Molecular Orbital Calculation of Preferred Conformations of Acetylcholine, Muscarine, and Muscarone

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SUMMARY

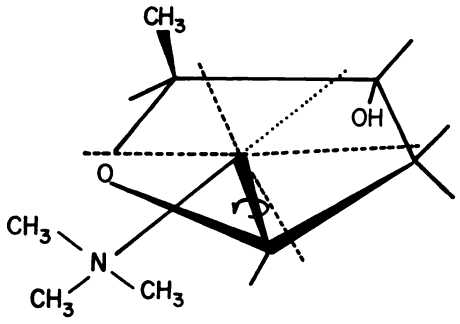
Using extended Hückel theory molecular orbital calculations, the preferred conformations of acetylcholine, L-(+)-muscarine, and D-(—)-muscarone have been predicted from total-energy minimization as a function of geometry. The calculations for muscarine and acetylcholine reveal a preferred conformation strikingly similar to conformations reported in the literature based upon crystal X-ray analysis. A comparison of calculated preferred conformations of L-(+)-muscarine and D-(—)-muscarone leads to the observation that the heteroatoms in the two molecules bear spatial relationships similar to each other. It is concluded that D-(—)-muscarone approaches the muscarinic receptor with the opposite face than does L-(+)-muscarine. From a consideration of the calculations on the three compounds, a complementary pattern of forces representing the muscarinic receptor is proposed.

INTRODUCTION

For many years, investigators have endeavored to map the active sites of the muscarinic receptor. The bulk of this work has centered around chemical modification, in an attempt to simulate the important features of acetylcholine and then to depart from this model in a regular manner, in order to assess the importance of the structural feature being varied. The problem is, of course, compounded by the fact that acetylcholine is not conformationally rigid in a classical sense. As a result, it has been necessary to constrain the functional groups of acetylcholine into a rigid structure in order to measure unequivocally their interatomic distances and relationships. Comparative cholinergic activities of these molecules have been used to predict the pharmacologically active conformation of acetylcholine and, hence, the complementary pattern of forces to be found on its receptor.

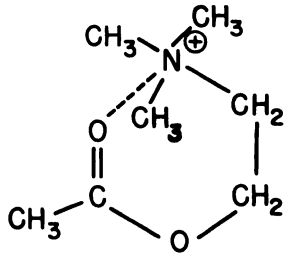
Clearly the rigid-conformation model possesses inherent weaknesses. In the process of spatially fixing the key functional groups to simulate various confor mations of acetylcholine, several additional atoms must be added to the molecule. The neglect of the possible interaction of these atoms with the receptor is questionable. In spite of the limitations inherent in the rigid-conformation approach, over the years there have evolved a number of useful empirical rules governing the structures of active muscarinics. These are typified by the 5-atom rule and by several assumptions regarding optimal distances between the nitrogen and oxygen atoms (1, 2).

The approach to the mapping of the cholinergic receptor has been made somewhat easier by the consideration of muscarine (I) a cholinomimetic with a much higher degree of conformational rigidity (3). This molecule has been regarded as having a side chain free to rotate about the bond joining it to the ring. The stereospecificity among muscarine and its isomers is high, suggesting a high degree of molecular complementarity with the receptor.



L-(+)-Muscarine conformational variations
(I)

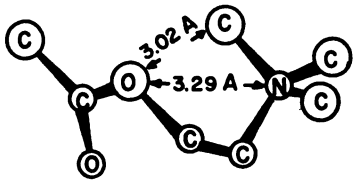
A physical approach to establishing the preferred conformation of acetylcholine has been attempted by several investigators. On the basis of infrared spectroscopic results, Fujita and Fellman proposed a cyclic conformation (II) (4). They subsequently modified this view and accounted for observed phenomena by assuming an inductive effect of the quaternary nitrogen on the carbonyl absorption peak (5, 6). Canepa and Mooney offered infrared evidence against these proposals (7).



(II)

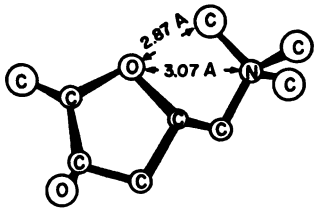
Another contribution toward the problem of acetylcholine and muscarine has been reported by Canepa, Pauling, and Sörum (8), and by Jellinek (9), respectively. They have determined the structures of these two molecules in the crystal by X-ray analysis (III and IV). These solid-state analyses reveal an astonishing similarity between the heteroatoms in these two molecules, so far as spatial relationship is concerned. It must be borne in mind, however, that the X-ray measurements are on solids; hence, they may not accurately reflect the conformations in biological media. Nevertheless, the close similarities with respect both to solid-state structure and to pharmacological activity in biological fluid are highly encouraging.

