On the Relationship between Electronic State of Molecules and their Biological Actions. I. The Nicotinic Action of Choline Phenyl Ether and the Enzymatic Decomposition of Phenyl Acetate

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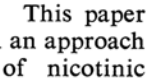
Several attempts have been made in the quantum-chemical approach for explaining the carcinogenic activity of hydrocarbon molecules since Schmidt's earlier work[1], and some indices correlating the electronic configuration of these molecules with their carcinogenic activity were proposed by these investigators[2-4]. Because of complexities in biological processes involved, however, there remain certain ambiguities concerning their legitimacy from the biochemical point of view, even though their apparent usefulness was established. Indeed, there have been some disputes as to which quantum-chemical index is most effective in predicting the carcinogenic activity of hydrocarbon molecules[5]. It seems rather appropriate at present, therefore, that such a quantum-chemical approach is made in the biological research field on the pharmacological actions or on enzymatic reactions, for their mechanism is simpler and can be analyzed further in detail. But such an attempt has not yet appeared. This paper is principally concerned with such an approach of explaining the effectiveness of nicotinic action of choline phenyl ether derivatives and the substrate specificity of choline esterases by applying the frontier electron method of Fukui et al.[6] A preliminary account of some of their results has already been reported[7].

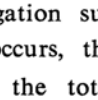
A Perturbation Theory in the LCAO Treatment of Conjugated Molecules Applicable to the Active Center of Enzyme or Receptor

The perturbation theory in the simple LCAO-MO method has been extensively developed by several investigators (Wheland[8], Coulson and Longuet-Higgins[9], Dewar[10], Mulliken and his co-workers[11], and Fukui et al.[6]) and has been applied successfully to the problem of chemical reactivity of molecules containing π-electrons. Long since, some physiologists and pharmacologists assumed tacitly that the drugs combined with so-called "receptors" of living organism as substrates did with active sites of an enzyme. Provided that the substrate or drug molecules contain π-electrons, it seems highly probable that such a complex formation is due to hyperconjugation of the π-part of the active site of enzyme or receptor to that of substrate or drug molecules.

On the other hand, recent developments in MO theoretical treatment of chemical reactivity in conjugated molecules have made a great contribution to the study of the mechanism of aromatic substitutions. One of these analyses was a theoretical treatment of hyperconjugation by Mulliken et al.[11] which occurred with the CH_2 group on $C_6H_7^+$ ion  . In this ion, the H_2 group was considered to be an atom (pseudo-atom) that has a (ls-ls) π-type orbital (quasi-π orbital). Such a treatment was also applied to a transition state of aromatic substitution by Fukui et al.[6] These workers pointed out an importance of hyperconjugation which occurs with the CBX group on  and derived an effective reactivity index, "superdelocalizability", from the stabilization energy due to this hyperconjugation. These treatments appear to be applicable to analysis of the transition state of enzyme-substrate or drug-receptor complex, to some extent at least. Adopting the method of perturbation theory proposed by Fukui et al.[6], therefore, the present authors attempted in this section a general formulation of the effect of conjugated molecules on the perturbation energy of their separating the properly assumed mesomeric group, its applicability to some biological

1) O. Schmidt, *Z. physik. Chem.*, **42**, 88 (1939).
2) A. Pullman and B. Pullman, *Rev. Sci.*, **84**, 145 (1946); "Concerisation par les Substances chimiques et Structure moleculaire" Mass on et Cei, Paris (1955).
3) C. A. Coulson, *Adv. Cancer Research*, **1**, 1 (1953).
4) C. Nagata, K. Fukui, T. Yonezawa and Y. Tagashira, *Cancer. Res.*, **15**, 233 (1955).
5) K. Fukui, T. Yonezawa and C. Nagata, *J. Chem. Phys.*, **31**, 550 (1959).
6) K. Fukui, T. Yonezawa and C. Nagata, This Bulletin, **27**, 423 (1954); *J. Chem. Phys.* **27**, 1247 (1957).
7) Y. Shinagawa and A. Inouye, *Kagaku*, **29**, 608 (1959); Y. Shinagawa, A. Inouye and T. Ban, ibid., **29**, 659 (1959).
8) G. W. Wheland, *J. Am. Chem. Soc.*, **64**, 900 (1940).
9) C. A. Coulson and H. C. Longuet-Higgins, *Proc. Roy. Soc.*, **A191**, 39 (1947).
10) M. J. S. Dewar, *J. Am. Chem. Soc.*, **74**, 3355 (1952).
11) L. W. Pickett, N. Muller and R. S. Mulliken, *J. Chem. Phys.*, **21**, 1400 (1953).

