ refers to the ratio of acid catalyzed hydrolytic rate constans of esters, RCOOEt, to that of MeCOOEt (9). The bulkier the substituent, the more negative the E_S value. By definition, $E_S(Me) = 0$.



(1)

$$E_S \equiv \log (k_R/k_{Me})_A \quad (3)$$

Since the E_S value is determined by the relative activation free energy from the unsaturated initial state to the saturated transition state of the ester hydrolysis, Hancock and his coworkers considered that a hyperconjugation effect of α-hydrogen may contribute to the estimate of E_S values (10). To separate the hyperconjugation effect from the "true steric effect", they defined the parameter E_S^C(corrected steric) as eq 4, assuming that the hyperconjugation effect is proportional to the number of α-hydrogen atoms, n_H. By definition, $E_S^C(Me) = 0$.

$$E_S^C \equiv E_S - 0.306\ (3 - n_H) \quad (4)$$

Relevant E_S^C values are listed in Table 1.

TABLE 1. Steric parameter E_S^C of common substituents

Substituent	E_S^C	Substituent	E_S^C	Substituent	E_S^C
H	0.32	*s*-Bu	-1.74	Et_3C	-4.72
Me	0.00	*t*-Bu	-2.46	*n*-Oct	-0.64
Et	-0.38	*n*-Pent	-0.71	Benzyl	-0.69
n-Pr	-0.67	*i*-Pent	-0.66	Phenethyl	-0.69
i-Pr	-1.08	Et_2CH	-2.59	$1/2\,(CH_2)_4$	-0.19[a]
n-Bu	-0.70	neo-Pent	-2.05	$1/2\,(CH_2)_5$	-0.38[a]
i-Bu	-1.24	*cyc*-Hex	-1.40	$1/3\ CH[(CH_2)_2]_3$	0[a]

a) The value per unit ligand evaluated from equilbrium constants of piperidine, pyrrolidine and quinuclidine derivatives for hydrogen bond formation with $CHCl_3$ and charge-transfer complex formation with I_2 using eq 7 and 8. The uncertainty of these values is about ± 0.1.

The particular use of the E_S^C constants has been viewed with skepticism by some workers (11, 12). Especially, the use of the constant susceptibility factor, 0.306, for the hyperconjugation effect per α-hydrogen has been criticized as being highly unlikely (12). In spite of theoretical uncertainties, E_S^C appears to be a more suitable parameter for the steric effect than E_S in certain reactivities. This is illustrated in the following examples.

USE OF E_S^C VALUES FOR PHYSICAL-ORGANIC PROCESSES

We have assumed that the steric effect of substituents R of type $CR_1R_2R_3$ can be expressed in terms of the steric effect of component substituents R_1, R_2 and R_3. With increasing substitution at the α-carbon, the total steric effect of substituents R has been observed to increase telescopically in such series as Me, Et, *i*-Pr and *t*-Bu (9). Thus, the simple addition of steric parameters for α-substituents is inadequate to represent the situation. We have found that the E_S^C parameter for 24 primary, secondary and tertiary alkyl groups can be formulated as eq 5 (Note a) by the linear combination of E_S^C parameters of component α-substituents (13).

$$E_S^C(CR_1R_2R_3) = \underset{(\pm 0.516)}{3.429}E_S^C(R_1) + \underset{(\pm 0.252)}{1.978}E_S^C(R_2) + \underset{(\pm 0.118)}{0.649}E_S^C(R_3) - \underset{(\pm 0.195)}{2.104}$$

$$n = 24 \quad s = 0.191 \quad r = 0.992 \qquad (5)$$

The R_1, R_2 and R_3 substituents are classified according to the relative magnitude of their E_S^C value so that $E_S^C(R_1) \geq E_S^C(R_2) \geq E_S^C(R_3)$, i.e., R_1 is the smallest while R_3 is the bulkiest. The E_S^C value of the α-hydrogen substituent of primary and secondary alkyl groups is taken as $E_S(H) - 3 \times 0.306 = 0.32$. In deriving eq 5, a few component substituents were considered as being conformationally restricted. Their effective steric effect was represented not by their original E_S^C but the values for substituents whose geometry is similar to the restricted conformation. Eq 5 can be regarded as meaning that the substituent effect in acidic hydrolysis of aliphatic esters can be separated into the steric and the "hyperconjugation" components. The analysis with the use of E_S instead of E_S^C in eq 5 was shown to give a much poorer result.