With the evidence from these two sources in hand, it seems logical to consider the



Acetylcholine crystal structure from X-ray analysis
(III)

preferred conformation of these two molecules on the basis of theoretical principles. Recent development of quantum mechanical methods applicable to nonaromatic molecules has made such an approach possible. The extended Hückel theory, developed by Hoffmann, has shown considerable



Muscarine crystal structure from X-ray analysis
(IV)

success in calculating preferred conformations of numerous hydrocarbons (10). The method determines total energy from a single parameterization, with the inclusion of all nonbonded interactions. As a result, the total energy calculated is a function of the assigned geometry, and the preferred conformation is thus predicted to be for the lowest energy model. We have recently reported the first successful use of this method in a fairly complex molecule containing two heteroatoms (11). The calculations correctly predicted the dihedral angle of phenylsydnone as found in the crystal.

CALCULATIONS

The calculations have been described in detail elsewhere (10), but briefly the ex-

pansion of a molecular orbital as a linear combination of atomic orbitals is

$$\Psi_i = \sum_j C_{ij}\phi_j$$

Upon minimizing the total energy, there is formed a set of Hückel equations, where Ψ is

$$\sum_{i=j}^{n} [H_{ij} - ES_{ij}]C_{ij} = \phi$$

the molecular orbital wave function, ϕ is the atomic orbital wave function, H the Hamiltonian operator, E the energy, S the overlap integral, and C the orbital coefficient. The calculations are performed for the valence electrons in s and p orbitals, using Slater orbitals as a basis set. The input parameters consist of the Slater exponent and the Coulomb integral, as shown in Table 1. All overlap integrals are considered. The resonance integrals are approximated by the equation

TABLE 1
Extended Hückel Theory Parameters

Coulomb integrals	
Electron	Value (eV)
N 2s	−26.00
N 2p	−13.40
O 2s	−35.30
O 2p	−17.76
C 2s	−21.40
C 2p	−11.40
Slater exponents	
Atom	Value
H	1.000
C	1.625
N	1.950
O	2.275

$$H_{ij} = 0.5K(H_{ii} + H_{jj})S_{ij}$$

with K set at 1.75. The total Hückel electronic energy is computed to be the sum of orbital energies of n electrons in $n/2$ filled orbitals,

$$\epsilon = 2\sum E_i.$$

The total molecular energies can be written as

$$E = 2\sum E_i + \sum_{n,n'} Enn' - \sum_{e,e'} Eee'$$

where Enn' and Eee' are nuclear-nuclear and electron-electron repulsion energies. The success of the calculations in predicting preferred conformations from minimum energies lies in the fact that the method of selecting the H_{ij} values must simulate, within the calculated electronic energies, the contribution of nuclear repulsions to the total energy (10, 12). Thus, the nuclear-nuclear and electron-electron repulsion energies cancel approximately, and the simple sum of one-electron energies behaves similarly to the true molecular energy (13). A characteristic of the extended Hückel theory is the frequently correct prediction of equilibrium conformation with an exaggeration of barrier heights due to overestimation of nonbonded interactions.

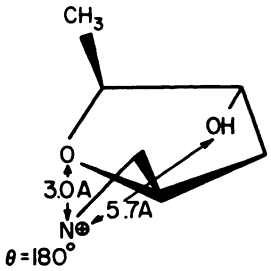
This delicate balance of repulsion energies is less likely to hold for charged species such as considered in the present study. Nevertheless, success has been achieved in conformational studies using neutral molecule approximations for ions (12, 14, 15).

For the calculations of the cholinergics in this study, it was necessary to input precise three-dimensional coordinates for each atom. This otherwise very difficult task was accomplished with ease and great speed by means of a computer program that was obtained from the Indiana University Quantum Chemistry Program Exchange. The program performs a series of vector summations with previously determined planes of atoms as references. The program can be keyed to output the atom coordinates on punched cards, in the fields required for the extended Hückel program. The bond angles and lengths used in the coordinates program were derived from X-ray analyses and, where not available, were assumed to be of conventional dimension. Bond lengths were assumed not to vary in the different conformational models. As a cross-check of the three-dimensional coordinates calculated by

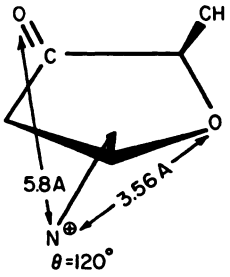
the program, all interatomic distances are also output and can be compared with measured values from wire models.

