Secondly, what about higher polymers of gas phase LiH? Matrix isolation techniques have been used to identify[5] a polymer of LiF. A trimer of LiH probably would be difficult to observe in the gas phase (very unfavorable entropy), but an intuitively reasonable structure would be a benzene-like hexamer with alternating lithium and hydrogen atoms. Just as in $(LiH)_2$, the three lithiums would be closer to the center of the molecule than the hydrogens because there are no valence orbital nodes between the lithiums and two between the hydrogens. Ethyllithium forms a gas-phase dimer,[22] but methyllithum appears to be predominantly a tetramer in solution studies, with the Li and methyl groups forming two interlocking tetrahedra.[23] Thus there is precedent for the existence of $(LiH)_4$ in a similar structure with H replacing CH_3.

Acknowledgments. One of us (P. A. K.) is most grateful to Leo Brewer for instruction in the thermodynamics of high-temperature species and for pointing out the role of high H_2 pressure in alkali hydride studies. P.A.K. also thanks Joe Cambre, Bill Hunt, and Lester Andrews for helpful comments.

---

[22] E. Weiss and E. A. C. Lucken, *J. Organometal. Chem.*, 2, 197 (1964).

[23] L. D. McKeever, R. Waack, M. A. Doran, and E. B. Baker, *J. Amer. Chem. Soc.*, 91, 1057 (1969).

Comparison of Semiempirical Classical and Semiempirical Quantum Mechanical Calculations on Acetylcholine

Mark Froimowitz* and Paul J. Gans

Contribution from the Department of Chemistry, New York University, New York, New York 10003. *Received March 18, 1972*

Abstract: The results of a semiempirical classical calculation on acetylcholine, in which the conformational energy is taken to be the sum of nonbonded and electrostatic pair-wise interactions, are compared to both semiempirical quantum mechanical INDO (intermediate neglect of differential overlap) results and to experimental data for the same molecule. It is found that both the classical and quantum mechanical studies give similar results and that both correlate well with experimental data. In addition, our study points out a difficulty in the INDO method in that it allows nonbonded atoms to approach almost to bonding distances before giving repulsion.

The importance of the acetylcholine molecule in the transmission of electrical signals through the neural system has stimulated much experimental and theoretical research on its allowed conformations. The theoretical studies have included semiempirical classical calculations by Liquori, Damiani, and De Coen,[1] in which they included nonbonded interactions and torsional potentials but did not include electrostatic interactions, and a recent follow-up by Ajò, *et al.*,[2] in which electrostatic interactions were included; and semiempirical quantum mechanical calculations by Kier[3] using the extended Hückel method and by Beveridge and Radna[4] using the INDO (intermediate neglect of differential overlap) method. The principal reason that our present study was initiated was to get a more quantitative idea of the accuracy that one can achieve using classical type potential functions by comparing our results both with the above quantum mechanical results, especially the very extensive study by Beveridge and Radna, and with experimental data for acetylcholine. This purpose is especially relevant at this time due to two recent papers. The first by Pullman and coworkers[5] criticizes semiempirical classical calculations of dipeptides on the basis that such calculations failed to predict certain experimental results while their PCILO (perturbative configuration interaction using localized orbitals) method did. The second by Tonelli[6] compares experimental nmr results to theoretical results obtained from dipeptide maps generated both classically and quantum mechanically. Tonelli found that classical and extended Hückel results agreed with experiment while the PCILO method of Pullman yielded different results.

Calculation Parameters

In order to facilitate comparison of our results with the INDO results of Beveridge and Radna,[4] hereafter referred to as BR, we used exactly the same structural parameters. These were taken by BR primarily from the X-ray crystallographic results of Canepa, *et al.*,[7] except for parameters involving the hydrogen atoms which were not determined. The hydrogen–carbon bond distances were set at 1.09 Å and the H–C–C bond angles were set at 109.47°. In addition, the methyl hydrogens of the trimethylammonium group were oriented for minimal steric repulsions.

---

* Address correspondence to author at the Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154.

[1] A. M. Liquori, A. Damiani, and J. L. De Coen, *J. Mol. Biol.*, 33, 445 (1968).

