# The Use of Molecular Orbital Calculations as an Aid to Correlate the Structure and Activity of Cholinesterase Inhibitors

W. Brock Neely

*The Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan*

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SUMMARY

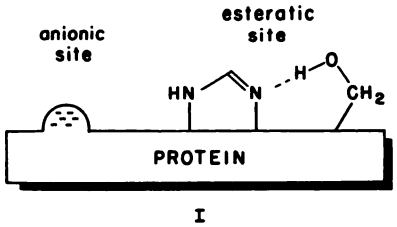
This study is an attempt to use molecular orbital (MO) calculations as an aid in correlating chemical structure with the ability of the material to act as a cholinesterase inhibitor. Several different carbamates and organophosphates were examined in this fashion. The interpretation of the results illustrates the utility of MO theory in correlation studies. In addition, it suggests a new way of looking at the action of certain inhibitors of cholinesterase.

Our previous work with a series of organophosphates as cholinesterase inhibitors gave us an interest in the area of structure-activity relationships (1). This interest stimulated a desire for a closer look at the correlation that existed between the structure of the chemical and its biological activity. A great deal of information is known about the nature of the catalytic site in cholinesterase. The books by O'Brien (2) and Heath (3) as well as articles by Wilson *et al.* (e.g., 4) give an excellent review of this subject. What was needed was a technique for describing the structure of the inhibitor in greater detail. With the advent of high-speed computers, there has been an explosive growth in the utility of molecular orbital (MO) calculations to problems of biological interest (5). Consequently, the application of MO theory appeared to be the best candidate for satisfying this need.

### *The Enzyme Model*

The evidence we have indicates two essential sites for the enzyme molecule, these are the anionic and the esteratic site. Not much is known about the anionic site other than that it is situated at an appropriate distance from the esteratic site for maximum binding of the acetylcholine substrate (4). The esteratic site, on the other hand, has been investigated rather thoroughly. It is not my purpose to examine all the evidence for or against any proposed model. What I would like to do is to accept the model that has received the strongest support and continue from there [see the book by Heath (3) for a summary of this area]. With this provision we see that the esteratic site consists of the serine hydroxyl plus a neighboring imidazole group from histidine (I). The proximity of these two amino acids is probably a result of the tertiary structure of the protein (6) rather than of being adjacent to each other on the peptide backbone.

The only thing we can say about the anionic site is that it must be an area of high electron density.




I

In a paper by O'Brien (7) it was suggested that the distance between the ester-

atic and the anionic site might vary depending on the species from which the cholinesterase was isolated. He then went on and made some rough calculations as to what these distances might be. The results indicated that for fly head cholinesterase the distance was 4.5–5.9 A and for erythrocyte cholinesterase it was less than 4.5 A. Friess and Baldridge (8) arrived at a value of 2.5 A for electric eel cholinesterase. Using this as a lower limit and assuming the values cited by O'Brien, Fig. 1 was constructed. The Dreiding-Stereomodels (distributed by G. M. Instrument Co., Greenville, Illinois) were used to estimate the maximum and minimum distances between the starred atoms. Realizing the drastic assumptions that were used in making this figure, I still would like to use the graph in the following discussion. Several interesting implications result which suggest further exploration. The use and interpretation of the figure will be presented in later sections of this paper.