RESULTS

The calculations on muscarine were made on a series of models involving the rotation of the side chain over the ring through 360 degrees (see Fig. 1). Angular increments of 60 degrees were chosen for convenience. For each model conformation, the onium group was also rotated to minimize the energy due to *N*-methyl group interactions with ring atoms. The total energies are plotted (in Fig. 1) as a function of the angle, the zero angle being the eclipsing of the C—N bond with the ring C—C bond. The calculations reveal a very definite energy minimum at 180 degrees. This is precisely the conformation reported by Jellinek on the basis of his X-ray analysis (9). The calculated barrier to rotation away from this conformation is very large, undoubtedly far in excess of a reasonable value. Nevertheless, the true barrier height must be regarded as being quite considerable, since the rotation from 180 to 240 degrees passes from an ethane-like staggered to an ethane-like eclipsed conformation, with a calculated energy barrier of 6 kcal. This same change in conformation in ethane is calculated to be 4 kcal; the experimental value is about 2.8 kcal.



L(+) Muscarine



D(−) Muscarone

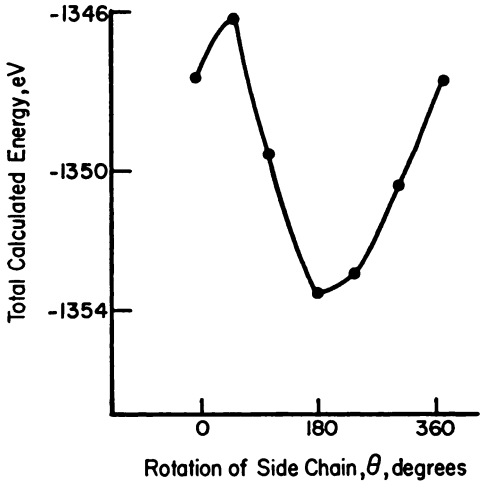


FIG. 1. *Conformational models of muscarine versus total calculated energy, E*

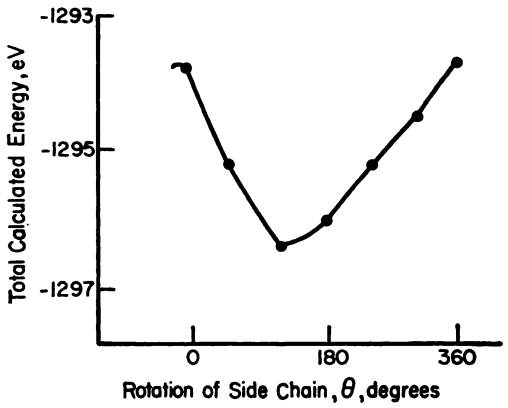


FIG. 2. *Conformational models of muscarone versus total calculated energy*

Interatomic distances calculated are close enough to X-ray measured values to be within experimental error. The 5-atom chain including the onium group comprises a single plane. An onium methyl group is close to the ring oxygen. The ring O to N distance is just 3 A.

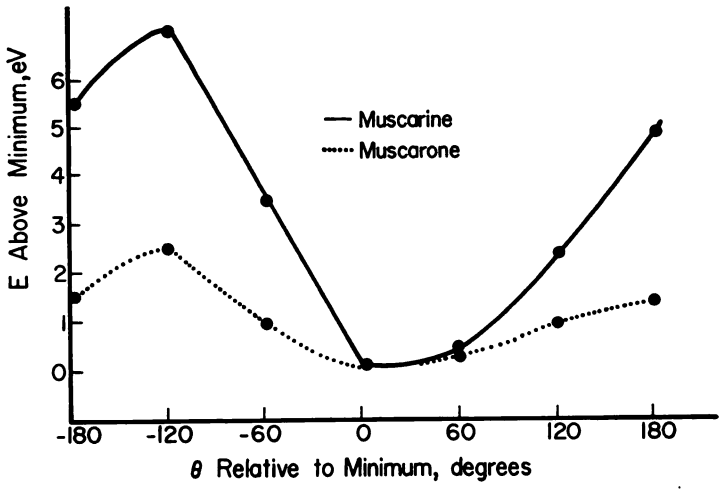


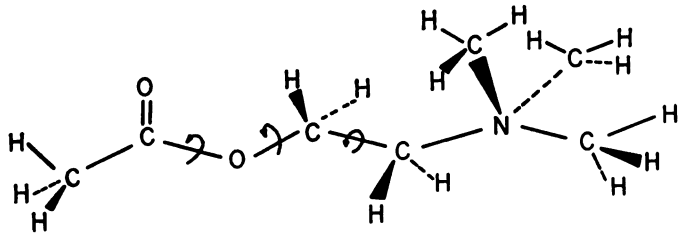
FIG. 3. *Energy versus conformation of muscarine and muscarone*

The calculations for the most active isomer of muscarone reveal an energy minimum for the conformation shown in Fig. 2. The N to carbonyl O distance is calculated to be 5.8 A, while the N to ether O distance is 3.56 A. No X-ray studies are available for muscarone with which to compare these calculated results.