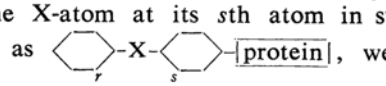
reactions being demonstrated in the following section.

Consider a certain atom X in an active center of enzyme or receptor approaching to the rth atom in the π-part of a substrate molecule. Provided that a hyperconjugation such as the CHX group on  occurs, the secular equation of the π-part of the total system containing the quasi-π orbital is, in a simple LCAO-MO treatment, written as

$$D_1(\lambda)=(h-\lambda)\Delta_1(\lambda)-\gamma_1^2\Delta_{1rr}(\lambda)=0 \qquad (1)$$

where

$$\Delta_1(\lambda)=\begin{vmatrix} a_{11}-\lambda & a_{12} \cdots\cdots a_{1n} \\ a_{21} & a_{22}-\lambda \ \ldots a_{2n} \\ a_{n1} & \cdots\cdots\cdots\cdots a_{nn}-\lambda \end{vmatrix}$$

and $\Delta_{1rr}(\lambda)$ is the minor of $\Delta_1(\lambda)$ corresponding to the element $a_{rr}-\lambda$, a_{ii} and $a_{ij}(i\neq j)$ are the well-known Coulomb and resonance integrals respectively, h and γ_1 are the Coulomb integrals of a properly assumed pseudo-atom and the resonance integral between this pseudo-atom and the rth atom of the substrate molecule respectively. All the quantities which represent energy are expressed in units of $(-\beta)$, all the overlap integrals being neglected. As stated above, however, the hyperconjugation of the π-part in the receptor or enzyme should be further taken into consideration. Assuming that the π-part of enzyme or receptor, whose secular determinant is $\Delta_2(\lambda)=0$, conjugates with the X-atom at its sth atom in such a manner as  , we can write the perturbation determinant as follows:

$$D(\lambda)=(h-\lambda)\Delta_1(\lambda)\Delta_2(\lambda)-\gamma_1^2\Delta_{1rr}(\lambda)\Delta_2(\lambda) -\gamma_2^2\Delta_1(\lambda)\Delta_{2ss}(\lambda)=0 \qquad (2)$$

where γ_2 is the resonance integral between the X-atom and the sth carbon atom in the active center. Considering the resonance integrals, γ_1 and γ_2 to be small perturbation terms, we can obtain the perturbation energy, ΔE.

When h is equal to λ_l, the highest occupied orbital of substrate, the perturbation energy is obtained as follows:

$$\Delta E=2\sqrt{-\frac{\Delta_{1rr}(\lambda_l)}{\Delta_1'(\lambda_l)}}\cdot\gamma_1+O(\gamma_1^2,\gamma_2^2)$$

and

$$-2\frac{\Delta_{1rr}(\lambda_l)}{\Delta'(\lambda_l)}=f_r^{(E)}$$

thence $\Delta E\simeq\sqrt{2}\,(f_r^{(E)})^{1/2}\cdot\gamma_1$

As shown in the following section, pharmacological potency or susceptibility to an enzymatic hydrolysis of homologous compounds has a fairly good parallelism to their f_r at a definite position, which is supposed, from the physiological point of view, to be involved in their reaction processes. The above relation might be said, therefore, to provide a quantum-chemical ground for such a supposition, suggesting a possibility that f_r is a fairly good index for estimating the magnitude of ΔE under a certain condition. On the other hand, superdelocalizability, which was considered by Fukui et al.[6] as one of the most useful index for the reactivity of π-electrons in a molecule was defiined as

$$S_r=\sum_{\text{occ}}\frac{2(C_r^{(i)})^2}{\lambda_i}$$

In the limit of $h\to 0$ and $\gamma_2\to 0$, therefore, it is apparent that ΔE is proportional to S_r. Thus the perturbation energy of complex formation is expected under a certain condition to vary nearly parallel to the superdelocalizablility of frontier electron in the substrate molecules.