Since the total steric effect of component substituents R_1, R_2 and R_3 on the reaction center in the transition state of ester hydrolysis is similar, if not identical, to that of three N-substituents of aliphatic amines on a certain electron acceptor or electrophile, we attempted to separate electronic and steric effects of N-substituents of amines $NR_1R_2R_3$ for various reactions by means of eq 6 where K is either the rate or equilibrium constant, $\Sigma\sigma^*$ is the sum of σ^* values for R_1, R_2 and R_3 and $E_S^C(R_1) \geq E_S^C(R_2) \geq E_S^C(R_3)$ (14). Examples are shown as eq 7 - 11.

$$\log K = \rho^*\Sigma\sigma^* + \delta_1E_S^C(R_1) + \delta_2E_S^C(R_2) + \delta_3E_S^C(R_3) + \text{constant} \qquad (6)$$

Hydrogen bond formation with $CHCl_3$ in *cyc*-hexane at 35° (14):

$$\log K = \underset{(\pm 0.210)}{-0.499}\Sigma\sigma^* + \underset{(\pm 0.229)}{1.013}E_S^C(R_1) + \underset{(\pm 0.108)}{0.419}E_S^C(R_2) + \underset{(\pm 0.098)}{0.209}E_S^C(R_3) - \underset{(\pm 0.138)}{0.101}$$

$$n = 29 \quad s = 0.145 \quad r = 0.946 \qquad (7)$$

Charge-transfer complexation with I_2 in *n*-hexane at 20° (14):

$$\log K = \underset{(\pm 0.385)}{-2.265}\Sigma\sigma^* + \underset{(\pm 0.451)}{1.878}E_S^C(R_1) + \underset{(\pm 0.215)}{0.908}E_S^C(R_2) + \underset{(\pm 0.181)}{0.469}E_S^C(R_3) + \underset{(\pm 0.251)}{4.283}$$

$$n = 24 \quad s = 0.266 \quad r = 0.956 \qquad (8)$$

Hydrogen bond formation with PhOH in CCl_4 at 27° (14,15):

$$\log K = \underset{(\pm 0.148)}{-0.473}\Sigma\sigma^* + \underset{(\pm 0.144)}{0.624}E_S^C(R_1) + \underset{(\pm 0.107)}{0.328}E_S^C(R_2) + \underset{(\pm 0.083)}{1.980}$$

$$n = 25 \quad s = 0.097 \quad r = 0.922 \qquad (9)$$

Association with Me_3B in gas phase at 100° (14,16):

$$\log K = \underset{(\pm 2.087)}{-4.878}\Sigma\sigma^* + \underset{(\pm 5.655)}{14.585}E_S^C(R_1) + \underset{(\pm 2.578)}{4.879}E_S^C(R_2) + \underset{(\pm 0.594)}{1.461}E_S^C(R_3) + \underset{(\pm 0.755)}{0.001}$$

$$n = 17 \quad s = 0.569 \quad r = 0.876 \qquad (10)$$

SN_2 reaction with EtI in Me_2CO at 35° (14,17):

Note a. Figures in parentheses are the 95% confidence intervals. n, s and r are, respectively, the number of data used in the correlation, standard deviation and multiple correlation coefficient.

$$\log K = \underset{(\pm 0.438)}{-0.801\Sigma\sigma^*} + \underset{(\pm 0.834)}{1.701E_s^c(R_1)} + \underset{(\pm 0.500)}{0.843[E_s^c(R_2) + E_s^c(R_3)]} - \underset{(\pm 0.502)}{2.546}$$

$$n = 13 \quad s = 0.388 \quad r = 0.967 \qquad (11)$$

Only in deriving eq 7 and 8, the *i*-Bu group in tertiary amines is considered to be conformationally restricted so as to mimick *neo*-Pent. Although some of the correlations are not as high as one would like, it seems reasonable to consider that the present procedure has a general applicability as a first approximation. Depending on the reaction type, the relative steric effect of components, as revealed by the coefficient of E_s^c terms, varies. Since the magnitudes of δ_2 and δ_3 values are similar, they are combined to reduce the number of independent variables in eq 11. In gerneral, $\delta_1 \geq \delta_2 \geq \delta_3$ indicating that the steric effect of the smallest component is most significant in determining the total steric effect.