In comparing these two compounds, it is evident in each case that deviation from the calculated equilibrium conformation produces a substantial energy barrier (see Fig. 3). However, there is a distinct difference in the shape of this potential curve as a function of rotation away from that of the preferred conformation. In the case of muscarine, the curve is quite steep. Only within a range of a 60-degree variation in one direction from the preferred conformation would there be expected any likelihood of a conformer existing, even under considerable perturbation pressure from extramolecular forces. Therefore, the calculations indicate a high degree of conformational fixation of muscarine within relatively narrow angular limits.

Muscarone, on the other hand, presents a somewhat different picture. The region of the energy minimum is broader than that for muscarine. It extends over about twice the angular radius and is found on both sides of the minimum energy angle. It can be concluded that muscarone can assume a greater number of conformations in the region of the energy minimum, in response to extramolecular perturbations.

The final calculation in this study was for the acetylcholine molecule (V). The variations considered were rotations about the methylene-methylene bond, the methylene-oxygen bond, and the oxygen-carbonyl carbon bond. To simplify the calculations, the acetyl methyl group was held rigid in the hydrogen-to-double bond eclipse. As in the two previous calculations, the onium methyls were rotated in each conformation



Acetylcholine
(V)

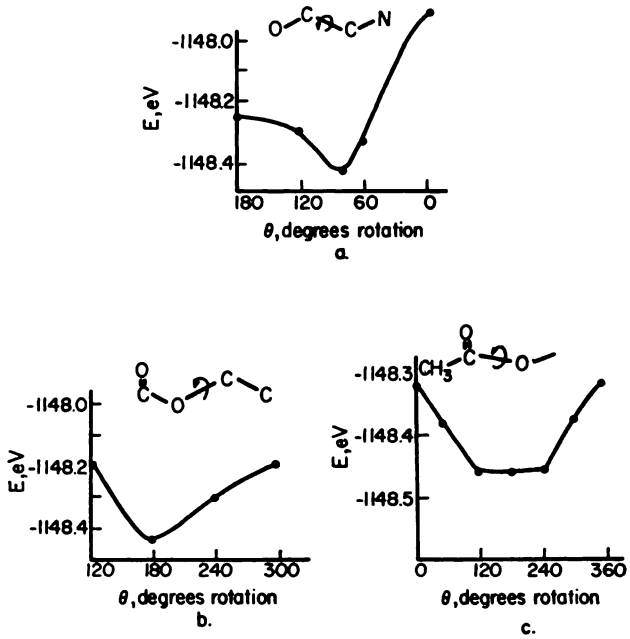
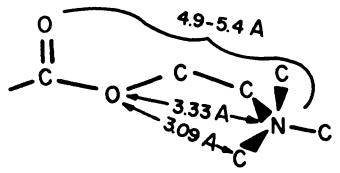


FIG. 4. *Rotational modes of acetylcholine versus total calculated energy*

to obtain the minimum energy. The results were quite striking (see Fig. 4).

The minimum for rotation about the methylene-to-methylene bond was found at an angle of 80 degrees from the staggered arrangement of the O and N atoms (Fig. 4a). This is exactly the conformation reported by X-ray analysis of the crystal. The interatomic distance calculated between the O and N atoms (VI) is within the experimental error of the X-ray measurement (8).



(VI)

The minimum energy from the calculation of the other two rotors was found for the completely staggered conformation, that is, where the methyl, the ester trio, and the two methylene groups are coplanar (Fig. 4, b and c). The X-ray analysis of the crystal shows a small rotation away from complanarity of the two methylenes with the ester group. Nevertheless, the agreement is good. The N to ether O distance is calculated to be 3.33 A as compared with the X-ray crystal value of 3.29 A.

An analysis of the potential surfaces for the three rotational patterns reveals distinctly less rigidity than that of muscarine and muscarone. The rotation about the methylene-methylene bond presents a barrier only one-tenth the magnitude calculated for the other compounds. The region about the minimum is shallow for all angles except the O-N eclipsed conformation. The methylene-ether O rotational pattern is steep except around the minimum (Fig. 4b). The final rotor varied, the carbonyl carbon to oxygen, produced an indistinguishable minimum over 120 degrees around the staggered conformation (Fig. 4c). It is evident that the calculated barrier to rotation of the trimethylaminomethyl group around the methylene-methylene bond in acetylcholine (Fig. 4a) is considerably lower than those for the rotations of comparable groups in muscarine and muscarone.