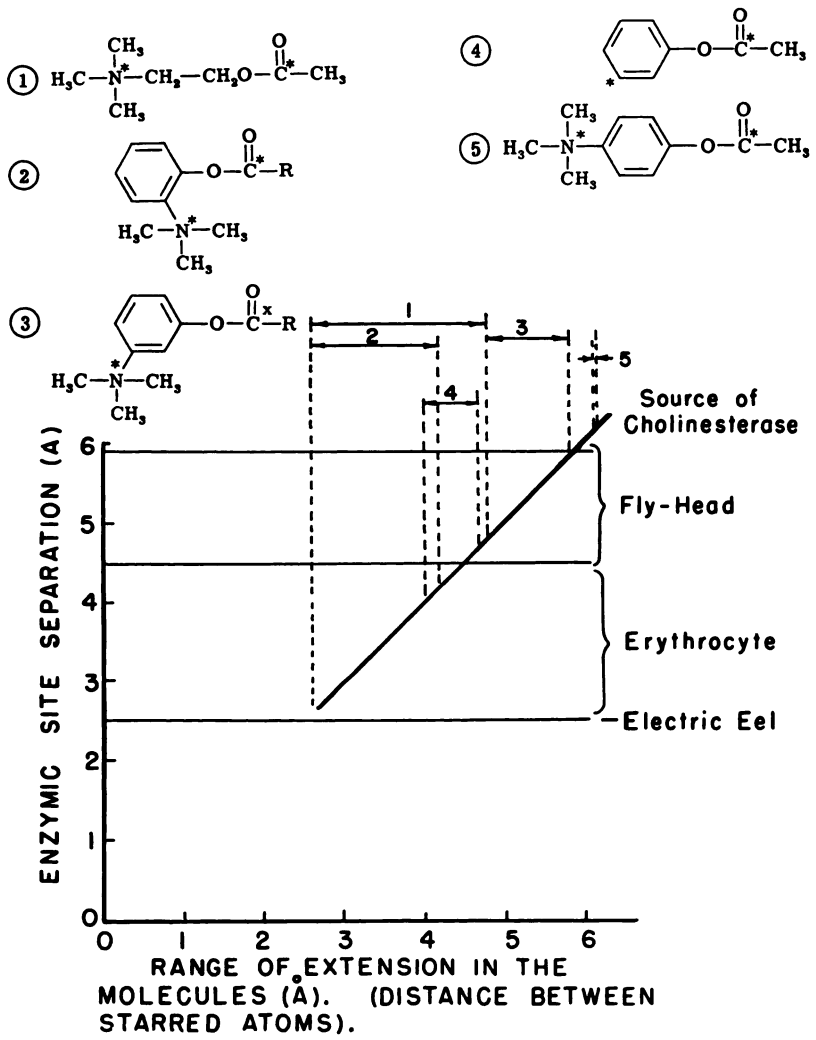
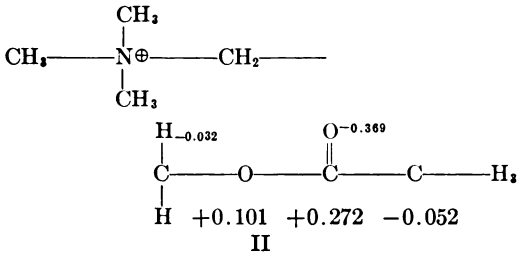



FIG. 1. *A comparison of the range of distances between the esteratic and anionic site for various cholinesterase systems and the distances between the carbonyl C and the quarternary for various inhibitors*

*Action of the Enzyme on Substrate and Inhibitor*

Using the values in Table 1, Pullman and Pullman (5) derived the following values for the net charges of the critical atoms in acetylcholine (II), the natural substrate for the cholinesterase enzyme.




II

The plus means lack, and the minus means an excess, of electrons in the present convention. With this representation of the ground state of the molecule it can readily be visualized that an attacking nucleophile such as $OH^-$ will seek out the C of the carbonyl group as the best possible site. Once interaction of the electron clouds has occurred, there will be a general perturbation of the entire system and the reaction scheme shown in Fig. 2 will logically follow.

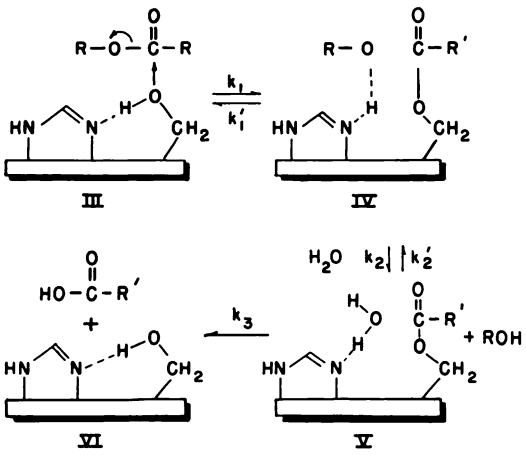



FIG. 2. *Possible model for the action of cholinesterase and its substrate*

The function of the imidazole group is to activate the serine hydroxyl making the oxygen a strong enough nucleophile to carry out the attack on the incoming ester (III). Once the ester linkage has been broken, we have liberation of the alcohol in (V). This makes the imidazole available to activate water, causing the subsequent release of the acyl group and the restoration of the active site for further catalysis (VI). This is an adaptation of the mechanism postulated by Spencer and Sturtevant (9) for the action of chymotrypsin.