[2] D. Ajò, M. Bossa, A. Damiani, R. Fidenzi, S. Gigli, L. Lanzi, and A. Lapiccirella, *J. Theor. Biol.*, 34, 15 (1972).

[3] L. B. Kier, *Mol. Pharmacol.*, 3, 487 (1967).

[4] D. L. Beveridge and R. J. Radna, *J. Amer. Chem. Soc.*, 93, 3759 (1971).

[5] B. Pullman, B. Maigret, and D. Perahia, *Theor. Chim. Acta*, 18, 44 (1970).

[6] A. E. Tonelli, *Macromolecules*, 4, 618 (1971).

[7] F. G. Canepa, P. Pauling, and H. Sörum, *Nature (London)*, 210 907 (1966).

In examining the acetylcholine molecule in Figure 1, it can be seen that its conformations are primarily a function of the four dihedral angles τ(O2–C6–O1–C5), τ(C6–O1–C5–C4), τ(O1–C5–C4–N$^+$), and τ(C5–C4–N$^+$–C3). However, in our study, as in that of BR, τ(O2–C6–O1–C5) is fixed at 0° and τ(C5–C4–N$^+$–C3) at 180°.[8] The bond C6–O1 has partial double bond character, and the group O2–C6–O1–C5 would therefore be expected to be planar. Crystallographic results over a number of analogs of acetylcholine all have the carbonyl oxygen cis to C5,[9] and *ab initio* quantum mechanical results for the group indicate a stabilization of about 2 kcal/mol for the cis over the trans form.[10] Similarly, for τ(C5–C4–N$^+$–C3) there is no large variation away from 180° in crystallographic studies over a number of analogs of acetylcholine, presumably because of steric hindrance due to the bulky methyl groups.[9] The dihedral angles used in our study are summarized in Table I.

Table I. Summary of Dihedral Angles Used Both in Our Study and in That of Beveridge and Radna

τ(H3–C3–N$^+$–C4) = 180.00°	τ(C5–O1–C6–C7) = 180.00°
τ(H1–C1–N$^+$–C4) = 58.74°	τ(O1–C6–C7–H7) = 0.00°
τ(H2–C2–N$^+$–C4) = 298.86°	τ(O1–C5–C4–N$^+$) = varied
τ(C3–N$^+$–C4–C5) = 180.00°	τ(C6–O1–C5–C4) = varied

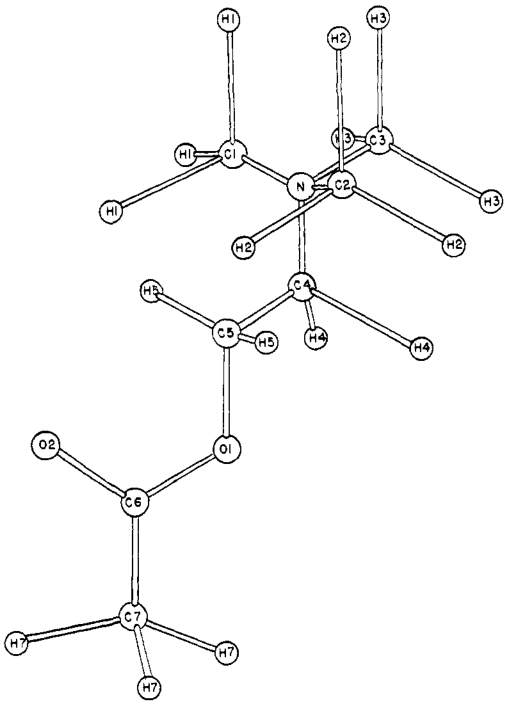

Figure 1. The fully extended conformation of acetylcholine showing the numbering system used in this paper.

The conformational energy, which was assumed to be pair-wise additive between all atoms which were three or more bonds apart, was divided into two distinct contributions, nonbonded interactions and electrostatic interactions.