It is obvious from the above discussions that, the larger the frontier electron density or superdelocalizability of the substrate or drug molecules, the larger the perturbation energy ΔE. Thence it would be expected that the susceptibility of homologous compounds to a certain enzyme or their pharmacological activity runs parallel with their frontier electron density or superdelocalizabillity. It will be shown in the following section, that the indices f_r or S_r have a correlation with the magnitude of drug actions or the rate of enzymatic hydrolysis.

Applications of the Frontier Electron Method

Electronic Structure of Choline Phenyl Ether and their Nicotinic Action.—Taking into account the above-mentioned results, the nicotinic action of choline ether derivatives was analyzed from the view-point of their electronic structure. From the pharmacological point of view, the group of $(CH_3)_3N\cdot CH_2\cdot CH_2-$ of these compounds is considered to react with anionic sites of chemoreceptor protein, while the difference in their potency would chiefly originate from the conjugated groups X-  -O- which is assumed to react with esteratic sites of the receptor[14,15]. Some workers suggested that the potency of these drugs in the depressant action was principally determined by electrostatic

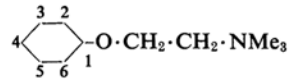
12) H. H. Jaffe, ibid., **20**, 279 (1952).
13) K. Fukui, C. Nagata and T. Yonezawa, *J. Am. Chem. Soc.*, **80**, 2267 (1958).
14) D. Nachmansohn, "Chemical Mechanism of Nerve Activity" in "Modern Trends in Physiology", Ed. by E. S. G. Barron, Academic Press, New York (1952); "Chemical and Molecular Basis of Nerve Activity", Academic Press, New York (1959).
15) R. B. Barlow, "Chemical Pharmacology", Methuen, London (1955).

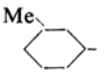
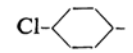
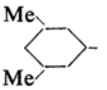
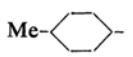
TABLE I

	Ester O	I	Br	Cl	CH_3	MeO	NO_2	N^+Me_3
k	2	1.5	1.8	2	3	1.8	$k_N=1$, $k_O=1$	0
δ	0	0.3	0.4	0.5	−0.1	0	0.2	−0.1
ρ	1	0.5	0.7	0.8	1	0.9	1	0

$\alpha_i=\alpha+k_i\beta$, $\alpha_\delta=\alpha+\delta\beta$, $\beta_{i,j}=\rho_{i,j}\cdot\beta$; $\lambda=(E-\alpha)/\beta$

TABLE II. NICOTIN-LIKE ACTIVITY OF ETHERS OF CHOLINE



	Activity*		$f_1^{(1)}+f_1^{(2)}$	$f_4^{(1)}+f_4^{(2)}$
	Suprarenals intact	Suprarenals ligated		
	337	268	0.629	0.613
	370	258	0.628	0.538
	220	192	0.570	0.596
	100	100	0.605	0.608
	13.1	13.5	0.542	0.729
	10.4	10.1	0.522	0.522
	5.2	5.2	0.410	0.907
	0.4	0.47	0.513	0.440

* Relative activity (molar) on blood-pressure of cats after atropin.

bonding between the negative charge on the atom of 1-position adjacent to the ether *O*-atom and the positive charge on the esteratic site[16]. As the present treatment of first approximation, therefore, the conjugated group X-  -O- alone was considered.

The value of integrals concerning the hetero-atom is given in Table I, in which the parameters k, ρ and δ are defined as follows:

$$\alpha_o=\alpha+k_o\beta,\ \alpha_{Cl}=\alpha+k_{Cl}\beta,\ \alpha_\delta=\alpha+\delta\beta$$

$$\beta_o=\rho_o\beta \quad \text{and} \quad \beta_{Cl}=\rho_{Cl}\beta$$

These values used for calculation do not differ much from those of other authors, which have lead to reasonable results in some other problems[12,13]. The Coulomb integral of an oxygen atom in an ether bond is taken as $\alpha+3\beta$, that of carbon atom attached to it being $\alpha+0.3\beta$. Solving the secular equations, the electron densities of the first and second highest occupied levels, $f_r^{(1)}$ and $f_r^{(2)}$, and the approximate superdelocalizability, $S'_r=f_r^{(1)}/\lambda_1+f_r^{(2)}/\lambda_2$, are calculated. These two levels are very close to each other.