Using this model for the action of the enzyme on acetylcholine [where R = $(CH_3)_3N^+CH_2CH_2$— and R′ = $CH_3$—] the organophosphates and carbamates (10, 11) have been considered to act in a similar fashion. One of the major differences between these two classes of inhibitors is the rate of the reactivation step, $k_3$. In the case of the phosphorylated enzyme, $k_3$ is very slow while the carbamates are hydrolyzed at a measurable rate (10, 11).

*Molecular Orbital Calculations*

The chemical reactions studied in biochemistry are determined by the behavior of electrons and the interaction of these electron clouds between different molecules. Furthermore, it is the mobile electrons in the molecule, the so-called $\pi$ electrons, that are responsible for many of the chemical and physical properties. Our interpretation of biochemical phenomena, consequently, is going to be just as good as our ability to describe the behavior of these $\pi$ electrons.

The best system that we have at present for such a description is the MO method. Using this technique, remarkable success has been achieved in interpreting the biochemical reactions of such molecules as the purines and pyrimidines (5). In essence, the method considers each mobile or $\pi$ electron as occupying a molecular orbital $\Psi$ extending over the whole framework. This orbital is approximated by a linear combination of all the available atomic orbitals $\lambda_r$ (1).

$$\Psi = \sum_r C_r \lambda_r \qquad (1)$$

These MO's are eigenfunctions of the Hamiltonian operator in the Schrödinger wave equation (2).

$$\mathbf{H}\Psi = E\Psi \qquad (2)$$

Substituting (1) into (2) and making use of the variation principle it is possible to find a set of coefficients $C_r$ which will give the best value for the energy of the MO. From this calculation the various electronic indices for each atom may be determined. In solving the secular determinant that is generated by the use of the variation principle, certain values for the coulomb and resonance integral must be used. The values used in the following calculations were taken from Pullman and Pullman (5) except where indicated and are listed in Table 1.

TABLE 1
*The parameters used for heteroatoms in the LCAO[a] calculations*

| Atom | Coulomb integral ($\delta$)[b] | Bond integral ($\eta$)[b] |
|---|---|---|
| C=N— | $\delta_n = 0.4$ | 1 |
| C—N— | $\delta_n = 1$ | 0.9 |
| C=N—$^{\oplus}$ | $\delta_n = 2$ | 1 |
| C=O | $\delta_0 = 1.2$ | 2 |
| C—O— | $\delta_0 = 2$ | 0.9 |
| C—Cl[c] | $\delta_{Cl} = 2$ | 0.4 |
| $C_1$—$C_2$≡$H_3$[d] ($C_1$ = aromatic C, and $C_2$≡$H_3$ = methyl group) | $\delta_{C_1} = -0.1$<br>$\delta_{C_2} = -0.2$<br>$\delta_{H_3} = -0.5$ | $\eta_{C_1-C_2} = 0.8$<br>$\eta_{C_2\equiv H_3} = 3$ |

[a] LCAO = Linear Combination of Atomic Orbitals.

[b] The $\delta$ and $\eta$ values were values by which the corresponding coulomb and bond integrals for the heteroatoms are increased or decreased as compared to the values for carbon and the C=C bond.

[c] In addition, an auxiliary inductive parameter is included for the attached carbon. This is given a value of 0.2 for the coulomb integral. All these values were taken from Streitweiser (12).

[d] These values were taken from Streitweiser (12).

The MO calculations will be confined to those groups that are aromatic in nature. The reason for this selection is twofold: (a) by far the greatest number of inhibitors are represented by this group and (b) at the present time these are the easiest materials on which to make the calculations. One more word of explanation; since many of the substituted aromatic groups are common to both the organophosphates and carbamates, it was decided to make the calculation on the corresponding alcohol rather than on the entire ester. While the perturbations in the electron cloud caused by a change in the R group will have some influence on both the carbonyl and the phosphorus groups, our present theories are not sensitive to such an effect. Consequently, the net charges calculated for the alcohol will be essentially the same for the corresponding ester. Table 2 gives the results of a series of such calculations along with the corresponding pK of the phenol. Figure 3 is a plot of the sum of the electron

