Ab Initio Calculations on Large Molecules Using Molecular Fragments. Electronic and Geometric Characterization of Acetylcholine[1a]

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Abstract: An *ab initio* procedure for molecular orbital determination, *i.e.*, the molecular fragment method, has been applied to the acetylcholine molecule. Specifically, the effect of conformation on total energy and on electronic structure has been investigated. Where possible, comparisons have been made to previous calculations and experimental data. The manner in which these observed trends relate to the biological activity, particularly to the mechanism of ester hydrolysis, has been discussed.

The important role that acetylcholine (ACh) plays as a chemical mediator in the transmission of nerve impulses has been recognized for many years.[2-4] However, only recently have theoretical tools progressed to the point of being able to contribute to the understanding of the specific way ACh performs its neurological function. Due to various theoretical and practical difficulties, all previously reported theoretical investigations on ACh[5-9] have been, at least in part, empirical, and the deficiencies of these procedures, especially in prediction of energy differences, have been documented.[10] De La Vega, Fang, and Hayes[11] have shown that both extended Hückel (EHT) and CNDO/2 procedures can lead to incorrect predictions of relative stability of conformers, especially on polar molecules. Additionally, it should be noted that EHT and INDO frequently have difficulty in electronic structure description, *e.g.*, establishing the correct ordering of filled molecular orbitals in benzene.[12] Consequently, in order to investigate the relationships between structure and reactivity of ACh reliably, it appears to be important to use techniques where less drastic approximations are employed.

This study provides the first *ab initio* investigation of various conformers of ACh. The method employed is an *ab initio* molecular orbital method for large molecules, that has been described in previous studies.[13-17] In these studies, the characteristics of this method as applied to saturated and unsaturated hydrocarbons, oxygen-containing, and nitrogen-containing molecules have been investigated. In general, the agreement between experimentally determined barrier height trends as well as shapes of rotational barriers and those predicted by the molecular fragment approach has been excellent. Additionally, the comparison between the ordering of valence molecular orbitals predicted by this method and more extensive *ab initio* calculations has shown very good agreement. Consequently, the procedure appears to be well suited for examination of the geometric and electronic structure of ACh.

Computational Procedure

Only a brief summary of the molecular fragment method will be presented here, since the details have been given previously.[12-14] The basis orbitals employed in this technique are those used by Frost[18] and are normalized floating spherical Gaussian orbitals (FSGO), defined as

$$G_i(\mathbf{r}) = (2/\pi\rho_i^2)^{3/4} \exp\{-[(\mathbf{r} - \mathbf{R}_i)/\rho_i]^2\} \qquad (1)$$

where ρ_i is the orbital radius and $\mathbf{R}_i$ is the position of the FSGO, relative to an arbitrary origin. The description of a π-type orbital is accomplished by a linear combination of two FSGO, *i.e.*

$$G_\pi = (G_u - G_d)/[2(1 - \Delta_{ud})]^{1/2} \qquad (2)$$

where G_u and G_d are symmetrically placed above and below the nuclear plane, on a line through the central atom, and Δ_{ud} is the value of the overlap integral between them. The optimum description of the various molecular fragments used in the investigation of ACh was obtained by variationally determining the nonlinear parameters of the FSGO with a direct energy search (shown in Table I).

Large molecule investigations were then carried out

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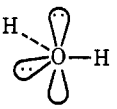
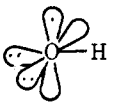
Table I. Molecular Fragments Employed[a]



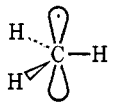
H_2O (oxy-π) sp^2 hybridization
O–H distance = 0.940 Å = 1.814 bohrs
π orbitals at ±0.10 bohrs
Optimized Gaussian parameters

	Distance	Radii	Energy, hartrees
O–inner shell	0.00083398[b]	0.24089502	−64.23958874
O–hydrogen	0.79678221	1.37684374	
O–lone pair	0.23835937	1.36888573	Scale factor
O–π	0.10000000	1.13643749	1.00302248



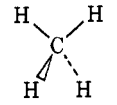
OH (sp–π) sp hybridization
O–H distance = $R_{H_2O}[R_{C=O}/R_{C-O}]$ = 0.817 Å = 1.548 bohrs
π orbitals at ±0.10 bohrs
Optimized Gaussian parameters