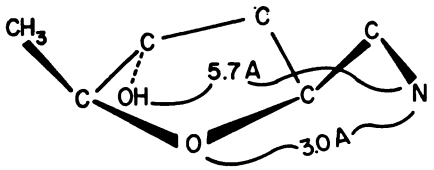
DISCUSSION

Four facts concerning the calculations must be clearly understood.

First, the extended Hückel theory molecular orbital calculations used, which employ a one-electron Hamiltonian for sigma and pi orbitals, are subject to the same criticism directed to all Hückel calculations. Second, the extended Hückel calculations balance nuclear-nuclear, electron-electron, and nuclear-electron interactions in a manner successful enough to predict preferred conformations in many cases but, at the same time, overestimate nonbonded interactions to produce very high calculated barriers. Third, these calculations use the valence-state ionization potential for all Coulomb integrals, including the positively charged nitrogen. Thus electron correlation is not treated. This problem has recently been recognized by inclusion of an iterative modification of the Coulomb integrals, reminiscent of the omega technique (16, 17). Fourth, prior to the matching of calculated molecular orbital properties with biophase reality, it must be recognized that the calculations are made on a conservative system, that is, on an isolated molecule.

With these facts in hand, it is now possible to discuss realistically the significance of the calculated results.

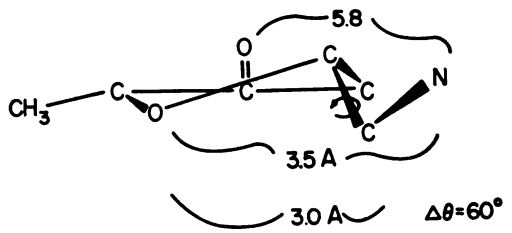
It is evident that calculated relative rotation barriers for muscarine (VII) and



L-(+)-Muscarine calculated in C_s (envelope) preferred conformation
(VII)

muscarone (VIII) indicate a high degree of fixation in the extended-onium-group conformation. This would tend to argue against the suggestion that the activity of D-(—)-muscarone is due to a folded conformation in which the N to carbonyl oxygen distance is comparable to the N to ether O distance in an extended-muscarine conformation (3). The calculations indicate that the N to ether O distance in both molecules is similar. It is also shown by the calculations that in muscarone, the N to ether O distance can be compressed to the muscarine N to ether O distance with a relatively modest expenditure of energy. The conclusion from these calculations is that the N and ether O play the same role in both molecules in regard to receptor interaction. This further implies that the role of the OH in muscarine is mirrored by the carbonyl oxygen in muscarone. In order for these three atoms to behave in the same manner, in regard to the receptor, it is necessary to conclude that D-(—)-muscarone approaches the receptor with the opposite face than does L-(+)-muscarine, relative to the onium side chain. This is quite possible since the methyl group is nearly in the ring plane of muscarone, due to the preferred C_2 half-chair ring conformation (18).

The calculations for acetylcholine indicate much greater conformational flexibility. Nevertheless, the calculated preferred conformation of acetylcholine (VI) places the three key atoms in relative positions similar to the previously described placement in muscarine and muscarone.



D-(—)-Muscarone calculated in C_2 (half-chair) preferred conformation
(VIII)

Within the spirit of the approximations made for the calculations and the results obtained, a pattern of forces representing the muscarinic receptor can now be proposed (see Fig. 5) which coincide with the three key atoms of these molecules.

In summary, a significant pattern emerges from the work just described. The three molecules discussed are all potent

muscarinic cholinergics; the X-ray analyses of two of the molecules show a strong similarity in the spatial arrangement of the heteroatoms; and, finally, the molecular orbital calculated preferred conformations agree very closely with each other and with the X-ray crystal values. There is no direct evidence, as yet, that solid-state and molecular orbital calculated conformations are

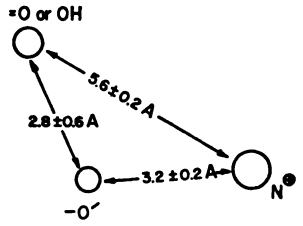


FIG. 5. *Proposed receptor pattern for muscarinics derived from calculations*

relevant to the environment *in vivo*. Nevertheless, the coincidence cited is definitely noteworthy and merits consideration in discussions of conformations of cholinergic molecules. Finally, these very interesting and encouraging results should serve as an impetus to further application of molecular orbital methods in the study of the conformation of other biologically important molecules.

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