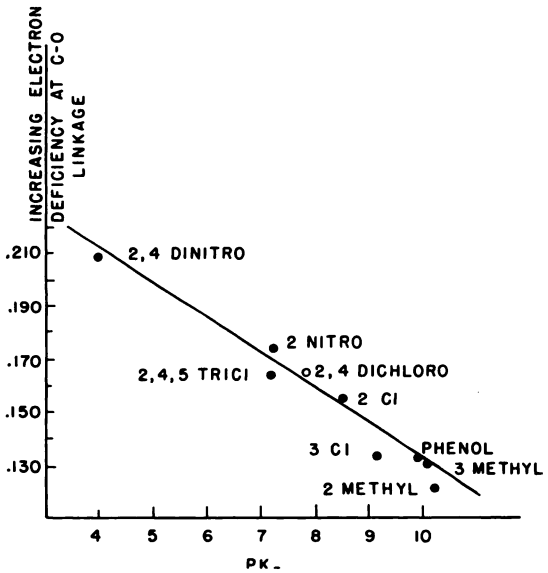


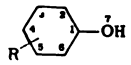

FIG. 3. *The correlation between the pK and the calculated electron deficiency at the C-O centers for various substituted phenols*

deficiency at the C (phenyl) and O atoms (a measure of the ease with which the phenol dissociates) and the pK (experimentally determined value of the same phenomenon).

In examining these data, I would like to make a few comments regarding the hyperconjugation effect of the methyl group. The parameters chosen for this calculation are those listed by Streitweiser (12) which probably overemphasize the magnitude of

TABLE 2

*Calculation of net electron charge on a series of substituted phenols. The experimentally determined pK is given in the last column*



| Substituent R | Net electron charge (These results were calculated by The Dow Computation Laboratory) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ | O | R | pK |
| | +0.056 | −0.049 | +0.003 | −0.036 | +0.003 | −0.049 | +0.076 | | 9.9 |
| 3-Cl | +0.055 | −0.029 | −0.063 | −0.016 | +0.002 | −0.037 | +0.076 | +0.015 | 9.2 |
| 2-Cl | +0.076 | −0.116 | +0.024 | −0.037 | +0.015 | −0.050 | +0.079 | +0.014 | 8.4 |
| 2,4 di Cl | +0.087 | −0.117 | +0.044 | −0.103 | +0.035 | −0.051 | +0.081 | +0.014[a] | 7.7 |
| 2,4,5-tri Cl | +0.086 | −0.105 | +0.043 | −0.084 | −0.031 | −0.030 | +0.080 | +0.015[b] | 7.2 |
| 2-$CH_3$ | +0.039 | −0.005 | −0.018 | −0.035 | −0.01 | −0.04 | +0.075 | −0.04 | 10.2 |
| 3-$CH_3$ | +0.057 | −0.062 | +0.045 | −0.055 | +0.004 | −0.070 | +0.076 | −0.04 | 10.1 |
| 2-$NO_2$ | +0.090 | −0.05[c] | +0.030 | −0.035 | +0.031 | −0.050 | +0.084 | +0.287[c] | 7.2 |
| 2,4 di $NO_2$ | +0.117 | −0.049 | +0.058 | −0.037 | +0.064 | −0.048 | +0.090 | +0.288[c,d] | 4.0 |

[a] Both chlorines have net charge of +0.014.
[b] All three chlorines have net charge of +0.015.
[c] This figure refers to net charge on the nitrogen.
[d] Both nitrogens have similar net charge.

the effect. However, as Streitweiser points out, the hyperconjugation of $\pi$-$\sigma$ conjugation becomes a significant energy factor in excited state. Suffice it to say that the calculations for the ground state of the molecule may be exaggerated; however, they indicate the potential and the direction of the effect when the molecule enters an excited state.

### *Carbamates*

The first group of inhibitors that will be discussed are taken from the paper by Kolbezen *et al.* (13). These are a series of *N*-methyl carbamates and are shown in Table 3 along with the inhibitor constants as calculated by these workers. Assuming that these compunds carbamylate the enzyme in a manner described by Wilson *et al.* (10, 11) then the rate of reactivation ($k_3$, Fig. 2) will be identical for the entire series. It follows from this identity that the difference in potency of these various carbamates must reside in the variation of either $k_1$, or the efficiency with which the intact molecule binds to the enzyme, or both. In examining the first four compounds

TABLE 3

*Anticholinesterase activity of a series of N-methylcarbamates of various phenols*[a]