	Distances	Radii	Energy, hartrees
O–inner shell	0.00057129[c]	0.24028227	−63.93304110
O–hydrogen	0.76467773	1.23671871	
O–lone pair (sp)	0.21614258	1.28753780	Scale factor
O–lone pair (π)	0.10000000	1.19741696	0.99725247
O–π	0.10000000	1.12242182	



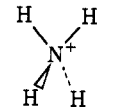
$\cdot CH_3$ sp^2 hybridization
C–H distance = $R_{C-H}[R_{C=C}/R_{C-C}]$ = 0.945 Å = 1.786 bohrs
π orbitals at ±0.10 bohrs
Optimized Gaussian parameters

	Distances	Radii	Energy, hartrees
C–inner shell	0.00000000	0.32682735	−33.38879442
C–hydrogen	1.13093139	1.51399487	
C–π	0.10000000	1.80399487	Scale factor
			0.98405892



CH_4 sp^3 hybridization
C–H distance = 1.090 Å = 2.060 bohrs
Optimized Gaussian parameters

	Distances	Radii	Energy, hartrees
C–inner shell	0.00000000	0.32784375	−33.98985962
C–hydrogen	1.23379402	1.67251562	
			Scale factor
			0.99722359



$^+NH_4$ sp^3 hybridization
N–H distance = 1.030 Å = 1.950 bohrs
Optimized Gaussian parameters

	Distances	Radii	Energy, hartrees
N–inner shell	0.00000000	0.27770068	−47.88418406
N–hydrogen	0.80547793	1.50046875	
			Scale factor
			1.00438066

[a] All units, unless explicitly given, will be atomic units. See ref 41 for a discussion. [b] The inner shell orbital lies on a line that bisects the HOH angle, displaced toward the two H nuclei. [c] The inner shell orbital lies along the O–H bond, displaced in the direction of the H nucleus.

by combining the appropriate molecular fragments and corresponding parameters within an SCF calculation. The molecular orbitals were taken as linear combinations of fragment FSGO, *i.e.*

$$\varphi_t = \sum_{A=1}^{p} \sum_{k=1}^{N_a} C_{kt}^{A} G_k^{A} \tag{3}$$

where the G_k^A are the previously determined fragment orbitals, and the C_{kt}^A are the coefficients that arise from the solution of the well-known SCF equations.[19,20] The elements of the charge and bond-order matrix, **P**, defined as

$$P_{rs} = 2\sum_{i}^{occ} C_{ir} C_{ts} \tag{4}$$

were used to monitor the convergence of the SCF calculation. Final convergence was assumed when

$$|P_{rs}^{(i+1)} - P_{rs}^{(i)}| \leq 0.00005$$

for all r and s. This corresponded in general to a root-mean-square error in the **P** matrix of 10^{-6} and a final energy value that was generally converged to ten figures.

The initial values of the P_{rs} were chosen in a special manner to aid the convergence of the SCF procedure. For molecular systems as large as ACh, the conditionally convergent SCF procedure may not reach an appropriate energy minimum with the unit matrix or other "standard" initial choices as starting values of the **P** matrix. To avoid this difficulty, starting charge and bond-order matrices that present a potential field for the electrons much more nearly like the proper convergence point were generated.[21] In particular, the initial **P** matrix for ACh was synthesized from converged **P** matrices for acetic acid (CH_3CO_2H) and choline ($HOCH_2CH_2N^+(CH_3)_3$). Taking the appropriate elements of the **P** matrices in this manner not only helps to ensure convergence, but also reduces the total amount of computer time spent doing SCF iterations.

Even though the actual amount of computer time used for this type of calculation is highly dependent upon the particular computer used, as well as the effectiveness of the programmer, reporting some measure of the computational effort is appropriate. For the specific case of ACh, about 14 min were spent in calculating and storing the various integral values, and each iteration took approximately 7 min on a Honeywell-635 computer, using double precision arithmetic.