For the nonbonded interactions, we have used a Lennard-Jones 6-12 potential function

$$U_{ij}^{\mathrm{nb}} = (a_{ij}/r_{ij}^{12}) - (b_{ij}/r_{ij}^{6}) \qquad (1)$$

which is the form most commonly used for this type of interaction in classical calculations, where[11]

$$b_{ij} = 3e\hbar\alpha_i\alpha_j/2m^{1/2}[(\alpha_i/N_i)^{1/2} + (\alpha_j/N_j)^{1/2}] \qquad (2)$$

$$a_{ij} = b_{ij}r_{\mathrm{min}}^{6}/2 \qquad (3)$$

and r_{ij} is the distance between atoms i and j. In addition, e and m are respectively the electron charge and mass, α_i is the atomic polarizability of atom i, N_i is the effective number of electrons of atom i as has been discussed by Pitzer,[12] and r_{min} is taken to be the interatomic distance at which the repulsive overlap part of the nonbonded interaction exactly cancels the attractive London forces.

At this point, there has been some diversity by different research groups in the parameters put into eq 2 and 3. We have taken most of the α_i's from Ketelaar[13] following the procedure used by Brant, *et al.*,[14] and the N_i's have been taken from Pitzer.[12] The polarizability of the N$^+$ atom was taken from Tessman, *et al.*,[15] by making the assumption that the polarizability of NH_4^+ can be broken into additive contributions from the nitrogen and hydrogen atoms. These parameters are listed in Table II. Also, we have set r_{min} equal to the sum of the van der Waals radii of the two atoms involved, as was done by Scott and Scheraga.[16] The van der Waals radii were taken from Bondi[17] and are also listed in Table II. The values that we have calculated for the constants a_{ij} and b_{ij} are listed in Table III.

Table II. Parameters for Calculating Constants Used in Nonbonded Potential Function

Atom	α, Å^3	N(eff)	van der Waals radius, Å
C carbonyl	1.23^a	5^d	1.70^e
C other	0.93^b	5^d	1.70^e
N$^+$	0.32^c	6^d	1.55^e
O carbonyl	0.84^b	7^d	1.50^e
O ester	0.70^a	7^d	1.50^e
H	0.42^b	0.9^d	1.20^e

[a] Reference 13 (following the procedure suggested in ref 14). [b] Reference 13. [c] Reference 15. [d] Reference 12. [e] Reference 17.

For the electrostatic interactions, we have made the assumption that partial charges due to electronic shifts can be considered to be point partial charges

---

(8) We have used the following convention for dihedral angles: τ(A–B–C–D) is the angle between the planes *A–B–C* and *B–C–D* with the eclipsed form being defined as 0°. Looking along *A–B–C–D*, a clockwise rotation of the plane *B–C–D* is considered positive.

(9) C. Chothia and P. Pauling, *Proc. Nat. Acad. Sci. U. S.*, **65**, 477 (1970).

(10) D. Hankins, private communication.

(11) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966); D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(12) K. S. Pitzer, *Advan. Chem. Phys.*, **2**, 59 (1959).

(13) J. Ketelaar, "Chemical Constitution," Elsevier, New York, N. Y., 1958, p 91.

(14) D. A. Brant, A. E. Tonelli, and P. J. Flory, *Macromolecules*, **2**, 228 (1969).

(15) J. R. Tessman, A. H. Kahn, and W. Shockley, *Phys. Rev.*, **92**, 890 (1953).

(16) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **42**, 2209 (1965).

(17) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).

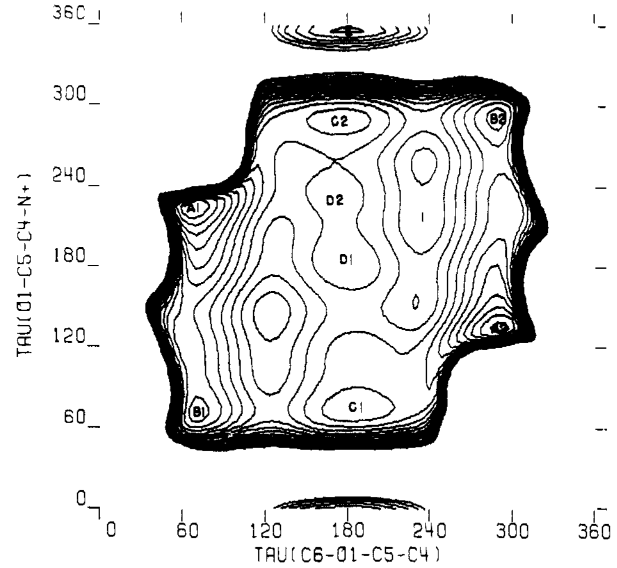

Figure 2. Calculated potential energy surface for acetylcholine with $D = 1.0$. Unlabeled extrema are maxima.