The calculated electron densities of two highest occupied levels of the positions 1 and 4, ($f_1^{(1)}+f_1^{(2)}$, $f_4^{(1)}+f_4^{(2)}$) are given in Table II, which demonstrates evidently that $f_1^{(1)}+f_1^{(2)}$ shows a distinct parallelism with the magnitude of activtiy of choline ethers observed on blood pressure of cats (Hey[16]). But the parallelism between $f_4^{(1)}+f_4^{(2)}$ and their activity is somewhat slighter, while neither $f_{(r)}^{(1)}+f_{(r)}^{(2)}$ at other positions nor their S'_r have such a correlation with their potency of nicotinic action. On the other hand, Imamura[17] has

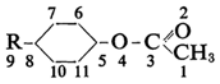
16) P. Hey, *Brit. J. Pharmacol.*, **7**, 117 (1952).

17) A. Imamura, personal communication.

TABLE III. SUBSTRATE SPECIFICITY OF BUTYROCHOLINESTERASE

R	Rate*	$f_5^{(E)}$	q_5	$s_5^{(E)}$	$s_3^{(N)}$	q_4
Me_3N^-	95	0.498	0.949	0.821		1.724
H	91	0.495	0.946	0.973	1.608	1.762
Me	65	0.459	0.984	0.944	1.520	1.764
MeO	51	0.454	0.987	0.955	1.521	1.764
NO_2	36	0.426	0.886	0.671	1.720	1.756
Cl	28	0.442	0.927	0.757	1.553	1.760
Br	28	0.440	0.932	0.770	1.539	1.760
I	28	0.437	0.932	0.765	1.539	1.766
tert-Bu	15	0.419				

* Percent maximum rate of hydrolysis of Ach.



recently made calculation of S_r on these compounds and found a fairly good parallelism between S_4 and their activity, a fact which suggests that the approximation of S'_r is not so satisfactory.

The results appear, however, to suggest that ethers of choline form weak bondings with the active sites of the receptor at 1- and 4-position, this process being rate-determinant. In this case, the energy level of substrate's frontier electron would be considered to be close to that of the active center in the receptor. The correlation of f_1 with their potency is not a so unexpected one, for this position corresponds to that of acetylcholine which is bound to the esteratic site of Nachmansohn's acetylcholinesterase model. On the other hand, the correlation of f_4 or S_4, though somewhat less than that of f_1, may be considered to provide support to Hey's view[16].

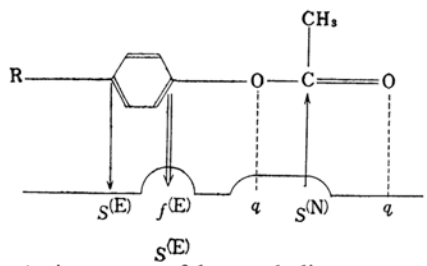
The quantity f_r, as well as S_r, are considered as an index for discussing the reactivity in electrophilic reaction. But, as stated above, the condition for f_r to correlate with ΔE differs from that for S_r. Taking Imamura's results into account, it might be said, therefore, that the two positions of drug molecules would be attached to two positions of the receptor different from each other in their nature. Such a speculation does not conflict with the suggested concept of the esteratic site of the receptor.