Results

Since it has been shown that, at least in crystal structures, the bond distances and angles are constant within 5% for two different crystal structures of ACh,[22,23] a constant nuclear framework has been chosen. In particular, rather than use the X-ray data for one arbitrarily selected conformer throughout the calculations, average values for the bond distances were taken.[24] The bond angles were chosen by assuming idealized hybridization for the heavy atoms, *e.g.*, sp^2 and sp^3

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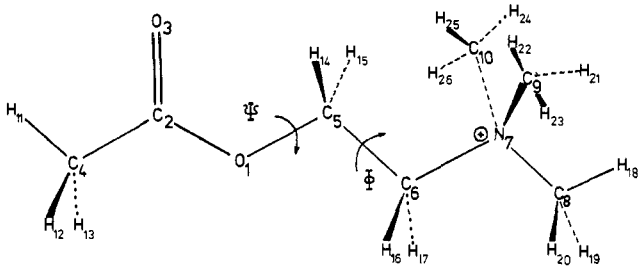
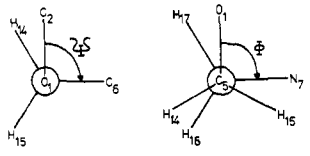


Figure 1. Atomic numbering scheme.



Bond Distances

	Å	bohrs[a]
C-H's	1.094	2.067
C_4-C_2	1.506	2.846
C_2-O_3	1.233	2.330
C_2-O_1	1.312	2.479
O_1-C_5	1.426	2.695
C_5-C_6	1.537	2.905
N_7-C's	1.479	2.795

a. See reference 39.

Figure 2. Bond distances and dihedral angles.

for the carbon atoms. This has been shown in previous studies[14,15] to make only minor differences when compared to studies using experimental distances and angles. However, these choices make it more difficult to make detailed comparisons with other theoretical investigations that employ differentg eometries,[8,9] as will be discussed later. The particular values chosen are given in Figures 1 and 2. It should also be noted that the methyl groups on the quaternary nitrogen were fixed at an orientation that minimized steric repulsions with the C_5–C_6 bond.

It has been reported that, for both acetylcholine bromide[22] and acetylcholine chloride,[23] as well as for analogs of ACh,[25] the X-ray crystal structures show little deviation from an O_3–C_2–O_1–C_5 dihedral angle of 0° and a C_5–C_6–N_7–C_8 angle of 180°. Therefore, the overall conformer can unequivocally be given by the two dihedral angles defined in Figure 2, C_2–O_1–C_5–C_6 (Ψ) and O_1–C_5–C_6–N_7 (Φ), and will be denoted as $\{\Phi,\Psi\}$.

This study is intended to investigate the manner in which an *ab initio* technique can be applied to a biologically important problem of conformational and electronic structure analysis. Therefore, calculations will be made on conformers that have been implicated in biological activity, as well as making detailed comparisons with the rotational curves obtained by previous theoretical procedures. In particular, the slices of the energy surface where Φ is fixed at 50° and Ψ is varied, and where Ψ is fixed at 180° and Φ is varied, have been investigated.

Figure 3 presents the results for the variation of Ψ

(25) P. J. Pauling, "Structural Chemistry and Molecular Biology," W. H. Freeman, San Francisco, Calif., 1968, p 555.

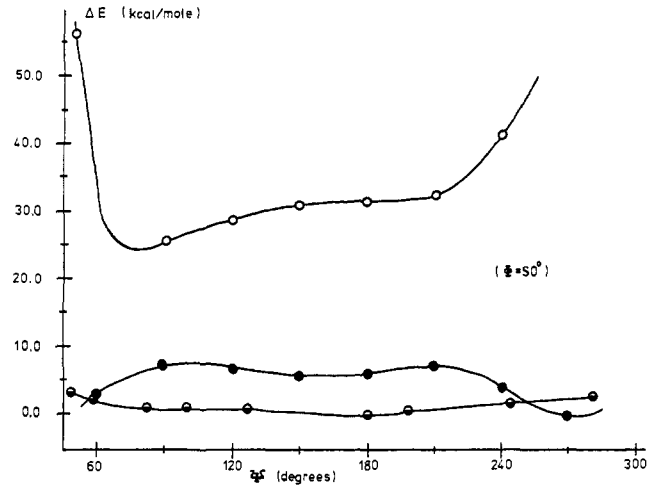


Figure 3. Total energy *vs.* X (Φ = 50°): (○) *ab initio;* (●) INDO; (◒) PCLIO.