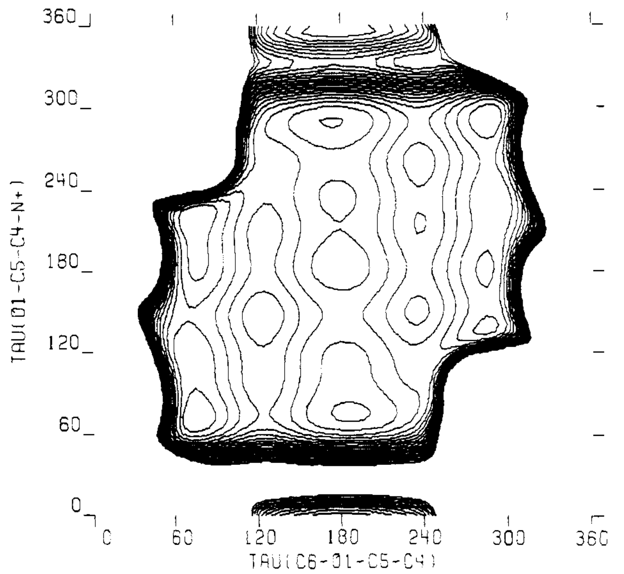

Figure 3. Nonbonded contribution to calculated potential energy surface.

Table III. Constants for Nonbonded Potential Function

Pair of atoms		a_{ij}, kcal Å^{12} mol^{-1}	b_{ij}, kcal Å^{6} mol^{-1}
C carbonyl	C other	3.45×10^6	447
C carbonyl	N$^+$	1.15×10^6	196
C carbonyl	H	4.72×10^4	159
C other	C other	2.80×10^5	363
C other	N$^+$	9.59×10^4	163
C other	O carbonyl	1.95×10^5	364
C other	O ester	1.69×10^5	315
C other	H	3.77×10^4	127
N$^+$	O carbonyl	6.78×10^4	169
N$^+$	O ester	5.97×10^4	148
N$^+$	H	1.15×10^4	53.2
O carbonyl	H	2.40×10^4	124
O ester	H	2.06×10^4	106
H	H	4.47×10^3	46.7

centered at the atoms. The electrostatic potential is then

$$U_{ij}^{\mathrm{el}} = q_i q_j / D r_{ij}$$

where q_i is the partial charge of atom i, D is the dielectric constant of the medium, and r_{ij} has the same meaning as above. We have taken our partial charges from those calculated by BR and they are listed in Table IV. We have done calculations both with $D = 1.0$, which is appropriate for an isolated molecule, and $D = 1.77$, which would be the dielectric constant of water at high (optical) frequencies.

Table IV. Partial Charges for Electrostatic Potential Function[a]

Atom[b]	Partial charge[c]	Atom[b]	Partial charge[c]
C6	0.49	O2	−0.37
C5	0.20	O1	−0.26
C1, C2, C3, C4	0.12	H5	−0.01
C7	−0.05	H other	0.03
N$^+$	0.11		

[a] Taken from the [180°, 180°] conformation shown in Figure 5 of ref 4. We have done some rounding and smoothing of charges on approximately equivalent atoms to simplify our input. [b] With numbering from Figure 1. [c] In units of electron charge.

In addition to these contributions to the conformational energy, it is common to add a torsional potential function so as to reproduce the correct experimental barrier for rotation about the various dihedral angles. However, we have not included such a potential for two reasons. Firstly, there is no theoretical basis for such a torsional potential. In high accuracy *ab initio* quantum mechanical calculations, the correct barrier energy falls out naturally. Nevertheless, it is not clear that a "good" classical calculation does not already include the classical analog of the sources of the barriers in the nonbonded and electrostatic potential energy functions. The second reason is that the uncertainty of our conformational energy map is probably larger than the relatively small contribution from a torsional potential energy. We estimate that this contribution would not add more than 1 or 2 kcal/mol to any conformation, though there is no experimental evidence indicating the height of such barriers in acetylcholine.