Electronic Structure of Phenyl Esters and Substrate Specificity of Cholinesterase.—The electronic structure of choline phenyl ethers has a similarity to that of phenyl acetate, a substrate of cholinesterase, and so we calculated them by the method of LCAO. As indices of reactivity, electron density q_r, frontier electron density f_r and superdelocalizability S_r are calculated by solving the secular equations crresponding to Eq. 1. The values of indices and the rate of hydrolysis by butyrocholinesterase partially purified from human plasma (Whittaker[18]) are given in Table III. A distinct parallelism between frontier electron density f_5 and the magnitude of rate of hydrolysis is clearly seen, only an exception is the case on nitrophenol ester. S_5 shows a slighter correlation with their rate of hydrolysis, while neither f_r nor $S'_r=f_r^{(1)}/\lambda_1$ at any other position in the molecules have such a correlation with their substrate specificity of butyrocholinesterase. In this caes, approximate superdelocalizability S_r' was also found to vary not always parallel to S_r so that their appoximation can said to be not so satisfactory as already suggested in the section above. In view of their similarity to the choline ether derivatives in the molecular structure and pharmacological nature, the position 5 of these compounds corresponds to the position 1 of the latter and so the correlation found on f_5 is not a so unexpected one. The possible sites of an ester molecule making bond with the active center of butyrocholinesterase would be probably such as illustrated in Fig. 1, which can be referred to Nachmansohn's model of true cholinesterase. In this case, the position which should be considered at first as rate-determinant would be position 5.

On the other hand, parallelism was not so striking between the magnitude of rate of hydrolysis of phenyl acetates by acetylcholinesterase and the indices of their electronic structure as in the case of butyrocholinesterase. It is found, however, that the rate of hydrolysis have a slight correlation with indices on the position 8 (except the NO_2-derivative), whose distance to ether oxygen atom is close to that of quarternary nitrogen atom in Ach. This fact seems concordant with Nachmansohn's model of acetylcholinesterase, in which the distance between nitrogen atom and ether oxygen atom can affect the rate of enzymatic hydrolysis. Nevertheless the bonding between the active center and the position 8 in phenyl acetate may

18) L. A. Mounter and V. P. Whittaker, *Biochem. J.*, **54**, 551 (1953).

Phenyl acetates



Active center of butyrocholinesterase

Fig. 1a. The possible sites of ester molecule masking bond with active center of butyrocholinesterase.

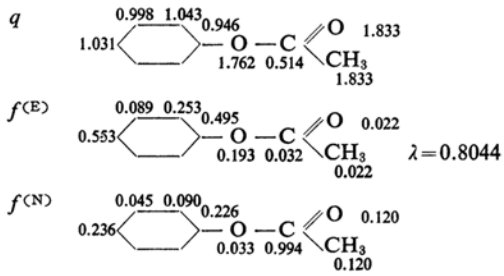
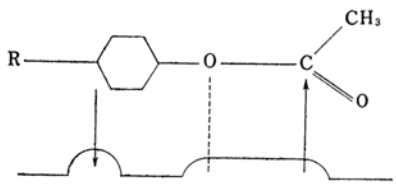


Fig. 1b. Molecular diagram of phenyl acetate.

TABLE IV. ACETOCHOLINESTERASE

R	% rate of hydrolysis	$S_8^{(E)}$	$f_8^{(E)}$	$S_8^{(N)}$	$S_3^{(N)}$
H	60	0.962	0.552	0.781	1.536
I	48	0.948	0.486	0.756	1.539
MeO	47	0.928	0.431	0.755	1.521
Me	45	0.938	0.492	0.765	1.520
NO_2	40	0.827	0.475	0.654	1.720
Cl	18	0.909	0.492	0.754	1.541
Br	15	0.916	0.488	0.752	1.539



Active center

be not so simple as that of Nachmansohn's model. Based on Wilson's results, Nachmansohn maintained that Coulomb force between quarternary nitrogen and active center is one of the essential factors on the bonding of enzyme-substrate complex, but in our results electron density q_8 has no clearer parallelism to the rate of reaction.

Wilson[19] employed the Michaelis-Menten constant as the basis of estimating contribution of electrostatic energy to enzyme-substrate (or -inhibitor) complex formation. As pointed out elsewhere[20], however, use of this constant involves a certain risk of erring in calculation of free energy change of such a complex catalytic reaction by a factor of ν (ν is the stoichiometric number in the rate-determinant process). It is a well-known classical theorem that $\vec{k}/\overleftarrow{k}=K$, where $\vec{k}$ and $\overleftarrow{k}$ are the forward and backward rate constants and K is the equilibrium constant. This theorem has been applied hitherto to the Michaelis-Menten relation of enzyme reaction,

$$E+S \underset{\overleftarrow{k}}{\overset{\vec{k}}{\rightleftarrows}} ES \xrightarrow{k} E+P$$