(Φ = 50°) as predicted by the *ab initio* procedure, as compared to that predicted by INDO[8] and PCLIO.[9] Each curve has as its zero the energy corresponding to the global minimum as calculated by that particular method: INDO {50, 270},[8] PCLIO {60, 180},[9] EHT {80, 180}.[7] It is seen that the rotation curve calculated by Pullman, *et al.*,[9] is extremely flat and has a very shallow minimum at {50, 180}.[7] The INDO curve[8] has larger barriers (~8 kcal/mol) and has three minima, {50, 270}, {50, 180}, and although not shown {50, 50}. The *ab initio* rotation curve has considerably larger energy differences (~30 kcal/mol), as was expected because of the results of earlier rotation studies,[13,14,17] but shows only one minimum at approximately {50, 70}. While it is obvious that the overall shape of the three curves differ significantly, it should also be pointed out that the *ab initio* curve is shifted up by approximately 20 kcal/mol, *i.e.*, no point along that curve is closer than 20 kcal/mol above the lowest energy conformer. In contrast to that, the two lowest energy conformers calculated by Beveridge and Radna (INDO)[8] lie directly along this curve. Pullman, *et al.*[9], (PCLIO) obtain a global minimum for their energy search that corresponds to a {60, 180} conformer, which is just off the curve that is plotted.

The various curves that are obtained when Φ is varied and X is held constant at 180° are shown in Figure 4. Included there, along with plots from the three previously mentioned techniques, are the results of Kier's EHT investigation.[7] Again the PCLIO method predicts small energy differences. However, in this case, the INDO results are nearly the same. In fact, those two curves are very similar, with the upward shift due to the fact that the PCLIO global minimum, {60, 180}, lies directly on the plot. The EHT results show a marked increase in rotation barriers (~14 kcal/mol), while still maintaining its overall minimum at {80, 180}. Again the *ab initio* curve has the largest energy differences (>50 kcal/mol). However, the lowest energy conformer of ACh of those included in this study appears on this plot. This minimum is at {180, 180} and corresponds to the fully extended conformer. This, of course, is the lowest conformer expected if only steric repulsion considerations are made.

A summary is given in Table II, which briefly compares the global minima of the semiempirical proce-

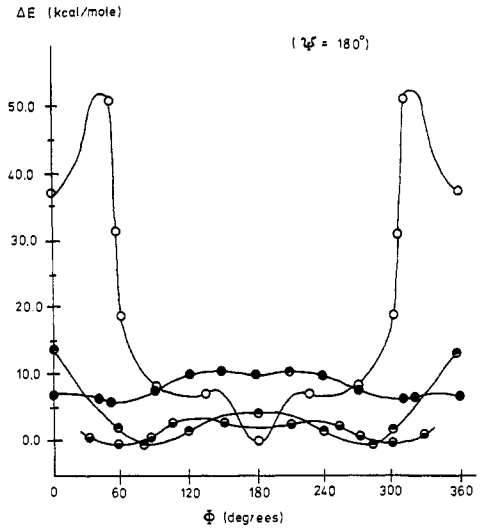


Figure 4. Total energy *vs.* Φ(X = 180°): (○) *ab initio;* (●) INDO; (◒) PCLIO; (◓) EHT.

dures to the energies calculated for the same conformers by the current *ab initio* technique. The results of the empirical potential[5,6] procedure were not presented in

Table II. Relative Energy Differences for ACh Conformers

Conformer (Φ, Ψ)	ΔE^g (this study)	Comments
{180, 180}	0.00	Minimum from this work
{180, 150}	0.12	Implicated in hydrolysis[a] and nicotinic[b] activity
{77, 79}	4.77	ACh bromide crystal structure[c]
{85, 167}	8.09	ACh chloride crystal structure[d]
{60, 180}	18.94	Minimum from ref 9
{80, 180}	10.00	Minumum from ref 7
{90, 180}	8.29	Implicated in muscarinic activity[e]
{75, 180}	9.19	Implicated in nicotinic activity[f]
{50, 270}	163.47	Minimum from ref 8

[a] See ref 39. [b] See ref 40. [c] See ref 22. [d] See ref 23. [e] See ref 38. [f] See ref 37. [g] kcal/mol.