Results and Discussion

The calculation for acetylcholine was done by varying τ(O1-C5-C4-N$^+$) and τ(C6-O1-C5-C4), hereafter referred to as [τ_1, τ_2], in 5° increments holding all other dihedral angles constant. The resulting 73 × 73 point grid was interpolated and plotted directly by a library routine CONTOUR available at the Courant Institute Computing Center at NYU. The resulting figures are displayed in Figures 2–5. The spacing of the contour levels is 0.5 kcal/mol in all four figures. In addition, in Figures 2, 3, and 5, the absolute minimum is taken as zero and the contour levels range up to 10 kcal/mol. The blank areas around the edges of these figures can then be thought of as being inaccessible to the molecule because of their high energy. For Figure 4, the above procedure could not be done since the energy goes to minus infinity for the overlap of atoms with partial charges of opposite sign.

Our primary result is presented in Figure 2, which was produced with the dielectric constant set to 1.0.

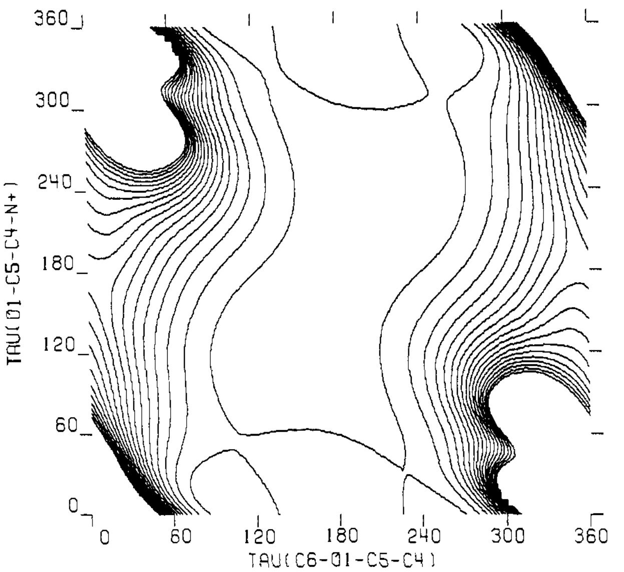

Figure 4. Electrostatic contribution to calculated potential energy surface with $D = 1.0$.

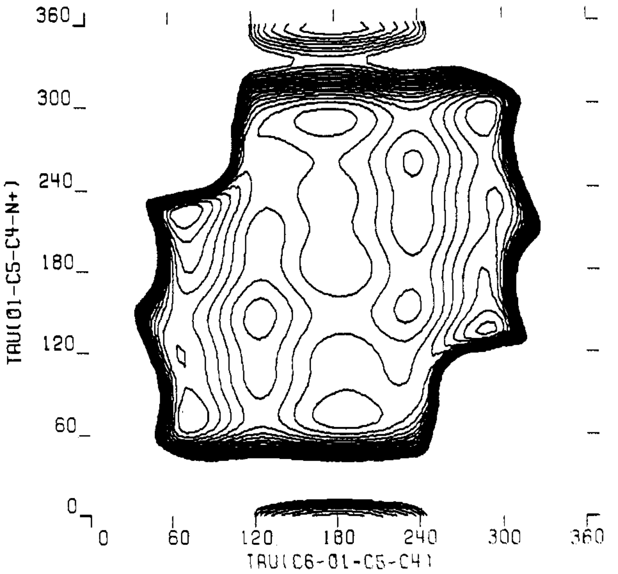

Figure 5. Calculated potential energy surface for acetylcholine with $D = 1.77$.

One notices that there is an approximate plane of symmetry running diagonally through the map with a total of nine minima, four mirror-image pairs plus a minimum that is not reflected. These minima are summarized in Table V.