The steric effect of the component substituents could be exerted intramolecularly on the reaction intermediate. This is particularly the case for steric effects in the ester hydrolysis and the SN_2 reaction analyzed by eq 5 and 11, respectively. However, for association equilibria with various electron acceptors as correlated in eq 7 - 10, the effect could be intermolecular. For such reactions, it is impossible to definitely identify which of these two types of steric effect is operative. Probably, it is inadequate to consider that the steric effect as analyzed with E_s^c values is solely of the intramolecular type.

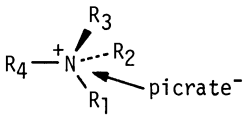
In fact, we have found more recently that the definitely intermolecular type of steric effect is indeed analyzable with E_s^c constants. We have determined the ion-pair formation-partition equilibrium constant with picrate anion for 58 primary, secondary, tertiary and quaternary ammonium ions (14). In aqueous media of pH 5 - 6, the ammonium ions and picrate are considered to exist almost completely as unpaired counter ions. When the aqueous solution is mixed with an immiscible organic solvent, the ions are partitioned into the organic phase as the ion pair. The ion-pair formation-partition equilibrium constant, K, using 1-octanol as the organic solvent is correlated with substituent parameters. Eq 12 is derived from the set of primary, secondary and tertiary ions and eq 13 is from the set of quaternary ions only. The four N-substituents are classified as $E_s^c(R_1) \geq E_s^c(R_2) \geq E_s^c(R_3) \geq E_s^c(R_4)$. Hydrophobic substituent parameter, π, is evaluated by taking the H substituent as the reference, 0.5 for additional CH_2 unit, -0.2 for the chain branching and -0.09 per a cyclic carbon (18), and summed for component four substituents. n_H is the number of N-hydrogen substituents. For the set of primary, secondary and tertiary ions, R_1 is always H so that the $E_s^c(R_1)$ term does not appear in eq 12. For the set of quaternary ions, the n_H term is lacking because $n_H = 0$. The susceptibility coefficients of component substituent effects are almost identical between eq 12 and 13 suggesting that the steric requirement for the ion-pairing of primary, secondary and tertiary ions does not differ from that for the quaternary ions. Thus, the hydrogen bonding association for primary, secondary and tertiary ions of the type $\equiv\overset{+}{N}\text{-}H\cdots\overset{-}{O}C_6H_2(NO_2)_3$ is not likely to occur in rather polar hydroxylic solvents. Since the steric effect of bulkiest R_4 substituents is not significant, the ion-pairing is perhaps achieved in such a manner that the counter anion must approach from the least hindered side of the ammonium ions (2). The n_H term accounts for the effect of hydrogen bonding with solvents. The positive coefficient of this term may indicate that the greater the number of NH hydrogen, the more stable the hydrogen bonding with the more basic 1-octanol favoring the ion-pair partitioning into the organic phase.

$$\log K = \underset{(\pm 0.082)}{0.916\Sigma\pi} + \underset{(\pm 0.244)}{0.798E_s^c(R_2)} + \underset{(\pm 0.145)}{0.276E_s^c(R_3)} + \underset{(\pm 0.092)}{0.428n_H} - \underset{(\pm 0.283)}{1.419}$$

$$n = 27 \quad s = 0.115 \quad r = 0.988 \qquad (12)$$

$$\log K = \underset{(\pm 0.088)}{0.870\Sigma\pi} + \underset{(\pm 0.287)}{0.848E_s^c(R_1)} + \underset{(\pm 0.244)}{0.677E_s^c(R_2)} + \underset{(\pm 0.166)}{0.225E_s^c(R_3)} - \underset{(\pm 0.269)}{1.774}$$

$$n = 31 \quad s = 0.109 \quad r = 0.990 \qquad (13)$$



(2)