Table II for convenience, but may be summarized. (a) For the conformers {80, 180}, {180, 76}, {74, 182}, and {72, 75} no energy difference greater than 1.1 kcal/mol was found in the initial investigation,[5] or greater than 0.72 kcal/mol in the most recent one.[6] (b) The global minimum is {80, 180} or {72, 75}, respectively, for investigation 1[5] and 2.[6]

Also included in Table II are the relative energies of other conformers of ACh that have been implicated as being of some biological and/or chemical importance. Although the *ab initio* procedure, as previously noted, has given the largest differences for rotational curves, all the experimentally observed conformers in this table are within 9.2 kcal/mol of the minimum. Clearly, all such conformers have to be considered "reachable," especially in view of possible stabilizing interactions with solvents, other ions in a crystal lattice, and enzyme active sites.

Another chemically interesting feature that is reported here is the effect that conformational change has upon the electronic structure of ACh. Since ACh is devoid of symmetry for most conformers, the molecular orbitals are best specified by their shape and location in the molecule, and not by the irreducible representation to which they belong. Table III describes the three highest occupied molecular orbitals and first virtual orbital for the *ab initio* calculations on ACh, giving the orbital energy of each for the conformers presented. The most striking feature of these data is that the molecular orbitals do *not* significantly change their *shape* upon rather large conformational changes. One implication of this observation will be pointed out in the discussion. Another consideration of importance is the manner in which the molecular orbital *energies* change with change in conformer. As the molecule becomes more spherical (less extended), the valence molecular orbitals as well as the LUMO exhibit a decrease in energy that is nearly uniform. More will be said in the next section about the relation of these electronic structure effects to the hydrolysis of ACh by acetylcholinesterase.

Discussion

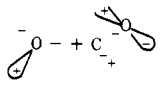
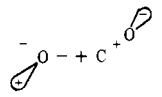
In the following discussion of the calculations just described, it is of interest first to recall that several assumptions and limitations have been necessitated in order to develop an *ab initio* method that is applicable to large molecules. In particular, the procedure uses quite small basis sets and assumes idealized geometries throughout. In addition, an incomplete variation of nonlinear parameters in the fragment calculations was sometimes employed, due to numerical instabilities. However, in order to assess the effect of these assumptions and limitations, extensive studies on prototype molecules have been carried out on hydrocarbons,[14,15] nitrogen-containing[17] and oxygen-containing[17] molecules. These studies indicate that, in spite of the limitations, these basis sets provide a remarkably well-balanced description of many aspects of electronic and geometric structure, and consequently will be assumed to provide an appropriate basis for the properties of ACh discussed below.

In summarizing the geometric structure predictions by the various methods, the first aspect of interest is the relative magnitude and general shape of the rotation barriers. While the empirical potential gives barriers that are probably unrealistically small, *e.g.*, <0.8 kcal/mol,[6] those calculated by INDO and PCILO are significantly larger (≃5 kcal/mol) and quite similar in at least one instance (see Figure 4). While EHT predicts a barrier of 14 kcal/mol for the rotation in Figure 4, the *ab initio* procedure gives even higher energy differences (>50 kcal/mol) for some of the conformers.

Even though the *ab initio* method may exaggerate somewhat the rotation barriers, as has been previously noted,[13,14,17] all of the experimentally known conformers of ACh, as presented in Table II, have calculated energies within 9.2 kcal/mol of the {180, 180} minimum. It is reasonable then, that ACh can assume any of these conformations in the course of interacting with solvent molecules or active sites, and the results of the *ab initio* investigations appear consistent with the available experimental data.