Table V. Summary of Minima Found in Our Study

Conformation	Rel energy, kcal/mol	Conformation	Rel energy, kcal/mol
A1 [225°, 70°]	0.00	C2 [290°, 175°]	3.70
A2 [135°, 290°]	0.29	D1 [185°, 180°]	4.11
B1 [70°, 70°]	2.74	D2 [235°, 170°]	4.27
B2 [290°, 290°]	2.75	E [355°, 180°]	7.37
C1 [75°, 185°]	3.65		

The first pair of minima that we discuss, B1 and B2, occur at [70°, 70°] and [290°, 290°] and are 2.74 and 2.75 kcal/mol, respectively, above the absolute minimum. The minimum B1 is very close to the [77°, 79°] geometry observed for acetylcholine by X-ray crystallography.[7] Minimum B1 would probably be preferred over minimum B2 for the crystalline state since it leads to a more compact molecule which should be incorporated more easily into a crystal lattice.

The second pair of minima, C1 and C2, occur at [75°, 185°] and [290°, 175°] and are 3.65 and 3.70 kcal/mol, respectively, above the absolute minimum. Again, there are experimental points associated with these minima. The conformation [70°, 180°] has been suggested by Chothia and Pauling[9] for the nicotinic action of acetylcholine and the range of conformations [60 to 120°, 144 to 213°] has been suggested by Chothia[18] for the muscarinic action of acetylcholine. In addition, an nmr study of acetylcholine in D_2O has suggested that the [±60°, 180°] conformation predominates in aqueous solution.[19]

Next we examine a pair of very broad minima, D1 and D2, at [185°, 180°] and [235°, 170°] which lie 4.11 and 4.27 kcal/mol, respectively, above the absolute minimum. Again, there are experimental points associated with these minima. The [150°, 180°] conformation has been implicated in the hydrolysis of acetylcholine by cholinesterase[20] and the conformation [180°, 180°] has been suggested for nicotinic action by Martin-Smith, *et al.*[21]

The fourth pair of minima include the absolute minimum A1 at [225°, 70°] and its corresponding mininum A2 at [135°, 290°] which lies 0.29 kcal/mol above A1. This pair does not correspond to any known experimental points. However, BR have found minima in the same region of conformation space, and it is unlikely that this is just a coincidence. While we do not claim that these conformations are the most stable ones for acetylcholine, the fact that they appear on both maps indicates that they are possible conformations for the molecule.

Finally, minimum E at [355°, 180°], which is 7.37 kcal/mol above the absolute minimum, does not correspond to any suggested experimental point. This is not surprising since it is a relatively narrow minimum with steep energy gradients around it which would tend to give it a low entropy and hence a high free energy and would, therefore, make it conformationally unimportant.

The origin of the minima in Figure 2, and indeed the whole structure of the energy surface, can be explained by separately examining the nonbonded and electrostatic energy surfaces. These are given in Figures 3 and 4, respectively. The nonbonded interactions clearly define the gross features of the combined nonbonded and electrostatic map in Figure 2, including all the minima that appear there. However, in the nonbonded energy map, the maximum energy difference between the minima, except for minimum E, is only of the order of 1 kcal/mol. The most significant

---

(18) C. Chothia, *Nature (London)*, **225**, 36 (1970).

(19) C. C. J. Culvenor and N. S. Ham, *Chem. Commun.*, 537 (1966).

(20) C. Chothia and P. Pauling, *Nature (London)*, **223**, 919 (1969).

(21) M. Martin-Smith, A. Small, and J. B. Stenlake, *J. Pharm. Pharmacol.*, **19**, 561 (1967).

feature is a broad, relatively flat, central valley. The electrostatic interactions, on the other hand, show a broad, essentially flat, *high* central plateau which is energetically unfavorable but becomes more favorable along the edges. This orders the various minima and forces them to occur at the "cliffs" of the nonbonded map where the electrostatic energy is most favorable. Thus, minima A1 and A2, which have the most favorable electrostatic energies, also have the lowest total energies.