Since three N-substituents R_1, R_2 and R_3 in ammonium ions are frontally situated for the access of counter anions, the total steric effect of N-substituents could be thought as being much enhanced compared with those in amines where substituents are located in the opposite side for the approach of electron acceptors. Eq 14, derived from the combined set of all classes of ions, however, shows that the relative significance of the steric effect of component substituents is similar to that observed in the hydrogen bonding equilibrium of amines with $CHCl_3$ shown as eq 7. Thus, the distance between two oppositely charged centers may not

$$\log K = \underset{(\pm 0.059)}{0.899\Sigma\pi} + \underset{(\pm 0.201)}{1.049E_s^c(R_1)} + \underset{(\pm 0.157)}{0.682E_s^c(R_2)} + \underset{(\pm 0.107)}{0.235E_s^c(R_3)} + \underset{(\pm 0.041)}{0.495n_H} - \underset{(\pm 0.187)}{1.852}$$

$$n = 58 \quad s = 0.114 \quad r = 0.989 \qquad (14)$$

be so close in the ion-paired complex. Of course, the coulombic force between counter charges is the driving factor for the ion pair formation. However, no electronic term is discernible in eq 14 showing that the positive charge distribution does not change significantly with the structural variation as far as alkyl ammonium ions are concerned.

USE OF E_S^C VALUES FOR INTERACTION OF AMMONIUM IONS WITH ACETYLCHOLINESTERASE

It is well known that the catalytic center of acetylcholinesterase comprises two types of binding site, the anionic and the esteratic sites (19). The interaction between positively charged quaternary nitrogen of acetylcholine molecule and the anionic site of the enzyme is suggested to play an important role in the binding of the substrate with the enzyme (19). To understand the role of this interaction, we attempted to analyze the inhibitory activity of simpler ammonium ions without the ester moiety. Using bovine erythrocyte acetylcholinesterase preparation, the inhibitor constant K_i (the dissociation constant of enzyme-inhibitor complex) was determined for 70 primary, secondary, tertiary and quaternary ammonium ions. Preliminary examinations suggested that each of the four N-substituents may exert hydrophobic as well as steric effects on the interaction with the enzyme specifically depending on the classification according to the relative bulk. We classified four substituents here in the same manner as did for the ion-pair formation. Unfortunately, it is impossible to definitely separate hydrophobic and steric effects from each other with the use of π_i and $E_S^C(R_i)$ values for each of the four N-substituent sets, since the internal correlation between these two constants within each set is fairly high.

Since the interaction of ammonium ions with the anionic site is regarded as being a sort of ion-pair formation, we expected that the total steric effect of N-substituents on the enzyme-inhibitor interaction would be quite similar to that on the ion-pair partition equilibrium with picrate anion. We used the steric constant terms in eq 14, $1.049E_S^C(R_1) + 0.680E_S^C(R_2) + 0.242E_S^C(R_3)$, together as a single steric parameter, $\Sigma\delta_i E_S^C(R_i)$, so that the internal correlations between independent parameters became negligible. As expected, eq 15 shows that the specific hydrophobic effect of substituents is really separable with the use of the model for the steric effect.

$$\log(1/K_i) = \underset{(\pm 0.169)}{0.545(\pi_1 + \pi_4')} + \underset{(\pm 0.229)}{1.075\pi_2} + \underset{(\pm 0.141)}{0.392\pi_3} + \underset{(\pm 0.214)}{0.827\pi_4''} + \underset{(\pm 0.425)}{1.152\Sigma\delta_i E_S^C(R_i)} - \underset{(\pm 0.112)}{0.195n_H} + \underset{(\pm 0.425)}{1.419}$$