Another aspect of interest is the overall lowest energy conformer predicted by the various methods. The empirical potential method predicts either an {80, 180} conformer[5] or a {72, 75} conformer,[6] if electrostatic interactions are included. The lowest energy structure obtained from EHT calculations is {80, 180}.[7] The global minimum found using the PCILO procedure is a {60, 180} conformer,[9] while INDO predicts the struc-

Table III. Valence Electronic Structure of ACh[a]

Molecular orbital	Conformer {180, 180} *ab initio* minimum	{180, 150} hydrolytic[b] and nicotinic activity[c]	{90, 180} muscarinic activity[d]	{75, 180} nicotinic activity[e]	ACh chloride[f]	PCLIO minimum[g]	ACh bromide[h]	INDO minimum[i]
 Antibonding π-type orbital	+0.374	+0.372	+0.365	+0.361	+0.366	+0.356	+0.348	+0.302
 Primarily lone pairs on oxygens	−0.265	−0.266	−0.273	−0.276	−0.272	−0.279	−0.287	−0.380
 Essentially isolated oxygen π orbitals	−0.311	−0.312	−0.320	−0.324	−0.320	−0.328	−0.333	−0.380
 Primarily lone pairs on oxygens	−0.381	−0.381	−0.391	−0.394	−0.391	−0.392	−0.400	−0.422

[a] The orbital energies have units of hartrees. For a discussion of atomic units, see ref 41. [b] See ref 39. [c] See ref 40. [d] An average conformer was selected from ref 38. [e] See ref 37. [f] See ref 23. [g] See ref 9. [h] See ref 22. [i] See ref 8.

ture to be {50, 270}.[8] The lowest energy description of ACh as obtained from the *ab initio* method used here is {180, 180}, the fully extended conformer. While the various methods give apparently disparate results as to the lowest energy conformer of ACh, there is essential agreement upon the geometrically flexible nature of the molecule. In addition, in all cases studied here, experimentally observed conformers were separated from the most stable conformer only by an amount of energy that could be readily supplied by, *e.g.*, interaction with solvent molecules. Consequently, the lack of unanimity in the prediction of a single most stable conformer should not necessarily be of concern. Instead, these various studies indicate that many conformers appear to be possible within reasonable energy ranges, and that one single optimum conformer should not be expected.

In addition, the use of different geometries makes detailed comparisons of minimum energy conformers and conformational energy curves extremely difficult. For example, the interaction of the various hydrogens with other atoms in nonextended conformations of ACh can be altered greatly by the choice of geometry and other factors (*e.g.*, allowing relaxation of quaternary nitrogen methyl group geometries and conformations during rotations). Thus, establishment of *the* minimum energy conformer will not in general be possible in studies such as these, due to the large number of degrees of freedom that are present, and different local minima using various procedures and geometries should not be unexpected. Consequently, comparisons among various methods that are made here must be done within the assumption that the differences in geometries and procedures for variation in geometry are small, and will not affect the results. ACh is a molecule where such assumptions are likely not to be entirely justifiable, and the discussions should be viewed accordingly.

Beveridge and Radna[8] presented hydrogen bonding from the methyl groups of the quaternary nitrogen to the ester oxygen as well as to the carbonyl oxygen as primary stabilizing influences for their global minimum. This hypothesis was originally suggested in crystal structures by Sutor,[26] and verification in solution has been attempted by infrared[27] and nmr[28] experiments. Although the results are not inconsistent with this explanation, conclusive data are still being sought. In fact, much of this evidence has been questioned by Donahue in a more recent review.[29] Additionally, a recent nuclear diffraction study[30] discounts this possibility in the solid state.

The INDO studies just discussed imply that the ability of the current procedure to describe the essential features of hydrogen bonding should be examined. The main question centers around the ability of the molecular fragment approach to describe hydrogen bonds, in spite of the lack of basis functions on hydrogen nuclei. More specifically, information about the intramolecular hydrogen bonding from methyl group hydrogens to oxygen as a function of the charge on nitrogen is desirable. To estimate this, the effect of protonation of the nitrogen atom in methylamine and trimethylamine on the electron density at the methyl hydrogens has been studied. The net charge of the methyl hydrogen becomes +0.017 more positive