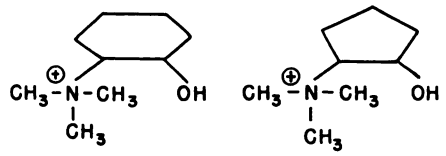
| *N*-Methylcarbamate | Inhibitor constant[b] |
|---|---|
| 1. *o*-Methylphenyl | $1.0 \times 10^{-4}$ |
| 2. *p*-Methylphenyl | $1.0 \times 10^{-4}$ |
| 3. Phenyl | $2.0 \times 10^{-4}$ |
| 4. *m*-Methylphenyl | $8.0 \times 10^{-6}$ |
| 5. *o*-Nitrophenyl | $5.0 \times 10^{-8}$ |
| 6. *o-tert*-Butylphenyl | $6.0 \times 10^{-6}$ |
| 7. *m-tert*-Butylphenyl | $4.0 \times 10^{-7}$ |
| 8. *m*-Dimethylaminophenyl methiodide | $1.6 \times 10^{-8}$ |

[a] Data taken from a paper by Kolbezen *et al.* (13).
[b] Molar concentrations for 50% inhibition of fly-brain cholinesterase.

in Table 3 it appears reasonable to assume that compound 4, the 3-methyl phenyl derivative, will have an increased activity over the other three because of the similarity of this structure to acetylcholine; i.e., the distance between the 3-methyl and the carbonyl carbon is the same as the methyl group and carbonyl carbon in acetylcholine. It is most encouraging to

examine the MO calculations in Table 2 which indicate that the 3-methyl phenol has a positive charge on C-3. This gives compound 4 (Table 3) an electronic similarity as well as a structural similarity to the natural substrate. As we have said before, the parameters used in the MO calculations probably overemphasize the effect in the ground state of the molecule. However, it is conceivable that the enzyme inhibitor complex perturbs the ground state of the molecule, making the effect even more pronounced than the calculations would indicate. Regardless of this, it demonstrates the validity of using MO calculations as an adjunct with other physical measurements in making correlation studies. This validity is further demonstrated with a nitroderivative (compound 5, Table 3), which is a poor inhibitor of cholinesterase. The data in Table 2 and Fig. 3 indicate that the 2-nitrophenol has a di-positive charge on the C—O atoms which enhances the stability of the anion and results in a greater ease of hydrolysis of the corresponding ester as compared to the phenol ester, where this di-positive charge is much smaller. This situation must lead to the following sequence of events. Once the carbamate has settled on the catalytic site, the ester is hydrolyzed giving the carbamylated enzyme which yields the intact enzyme through the step controlled by $k_3$ in Fig. 2. The explanation for the decreased activity of the nitro derivative resides in the fact that $k_1$ is greater for compound 5 than for the other derivatives listed in Table 3, with the result that the intermediates (IV) and (V) are formed which decompose to (VI). In view of this observation, any substituent on these carbamates which lowers $k_1$ and provides a better fit on the enzyme will give a higher inhibition constant.

Compound 7 in Table 3 is more active on fly head cholinesterase than compound 6 because of the better fit. This is substantiated in Fig. 1 where it is seen that the distance between the quaternary nitrogen in the 3 position falls into the site separation of fly head cholinesterase whereas the quaternary nitrogen in the 2 position should be more potent on erythrocyte or electric eel cholinesterase. Compound 8 is rather interesting since the quaternary nitrogen should facilitate the ease with which this particular carbamate hydrolyzes. Like the nitro compound (5, Table 3), compound 8 should also be inactive. Since the opposite situation is true, I would like to suggest that the corresponding phenol, namely, the 3-trimethylaminophenol, blocks the enzyme by binding at the anionic site through the quaternary nitrogen and hydrogen bonding between the phenolic hydroxyl and the imidazole group on the esteratic site. The inhibitors (VII, VIII) prepared by Friess and Baldridge *et al.* (8) help support this hypothesis.




VII VIII

These two compounds must act through a mechanism involving binding at both the anionic and esteratic sites since no hydrolysis can occur.

*Organophosphates*

The only organophosphates that will be discussed are a series of phosphoramidates that were presented in an earlier paper (1). Figure 4 is taken from that paper and shows the correlation between the cholinesterase inhibition and the infrared stretching vibration of the phenyl-O bond. As we discussed previously (1) the stretching vibration gives an indication of the strength of the phenyl-O bond and hence the ease with which the material hydrolyzes. It will be noted that for the most part, MO calculations agree with other physical measurements as to which organophosphate is the best enzyme inhibitor.