$$n = 50 \quad s = 0.269 \quad r = 0.942 \qquad (15)$$

The coefficient of the steric term is close to 1 indicating that the stereospecific fit of ammonium ions with the anionic site of acetylcholinesterase is almost identical in nature with that required for the ion-pair formation with picrate. The specific hydrophobic effect of substituents as revealed by eq 15 suggests that the hydrophobic nature of the enzymic milieu surrounding the anionic site is not uniform. The coefficient associated with each of the π terms probably indicates the hydrophobic nature of the enzyme surface corresponding to each of substituents. π_4' and π_4'' represent, respectively, the hydrophobicity of the main and the side chains of R_4 substituents. The non-uniform hydrophobic effect of the R_4 substituents was also ascertained by examining the K_i values for 8 primary and 8 alkyltrimethyl quaternary ions having variously branched R_4 substituents. The n_H term having negative sign probably indicates that the larger the number of NH hydrogen, the more is the association with the enzyme prevented by the hydration in the aqueous bulk phase. The hydrogen acceptor in the enzymic milieu, if any, seems to be less basic than water.

We can draw schematically the interaction of ammonium ions with the anionic site of acetylcholinesterase based upon the above information. The detailed discussion of the analysis summarized here will be published elsewhere (14).

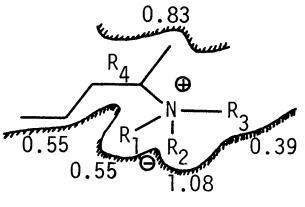


Fig. 1. Susceptibility of the enzyme around the anionic site to the hydrophobicity of N-substituents.

OTHER EXAMPLES OF THE USE OF E_S^C IN STRUCTURE-ACTIVITY STUDIES

The E_S^C parameter was first introduced by Hansch and Lien into biological structure-activity studies (20). They showed that the adrenergic blocking activity of β-haloalkylamines (3) are well analyzed in terms of parameters for N-substituents as eq 16 (20). Other earlier analyses made by Hansch and his coworkers are shown in eq 17 and 18.

$$-\log ED_{50}(\text{mole/kg cat}) = \underset{(\pm 1.82)}{3.57}\Sigma\sigma^* + \underset{(\pm 0.43)}{1.11}\Sigma E_S^C - \underset{(\pm 1.09)}{4.43}n_H + \underset{(\pm 1.40)}{11.91}$$

$$n = 10 \quad s = 0.235 \quad r = 0.986 \qquad (16)$$

Hydrolysis of *p*-nitrophenyl alkanoates (4) with serum esterase (21):

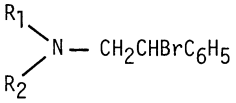
$$\log(\text{Relative Rate}) = \underset{(\pm 7.20)}{-15.65}\sigma^* + \underset{(\pm 0.56)}{2.76}E_S^C + \underset{(\pm 0.67)}{1.77}$$

$$n = 6 \quad s = 0.232 \quad r = 0.995 \qquad (17)$$

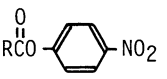
Inhibition of fly-head acetylcholinesterase by phosphoramidates (5) (21):

$$\log K_i = \underset{(\pm 1.30)}{1.08}\Sigma\sigma^* + \underset{(\pm 0.42)}{1.00}\Sigma E_S^C + \underset{(\pm 0.64)}{5.46}$$

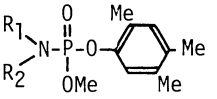
$$n = 8 \quad s = 0.309 \quad r = 0.970 \qquad (18)$$



(3)



(4)



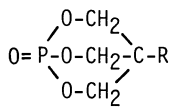
(5)

One of recent analyses performed in this laboratory is that for paralyzing activity of primary amines against American cockroaches. Aliphatic amines such as the cyclohexylamine derivatives are known to exhibit considerable insecticidal and acaricidal activities (22). *Iso*amylamine, produced by the decarboxylation of *L*-leucine which is accumulated in DDT-poisoned insects, has been reported to strongly paralyze houseflies (23). As a part of systematic studies of the toxicity of aliphatic amines, we have found that the toxicity of primary amines is related to E_S^C of the alkyl group (24). Eq 19 analyzes the minimum effective dose (MED, mole/head) which induces paralysis against cockroaches by injection in 30 min. No discernible effect other than steric appears in eq 19. The simple correlation coefficient between E_S^C and π values of substituents used here is only 0.31.