Another possible variable in our potential energy functions is the dielectric constant. Clearly, if one is doing the calculation for an isolated molecule, which is the way almost all quantum mechanical studies are performed, the dielectric constant should be equal to that of a vacuum which is 1.0. On the other hand, if the molecule is assumed to be in aqueous solution, the dielectric constant should vary between different pairs of interacting atoms, depending on how much water is instantaneously between the different pairs of atoms. This becomes a hopelessly complex situation, unless one makes the assumption that water is a macroscopic-type fluid that permeates the region about the molecule. The dielectric constant should then be equal to the square of the optical index of refraction since the water dipoles will not be able to align themselves to the rapid movement of the molecule. In that case, the dielectric constant will be equal to 1.77. As can be seen from Figure 5, changing the dielectric constant does not change the gross features of the acetylcholine map. The only change is that the energy differences between the minima are lowered. This is easily understood from the discussion of the previous paragraph. Increasing the dielectric constant will tend to wash out the electrostatic interaction and cause the nonbonded interaction to predominate even more than it does already.

The fact that the minima in the center of Figure 5 becomes energetically more favorable should be significant for the acetylcholine molecule in aqueous solution. The [±60°, 180°] conformation that has been suggested for acetylcholine in aqueous solution on the basis of an nmr study[19] leads to a much more open molecule than the [77°, 79°] conformation found in the crystal state[7] where acetylcholine is folded up. We would, therefore, expect more hydrogen bonding between the water and acetylcholine for the former conformation, particularly for the carbonyl and ester oxygens. This would be favorable from two points of view. First, there would be a lowering of the energy of the conformation due to water-acetylcholine interactions. Secondly, due to solvent shielding, there would be a decrease of the relatively unfavorable electrostatic interaction of acetylcholine with itself. Taking these two factors into account, we would not be surprised to see these minima become the absolute minima in aqueous solution.

Comparison with Other Theoretical Calculations

If one now compares Figure 2 with the conformational energy map produced by BR,[4] one notices two significant details. First, and most importantly, one observes that the general outlines of the two maps are very similar. Minima and maxima appear in about the same regions of the conformation space. This is especially true in the center where both maps contain a minimum at about [180°, 180°] and a double maximum at about [135°, 130 to 220°]. This is quite significant in that two relatively different approaches to calculating conformational energies give similar results. Semiempirical quantum mechanical calculations, while they have a theoretical basis, always leave an uncertainty in the calculated results because of the many approximations made to cut down the computation time. The fact that both approaches have given similar results *and predicted conformations that have been experimentally observed* indicates that both methods are at least semiquantitatively correct. This is especially important for the semiempirical classical calculation since it has even less of a theoretical base. Of course, more studies on different molecules must be made to confirm this conclusion.

Secondly, one notices that of the minima appearing at the edges of Figure 2, all have been shifted on BR's map into regions that would correspond to very high energies on our map. Upon checking these areas of conformation space, we found that the INDO method seems to allow a good deal of atomic overlap while at the same time giving energy stabilization. BR's minima and the short interatomic distances that we found for those conformations are listed in Table VI.

Table VI. Minima Found by Beveridge and Radna and Short Interatomic Distances Found in Our Study[a]

Minimum	Atom pair	Interatomic distance, Å	Sum of van der Waals radii for atom pair, Å
[50°, 270°]	C1–C6	2.69	3.40
	H1–O1	1.98	2.70
	H1–C6	1.65	2.90
	H1–O2	2.10	2.70
	H1–C7	2.35	2.90
	C2–O2	2.57	3.20
	H2–O2	1.57	2.70
	C5–O2	2.68	3.20
[50°, 50°]	C1–C6	2.67	3.40
	H1–O1	1.98	2.70
	H1–C6	2.15	2.90
	C4–C6	2.88	3.40
	C4–O2	2.66	3.20
	H4–O2	2.10	2.70
	C5–O2	2.67	3.20
[120°, 300°]	C1–O2	2.32	3.20
	H1–O2	1.86	2.70
	H1–O2	2.03	2.70
[40°, 180°]	H1–O1	1.88	2.70
	C5–O2	2.67	3.20
[200°, 330°]	C4–C6	2.74	3.40
	C4–O2	2.37	3.20
	H4–O2	2.19	2.70
	H4–O2	1.90	2.70
	C5–O2	2.67	3.20
[160°, 30°]	C4–C6	2.74	3.40
	C4–O2	2.37	3.20
	H4–O2	1.90	2.70
	H4–O2	2.19	2.70
	C5–O2	2.67	3.20

[a] We have only included interatomic distances that are more than 0.5 Å within the sum of the van der Waals radii of the two atoms involved.