(E enzyme, S substrate, ES enzyme-substrate complex)

The Michaelis-Menten constant given by $K_m=\vec{k}/\overleftarrow{k}$ was used to calculate the free energy change of this reaction by Wilson as $-\Delta F=RT\ln K_m$. It was already clearly demonstrated by Horiuti[21], that a theorem $(\vec{k}/\overleftarrow{k})^\nu=K$ was valid for any reaction having the stoichiometric number ν. Enzymatic reactions should have such complex rate processes that use of the Michaelis-Menten constant is allowable only for a special case of $\nu=1$. Hence, Wilson's results could not be regarded as a sufficient evidence for electrostatic bonding, other bonds such as hydrogen bonding being also allowable. Furthermore, γ-dimethylbutylacetate, which has no positive charge on the corresponding position, is hydrolyzed by acetylcholinesterase at almost the same rate as acetylcholine[15], a fact which seems also unfavorable to Nachmansohn's view. The difference of substrate specificity found between butyro- and acetylcholinesterase suggests that the quantum-chemical process involved in their reactions are different ones. The bonding mechanism of the latter complex seems to be more complex than the former one. The mechanism of enzymatic reaction proposed in the previous section may not be a rate-determinant in this case. It seems worthy to note here, however, that $S_8^{(N)}$ has better correlation with a rate of hydrolysis than other indices (Table IV), and so the negative charge on the position 8 should be considered at least to affect bonding of the complex.

19) I. B. Wilson, "The Mechanism of Enzyme Action", Ed. by W. D. McElroy and B. Glass, Johns Hopkin's Press, Baltimore, Maryland (1954).

20) A. Inouye and Y. Shinagawa, *Kagaku*, **30**, 481 (1960).

21) J. Horiuti, This Bulletin, **13**, 210 (1938); *Z. physik. Chem., N. F.*, **11**, 358 (1957).

Comment

Fukui and his co-workers introduced the frontier electron method and have obtained hitherto many fruitful results in their studies on reaction mechanism of aromatic hydrocarbons. As shown above, a fairly good correlation could be also proved between the π-electron density or its superdelocalizability of a certain position calculated by this method and nicotinic action of phenyl ether derivatives of choline or rate of enzymatic hydrolysis of phenyl acetate derivatives. The position, which was found to have such a correlation, is also expected from a pharmacological and chemical point of view. A possibility of such a correlation is demonstrated theoretically under an assumption that the active site of the receptor (or enzyme) has also π-electron system and a complex is formed between active sites and durgs by hyperconjugation through the π-electron system, their highest occupied level being very close to each other. This assumption is rather bold, but seems not so unreasonable: enzyme substrate complex or receptor-drug combination has been widely assumed in enzymological or pharmacological studies, and in the complex assumed, it is only required that π-electron can be transfered and hence its bonding is not needed to be a covalent one, hydrogen bonding being also allowable. In view of these facts the present invetigative approach might be said to demonstrate a possibility of explaining the drug actions at the molecular level. At least, the results suggest that the electronic configuration of molecules is important in quantitative interpretation of drug actions or substrate specificity of enzyme action and qualitative electronic explanation in these fields would be replaced by its systematic computation by an adequate quantum-chemical treatment in future.

Summary

1. Applying the frontier electron method of Fukui et al., it was attempted to explain the effectiveness of nicotinic action of some choline phenyl ether derivatives and the substrate specificity of some phenyl acetates in the enzymatic hydrolysis.

2. A fairly good correlation could be proved between the frontier electron density (f_r) or its superdelocalizability (S_r) of a certain position of these compounds and their potency or susceptibility to hydrolysis.

3. Assuming that the active site of receptor (or enzyme) has also π-electron systems and a drug-receptor (or -enzyme) complex is formed by hyperconjugation through π-electron systems, a general formulation of the effect of such a hyperconjugation on the perturbation energy of their system was attempted. It was demonstrated, qualitatively at least, by our calculations, that the larger the f_r of drug molecules or their S_r the larger the perturbation energy and so a possibility of above-mentioned correlation was predicted to some extent from the quantum-chemical basis.

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