$$-\log MED = \underset{(\pm 0.18)}{-0.54}E_S^C + \underset{(\pm 0.11)}{4.73}$$

$$n = 13 \quad s = 0.185 \quad r = 0.892 \qquad (19)$$

Another example is the analysis of toxicity exerted by bicyclic phosphate esters (6). This class of compounds with suitable 4-substituents (R) are extremely toxic to mammals (25). The 4-Et derivative is the toxic principle in the smoke produced on burning a noncommercial fire-retardant polyurethane foam (26). The analysis of toxicity in terms of LD_{50}(mole/kg mice) determined after 24 hrs of injection gave eq 20 (27, Note b).



(6)

$$-\log LD_{50} = \underset{(\pm 0.25)}{-1.25}\pi^2 + \underset{(\pm 0.91)}{3.51}\pi + \underset{(\pm 0.45)}{0.49}\sigma^* - \underset{(\pm 0.18)}{0.68}E_S^C + \underset{(\pm 0.74)}{2.65}$$

$$n = 18 \quad s = 0.246 \quad r = 0.976 \qquad (20)$$

As evident, there is an optimum hydrophobicity for this class of compounds to reach the site of action which was recently suggested as being synaptic sites of the central nervous system where they antagonize GABA (28). In eq 19 and 20, the sign of the E_S^C term is negative, indicating that the bulkier the substituents, the higher the perturbation with biomacromolecule participating in the critical process for the biological activity.

Note b. With additional substituents and omitting non-alkyl type substituents whose E_S^C value is ambiguous, eq 20 supersedes the previous correlation appearing in ref 27.

DISCUSSION

The above examples show that the E_S^C values are useful for separating the stereospecific factor from others and for evaluating its relative significance in biological activities. In these examples and several others, E_S^C is the best steric parameter. However, this does not mean that E_S^C is always superior to other steric parameters. In fact, the Taft "uncorrected" E_S value has been shown to be a suitable parameter for intra- as well as inter-molecular steric effects of substituents (mostly aromatic) in a number of examples including ortho substituent effects (29), hapten-antibody interactions (30), enzyme-substrate (31) and enzyme-inhibitor complex formations (32,33), and pharmacological (34,35) and pesticidal activities (36,37). Although it is not corrected for the "hyperconjugation effect", the E_S value is the better parameter than the E_S^C value in these cases. Charton advanced evidence that the E_S value is a real parameter for the steric bulk depending on substituent dimensions expressible in terms of van der Waals radii (38). For symmetric-top substituents as H and CX_3 (X = H, Me and Halogen) a linear relationship such as eq 21 was derived by Kutter and Hansch where $r_V(av)$ is the average van der Waals radius (34).

$$E_S = -1.839 r_V(av) + 3.484 \qquad n = 6 \quad s = 0.132 \quad r = 0.996 \qquad (21)$$

Thus, apart from the original definition and without the correction for the "hyperconjugation effect", the E_S value has its own significance as a steric parameter. If the steric effect belongs to the type depending on the "effective" van der Waals width of substituents, the E_S value should be applicable not only to aliphatic but also to aromatic systems.

Then, what is the real significance of the "hyperconjugation effect" in defining the E_S^C value? Since the use of the E_S^C value is justified empirically, it is likely to have its own significance. The term defining the "hyperconjugation effect", $-0.306(3-n_H)$, can be interpreted in another way. Proportional to the number of α-branches, the E_S^C value is more negative than the corresponding E_S by a factor 0.306. Thus, it emphasizes the effect of α-branching of alkyl groups more than E_S. For the E_S^C value of hydrogen, n_H is taken as zero so that $E_S^C(H) = 0.32$ while $E_S(H) = 1.24$. Thus, the E_S^C scale estimates the effective bulk of Me relative to that of H as much smaller than does the E_S scale. We tentatively postulate that the more strict the steric demands between interacting partners, the more suitable the use of E_S^C scale than the use of E_S (Note c).