While some of the overlaps might occur if the atoms were hydrogen bonding, as was reported by BR, there are just too many overlaps of atoms that do not hydrogen bond to each other for the molecule to have minima at those conformations. A similar result was found by Hankins and Kang[22] in their current INDO study of glycerylphosphorylcholine. They found an exaggerated stabilization of −70 kcal/mol when two antiparallel carbonyl dipoles were at a separation of 1.9 Å, which is almost the energy of a molecular bond. Apparently INDO, which is parametrized to give the correct interatomic distance for bonding, allows nonbonded atoms to approach almost to bonding distances before giving repulsive energies. This appears to be a significant difficulty in the INDO method.

Kier, in his study of acetylcholine by the extended Hückel method,[3] found only one minimum at [80°, 180°]. In addition, Kier varied the dihedral angle τ(C7–C6–O1–C5) and found a very broad minimum ranging from 120 to 240°. This is a curious result considering that the C6–O1 bond has partial double bond character and one would expect a planar structure with a high barrier to rotation about C6–O1.

In their semiempirical classical calculation, Liquori and coworkers,[1] using nonbonded interactions and torsional potentials, produced a very symmetrical conformational energy map with nine minima corresponding to different combinations of τ(O1–C5–C4–N$^+$) = 60, 180, 300° and τ(C6–O1–C5–C4) = 60, 180, 300°. In their follow-up, Ajò, *et al.*,[2] using partial charges generated by the CNDO/2 method and by the extended Hückel method, produced a conformational energy map very similar to ours though the differences in the energies of the minima are only of the order of 1–2 kcal/mol. The reason for these differences is that different nonbonded potential functions and partial charges were used. Besides using a different function for the repulsive term of the nonbonded interaction, they also used slightly different constants that were derived from experimental data on gases in the attractive term. The major difference, however, is that in the above two papers there was no differentiation between different kinds of carbon and oxygen atoms. One set of constants was used for both methyl and carbonyl carbons and another for ester and carbonyl oxygens. This is an approximation that we did not make. Finally, we did not use a torsional potential for the reasons outlined above.

Conclusions

The semiempirical classical calculations reported here lead to several significant conclusions.

First of all, the results of the calculation are substantially in accord with both experimental data on acetylcholine and the INDO results of Beveridge and Radna.[4] Of the four pairs of minima that were found that would be expected to be conformationally important, all but one are associated with conformations that have been suggested for acetylcholine on the basis of experimental observation. The remaining pair of minima, however, also appears in Beveridge and Radna's conformational energy map and, therefore, would not be expected to be a totally fictitious result.

Secondly, we have noticed that the INDO method may sometimes lead to unreasonable results. INDO, due to its neglect of many electron–electron repulsion terms, will allow significant overlap of atoms that are not bonded to each other. While this would be expected in situations where there is hydrogen bonding, INDO also allows atoms that do not hydrogen bond to approach very closely before finally giving repulsion.

Thirdly, our semiempirical classical calculation is of the order of 10,000 times as fast on the same computer as the INDO calculation of Beveridge and Radna. We calculated the 5329 points for each of our conformational energy maps using about 2 min of computer time per map. Beveridge and Radna, using the INDO method, needed 3 min of computer time to generate each of their *points.*[23] This enormous saving of computer time opens up many possibilities. One can do much more thorough conformational studies on larger molecules with much less expense than with semiempirical quantum mechanical calculations, apparently with about the same results. Further studies comparing these two methods are needed.

Acknowledgments. We thank Professor Max Goldstein and the staff of the Courant Institute Computing Center for both a grant of computer time and programming assistance. In addition, one of us (M. F.) thanks the National Science Foundation for a graduate fellowship. We are grateful to Professor David L. Beveridge and Richard J. Radna both for preprint material and for data which did not appear in their paper. It should also be noted that the compilation of experimental data that we used is essentially the same as that compiled by Beveridge and Radna. Finally, we thank Dr. David Hankins for many useful discussions and his infectious enthusiasm.

---

(22) D. Hankins and S. Kang, private communication.

(23) D. L. Beveridge, private communication.