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(29) J. Donahue, "Structural Chemistry and Molecular Biology," W. H. Freeman, San Francisco, Calif., 1968, p 443.
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for methylamine and +0.014 more positive for trimethylamine upon protonation, as obtained from a Mulliken population analysis[31] of the FSGO wave functions[12] determined by the current procedure. A more extensive *ab initio* calculation with an STO-3G basis set[32] gives +0.082 and +0.063, respectively. Thus, both procedures indicate that the hydrogens in question do become more positive upon protonation, as expected. Furthermore, the trend toward lower positive charge per hydrogen from methylamine to trimethylamine is exhibited in both procedures, indicating a spreading out of the charge as more methyl groups are added. Of course, these numbers in either instance should not be considered to be a precise description of the electronic environment near the hydrogen nuclei, especially in the light of the well-documented deficiencies of the Mulliken analysis.[33] An additional complicating factor in making these comparisons arises because the molecular fragment procedure employs orbitals in bonding regions as well as on nuclei, requiring further modifications[21] of the Mulliken procedure in order to extract charges on nuclei. Thus, considering the difficulties of making direct comparisons, it does appear that a description of the hydrogens in question that is comparable to that obtained by the use of more extensive basis sets is obtained using the molecular fragment method.

In other investigations, recent work in this laboratory has indicated that hydrogen bonding between water molecules[34] as well as those that stabilize polypeptide structures[35] can be adequately described. For example, the estimate of the stabilization in single stranded polyglycine due to hydrogen bonding has been found[35] to be 6.1 kcal/mol, indicating that quantitative, as well as qualitative, effects of hydrogen bonding in some systems can be extracted using the molecular fragment procedure. Thus, it seems likely that hydrogen-bonding effects in ACh, if present, would be revealed using the molecular-fragment procedure.

The different shapes of the barrier to rotation curves that are given in Figure 4 can be rationalized for the most part in terms of the differences in the manner in which hydrogen bonding is described in the various procedures. For example, at $\Phi \cong 60°$ ($\Psi = 180°$), the INDO and PCILO methods obtain at least a relative minimum, while the molecular fragment method predicts a relative maximum. Geometric considerations would suggest that, if a minimum were to occur at that point, hydrogen bonding would be expected to be an important contributor. Thus, the lack of a stabilizing hydrogen-bonding effect using the molecular-fragment procedure suggests that the PCILO and INDO methods may be overestimating the positive nature of the methyl hydrogens,[36] and thus causing an apparent minimum. The observed relative maximum in the molecular fragment description indicates that the methyl hydrogens do not have a large positive charge, thus causing the relative maximum through repulsive effects of the oxygen lone pairs with the methyl hydrogens. Since a closely related conformation, *i.e.*, {50, 270}, is a global minimum for the INDO calculation, the question of the adequacy of hydrogen-bonding description may be significant, and further investigations are certainly desirable.

A more detailed discussion of the nature of the electronic structure of ACh, as displayed in Table III, is also appropriate. The molecular orbitals given there have been classified according to their orbital energies and to the region of the molecule in which the principal contributors to it are situated. Initially it should be pointed out that, in accordance with the general assumption that reactions of interest for ACh involve the ester functionality, the four highest occupied molecular orbitals, as well as lowest unoccupied molecular orbital, all are located in the vicinity of the ester moiety in ACh.

Next, the manner in which the shape of the molecular orbitals depends upon specific conformations and its implication to the interactions of ACh with an active site[37-40] is of some interest. In particular, the magnitude of the contributions of the FSGO to each of the molecular orbitals of Table III is remarkably constant as a function of the two rotation angles. This suggests that the ACh molecule has a rather static charge distribution, which merely changes its orientation in space, and does little readjusting as the conformer is varied. The implication of this is that, if the ACh substrate molecule does, in fact, obtain a conformation that is complementary to the ACh enzyme active site, it is not primarily because of favorable changes in electronic structure of the substrate as a function of rotation but, rather, in order to suit the geometric requirements of the active site.

Upon closer examination there are, however, some subtle energetic considerations that also should be discussed. The general trend of the orbital energies[41] can be understood qualitatively in light of a coulombic attraction between the ester moiety and the quaternary nitrogen. As this interaction distance decreases, *i.e.*, the molecule becomes less extended, the electrons in the high-lying occupied orbitals are stabilized by an electrostatic interaction with this cationic center. A similar argument can be presented for the electron affinity of the lowest virtual orbital, and an appropriate energy trend is also correctly anticipated in this case.