In order to initiate the mechanism leading to the eventual biological effect, the bioactive compounds must "fit" certain macromolecular targets so as to interact with specific groups located with a particular spatial arrangement. When the specific groups are located within a narrow cleft on the topological architecture of macromolecules, the steric fit for bioactive compounds could be achieved by being engulfed into the cleft with proper orientation and conformation. When the interaction occurs on a broad cleft (or surface) of the macromolecules, the steric effect could be less specific in covering such sites. If the interactions with two or more distant receptor sites are simultaneously required, the distance between two or more functional units of bioactive molecules could be an important factor for the steric complementarity.

The E_S value is the parameter for the width of substituents. The E_S^C value is also considered as a "modified" width parameter. Thus, their application may be limited to interactions occuring on the broad cleft.

Verloop and his coworkers recently developed new steric parameters, "Sterimol substituent constant", representing the length and width of various directions for each of a number of substituents (39). Using specific width and/or length parameters simultaneously, they separated the steric effect and analyzed successfully directional nature of stereospecific requirements of bioactive compounds in certain examples (39). Hansch and his associates proposed to use group molar refractivity, MR, as a measure of the effective volume of substituents (40,41). Although it is also regarded as being a model for the non-hydrophobic attractive forces, the MR value has been successfully applied in a number of enzymic reactions (42, 43). The van der Waals molar volume of substituents was used as the volume-dependent steric parameter for the type of the effect accompanying the engulfment into the cleft for quite a few examples (44,45,46). Thus, depending upon the situation, it is necessary to select suitable set of parameters to delineate the steric effect of bioactive compounds.

With the use of physical-organic models and regression analysis, it is possible to separate the relative significance of stereospecific factor in overall action of bioactive compounds in certain cases. However, it is difficult to definitely identify particular processes where the stereospecificity is most critical with this procedure alone especially when for example

---

Note c. In this respect, the work by A. Babadjamian, M. Chanon, R. Gallo, and J. Metzger appearing in *J. Amer. Chem. Soc.*, 95, 3807 (1973) may be cited. Their result seems to support this postulation.

the whole animal body is used to evaluate the activity. Even so, we believe that the present procedure is an important point from which to start a search for a more rigorous "molecular" mechanism including stereospecificity of the action of bioactive compounds.

Acknowledgments - The author is indebted to Professor Minoru Nakajima for his support and to Professor Morifusa Eto for his supplying unpublished toxicity data of additional bicyclic phosphate ester derivatives. He gratefully extends his appreciation to Chiyozo Takayama for his cooperations including his skillful experimental work and to Yasuhiko Tagoh for his association in a part of the present project.