In contrast to the carbamates, reactivation of the phosphorylated enzyme is very slow. This means that those phosphate esters which are susceptible to nucleophilic attack make for the best inhibitors.

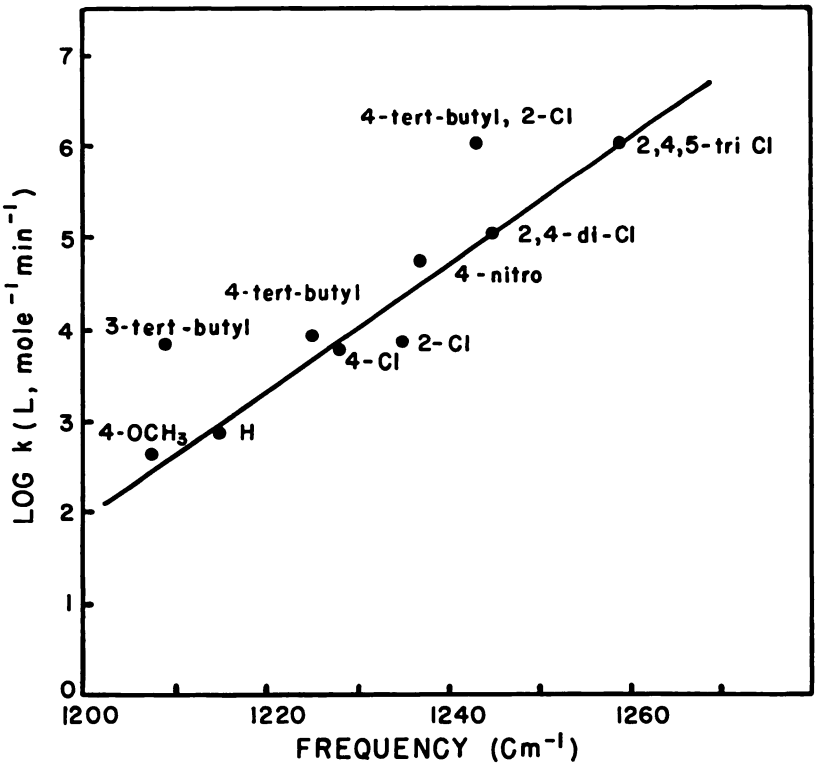



FIG. 4. *Relation of log k for cholinesterase inhibition and frequency of phenyl-O stretching vibration for the series of substituted phenyl phosphoramidates* (1)

The phosphoramidates possessing the 4-*tert*-butyl, 2-Cl phenyl, and the 3-*tert*-butyl phenyl in Fig. 4 are exceptions and accordingly are worthy of special mention. These two materials are more potent as inhibitors than the strength of the phenyl-O bond would predict. From Fig. 1 it seems reasonable that a tertiary butyl group in the 4 position is too far removed from the esteratic site for good binding. However, the distance between the 2-Cl and the 4-*tert*-butyl is just about right. Is it possible that in this case the phosphoramidate is a vehicle for bringing the substituted phenol to the active site and it is the 2-Cl, 4-*tert*-butyl phenol which actually is the inhibitor?

The increased activity might be due to phosphorylation of the esteratic site and also the type of binding that was previously discussed for the trimethyl amino phenyl carbamate. The activity of 3-*tert*-butyl phosphoramidate would substantiate this type of mechanism. In this case, it is the 3-*tert*-butyl phenol which has the correct dimension. The fact that 4-*tert*-butyl phosphoramidate does not possess enhanced activity again confirms this hypothesis. The distance between the 4-*tert*-butyl group and the hydroxyl are just too great. It will be interesting to compare these compounds for their activity on cholinesterases from different sources.

## *Conclusions*

This paper has had a threefold purpose. The first has been the demonstration of the validity of the simple Hückel Molecular Orbital Theory as an aid in correlation studies. It should be emphasized that MO theory should be used only in conjunction with other methods. The second purpose of this article has been to illustrate some interesting relationships between inhibitors of cholinesterase and their chemical structures. The questions raised by this investigation are currently being investigated.

Finally, the paper demonstrates the importance of utilizing as many disciplines of chemistry as possible in problems of biological importance. The combination of approaches to such questions often yields new concepts which can be very valuable in the ultimate solution of the problem.

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