The possible relationship that this feature of the electronic structure has to the mechanism of ester hydrolysis should be noted, especially since this hydrolysis has been studied carefully in solution,[42] and much current effort is being directed toward an understanding of the enzymatic hydrolysis.[43-45] However, in in-

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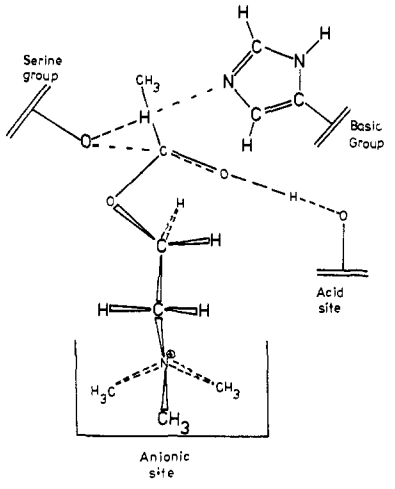

Figure 5. Model for proposed transition state for enzymatic hydrolysis of ACh.

terpreting these data, there is one underlying assumption that will be made that should be noted, *i.e.*, that the conformational energy differences in the transition state (T.S.$^{\pm}$) are, in all instances, simply exaggerated when compared to the reactant conformational energy differences in ACh. This assumption, if valid, assures that the differences in total energy of isolated (ground state) ACh as a function of conformer will establish the relative rates of hydrolysis. Specifically, the particular conformer of interest that is implied by this assumption is the fully extended {180, 180} structure. In addition to the implications derived from the current theoretical studies to support this assumption, the experimental evidence, for both enzymatic[39] and base-catalyzed hydrolysis,[42] also indicates that the conformer identified as {180, 180} is of importance.

Within the context of the previous assumption, the molecular orbital energies given in Table III can be analyzed for possible correlation with the mechanism of ACh hydrolysis. In particular, for the case of nucleophilic (base catalyzed) attack, it is the first unoccupied orbital for the various conformers of ACh that it important. As the LUMO lowers in energy, it becomes easier for electrons to attack the molecule, making such hydrolysis more facile. The LUMO for all conformers is an antibonding π-type orbital and does have significant "hole density" at the carbonyl carbon, which is the usually accepted site of nucleophilic attack. However, the LUMO orbital energy in the {180, 180} conformer is higher than in other conformers examined, indicating that nucleophilic attack is not favored in the {180, 180} conformation.

On the other hand, for electrophilic (acid catalyzed) attack, it is the HOMO and its associated energy that is of concern. This time, however, the electrons in this orbital need to be more accessible, or less stable, for the conformer to be labile toward this attack. Since the fully extended conformer is characterized as having the *highest* energy HOMO (and LUMO), the molecular orbital structure suggests that the mechanism for ACh ester hydrolysis may begin *via* electrophilic attack.

However, since electrophilic and nucleophilic hydrolyses are merely extremes of a spectrum of possible mechanisms, the above suggestion should not necessarily be interpreted that the mechanism is totally electrophilic. Rather, it is necessary only that, in the T.S.$^{\pm}$, a stronger bond is formed to the attacking electrophile than to the nucleophile. For enzymatic hydrolysis, this transition state might be represented as in Figure 5. It should be noted that the mechanism predicted by these theoretical data correlates well with a slightly modified version of the enzyme active site proposed by Beckett, *et al.*[46] This modification amounts to electrophilic attack on the carbonyl oxygen rather than on the ester oxygen.

It should also be noted that there is substantial evidence[47] to indicate that the hydroxide-catalyzed hydrolysis of esters in solution, studied for the ACh derivatives mentioned above, is a predominately nucleophilic reaction. On the basis of the current studies, this would indicate that the reaction in that instance is not controlled by HOMO and LUMO electronic effects, but that other features of the electronic and geometric structure of ACh contribute to the observation that the extended structure of ACh is an important one for reactivity. This point also serves to emphasize the fact that considerable work must yet be done to understand fully the relationship between enzymatic and solution hydrolysis.

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