REFERENCES

1. E. Fischer, *Z. Physiol. Chem.*, 26, 60 (1898).
2. A.J. Clark, *The Mode of Action of Drugs on Cells*, Williams and Wilkins, Baltimore, 1937.
3. C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, 86, 1616 (1964).
4. C. Hansch, *Accounts Chem. Res.*, 2, 232 (1969).
5. C. Hansch, *J. Med. Chem.*, 19, 1 (1976).
6. T. Fujita, *Adv. Chem. Ser.*, 114, 1 (1973).
7. T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, 86, 5175 (1964).
8. L.P. Hammett, *Physical Organic Chemistry*, 2nd Ed., McGraw Hill, New York, 1970, p.347.
9. R.W. Taft,Jr., *Steric Effects in Organic Chemistry*, M.S. Newman, Ed., Wiley, New York, 1950, p.556.
10. K. Hancock, E.A. Meyers, and B.J. Yager, *J. Amer. Chem. Soc.*, 83, 4211 (1961).
11. K. Bowden, N.B. Chapman, and J. Shorter, *J. Chem. Soc.*, 5239 (1963).
12. S.H. Unger and C. Hansch, *Progr. Phys. Org. Chem.*, 12, 91 (1976).
13. T. Fujita, C. Takayama, and M. Nakajima, *J. Org. Chem.*, 38, 1623 (1973).
14. C. Takayama, T. Fujita, and M. Nakajima, in preparation.
15. D. Clotman and Th. Zeegers-Huyskens, *Spectrochim. Acta*, 23A, 1627 (1967).
16. H.C. Brown, D.H. McDaniel, and O. Häfliger, *Determination of Organic Structures by Physical Methods*, Vol.1, E.A. Braude and F.C. Nachod, Eds., Academic Press, New York, 1955, p.634.
17. W.A. Henderson,Jr. and C.J. Schultz, *J. Org. Chem.*, 27, 4643 (1962).
18. A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
19. I.B. Wilson and E. Cabib, *J. Amer. Chem. Soc.*, 78, 202 (1956).
20. C. Hansch and E.J. Lien, *Biochem. Pharmacol.*, 17, 709 (1968).
21. C. Hansch, *Drug Design*, Vol.1, E.J. Ariëns, Ed., Academic Press, New York, 1971, p.329.
22. C.W. Kearns and C.C. Compton, *J. Econ. Entomol.*, 31, 625 (1938).
23. S. Tashiro, E. Taniguchi, M. Eto, and K. Maekawa, *Agric. Biol. Chem.*, 39, 569 (1975).
24. Y. Tagoh, unpublished analysis.
25. E.M. Bellet and J.E. Casida, *Science*, 182, 1135 (1973).
26. J.H. Patajan, K.J. Voorhees, S.C. Packham, R.C. Baldwin , I.N. Einhorn, M.L. Grunnet, B. G. Dinger, and M.M. Birky, *Science*, 187, 742 (1975).
27. M. Eto, Y. Ozoe, T. Fujita, and J.E. Casida, *Agric. Biol. Chem.*, 40, 2113 (1976).
28. N.G. Bowery, J.F. Collins, and R.G. Hill, *Nature*, 261, 601 (1976).
29. T. Fujita and T. Nishioka, *Progr. Phys. Org. Chem.*, 12, 49 (1976).
30. E. Kutter and C. Hansch, *Arch. Biochem. Biophys.*, 135, 126 (1969).
31. C. Hansch, E.W. Deutsch, and R.N. Smith, *J. Amer. Chem. Soc.*, 87, 2738 (1965).
32. C. Hansch, K.H. Kim, and R.H. Sarma, *J. Amer. Chem. Soc.*, 95, 6447 (1973).
33. T. Fujita, *J. Med. Chem.*, 16, 923 (1973).
34. E. Kutter and C. Hansch, *J. Med. Chem.*, 12, 647 (1969).
35. G.H. Hamor and E.J. Lien, *Il Pharmaco*, 24, 704 (1969).
36. T. Nishioka, T. Fujita, K. Kamoshita, and M. Nakajima, *Pestic. Biochem. Physiol.*, 7, 107 (1977).
37. A. Fujinami, T. Satomi, A. Mine, and T. Fujita, *Pestic. Biochem. Physiol.*, 6, 287 (1976).
38. M. Charton, *J. Amer. Chem. Soc.*, 91, 615 (1969).
39. A. Verloop, W. Hoogenstraaten, and J. Tipker, *Drug Design*, Vol.7, E.J. Ariëns, Ed., Academic Press, New York, 1976, p.165.
40. C. Hansch, A. Leo, S.H. Unger, K.H. Kim, D. Nikaitani, and E.J. Lien, *J. Med. Chem.*, 16, 1207 (1973).
41. W.J. Dunn, III., *Eur. J. Med. Chem.*, 12, 109 (1977).
42. C. Hansch and D.F. Calef, *J. Org. Chem.*, 41, 1240 (1976).
43. C. Hansch, J.Y. Fukunaga, P.Y.C. Jow, and J.B. Hynes, *J. Med. Chem.*, 20, 96 (1977).
44. I. Moriguchi and Y. Kanada, *Chem. Pharm. Bull.*, 25, 926 (1977).
45. M. Kiso, T. Fujita, N. Kurihara, M. Uchida, K. Tanaka, and M. Nakajima, *Pestic. Biochem. Physiol.*, 7, in press (1977).
46. A.E. Bird, *J. Pharm. Sci.*, 64, 1671 (1975).