A Molecular Orbital Calculation of the Preferred Conformation of Nicotine

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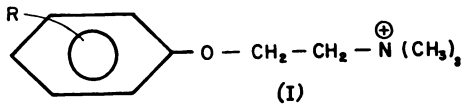
SUMMARY

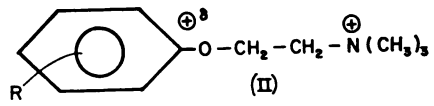
Using extended Hückel theory molecular orbital calculations, two equally preferred conformations of the nicotinium ion have been predicted from total energy minimization as a function of geometry. The calculated conformations are in good agreement with reported nuclear magnetic resonance (NMR)-predicted conformations of several nicotine derivatives. In only one predicted nicotinium ion conformation are there found to be two atoms, including the quaternary nitrogen atom, of comparable charge and interatom distance to those found in acetylcholine. Specifically these atoms in the nicotinium ion relate very closely to the quarternary nitrogen and carbonyl oxygen atoms of acetylcholine in its calculated preferred conformation. This observation permits the prediction that the two principal atoms necessary for nicotinic activity in the nicotinium ion are a quaternary nitrogen atom and a negatively charged atom about 4.85 A removed. As an adjunct to the study, an extended Hückel theory population analysis of acetylcholine in its calculated preferred conformation indicates a slightly negative total ($\sigma + \pi$) charge density on the ether oxygen atom.

INTRODUCTION

Two points of view prevail concerning the key electronic features that are necessary in a molecule eliciting nicotinic cholinergic activity. The first point of view, advanced by Hey (1, 2), is that activity is dependent upon the presence of a partial positive charge a suitable distance from a quarternary nitrogen. Hey based his opinion on observations of the activity of a limited series of substituted phenylcholine ethers (I) in which the ring substituents were graded according to their ability to withdraw or donate electrons mesomerically with respect to the ether oxygen. Substituents withdrawing electrons mesomerically were found to enhance activity. Hey presumed from this evidence that maximum nicotinic activity would be found in the choline ether series where polarized structures like (II) contribute appreciably. This was predicated upon the assumption that the nicotinic agent acetylcholine was similarly polarized (III) when absorbed at the receptor.



(I)



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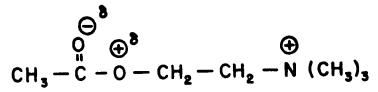

(III)

Barlow and Hamilton studied a series of nicotine analogs, pyridylmethyl and pyridylethyl dialkylamines substituted in the α, β, and γ ring positions (3). They

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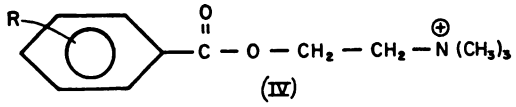
noted that β-substituted pyridine derivatives were generally more active. They reached the conclusion that a slightly positively charged secondary site was necessary for activity. The range of distances proposed for this site was 3.4–4.5 A from the onium group.

The second point of view, based on a study of esters of chlorine by Sekul and Holland (4, 5), is that a partial negative charge at some distance from the onium group is essential for nicotinic activity. This negative charge is assumed to be in a position analogous to the partial negative charge assigned to the carbonyl oxygen atom of acetylcholine (III). Coleman, Hume, and Holland (6) further justified this point on the basis of a study of a series of phenylcholine ethers; they attributed their findings to an inductomeric effect on the ring, the same substituent effect observed by Hey. They suggested that the partially negatively polarized ring provides a secondary binding feature in the molecule, presumably comparable to the partially negatively charged carbonyl oxygen of acetylcholine.

Ormerod (7) studied a series of substituted benzoycholine esters (IV) and



(IV)

observed that electron-releasing substituents enhance activity, presumably by increasing the partial negative charge on the carbonyl oxygen atom. Thus, the view that a secondary negative site is present in the molecule was supported. Triggle feels that the combined evidence of Hey, Ormerod, and Sekul and Holland suggests the need for two secondary sites, one partially negative and the other partially positive in character, such as found at the two oxygen atoms of acetylcholine (8).

Barlow and Hamilton have compared the activities of (+) and (—) nicotine in an effort to ascertain any significant difference (9). Such a finding would tend to implicate the presence of a third site in the molecule, imparting stereospecificity. They observed a varying degree of specificity from one group of receptors to another. This suggests some variation in receptor structure. They felt that differences in activity as an agonist between the two isomers could be a difference in efficacy rather than one of affinity. Barlow concludes that stereospecificity of nicotine may in fact be a result of a portion of the molecule located in a less active isomer functioning as a deterrent to affinity rather than the actual presence of a third pharmacodynamic group optimally placed in the more active isomer (10).

This work has been summarized by Barlow who leaves open the question of the nature of the secondary binding site (11).

It is evident, from an examination of the structures so far referred to, that there are limitations in the use of their structures to map the nicotinic receptor. These limitations arise from the fact that (a) the structures are not conformationally rigid, at least in a classical sense, and (b) an assignment of relative charge of key atoms has been based upon valence-bond intuition and/or π-electron-only molecular orbital considerations. Indeed, as will be shown shortly, the assignment of a positive charge to the acetylcholine ether oxygen is probably incorrect, tending to invalidate many assumptions upon which the charge of this atom is based.

There is now a quantum mechanical method available that will permit the assignment of conformation and charge in a molecule. The method, devised by Roald Hoffmann (12) and known as extended Hückel theory (EHT), uses a single parameterization to calculate the eigenfunctions and eigenvalues, with overlap and all nonbonded interactions included. As a result, the total energy, calculated for all valence electrons (σ and π), is a function of assigned geometry, the preferred conformation being presumed as that having the lowest total energy value. The method has met with considerable success in predicting preferred conformation of hydrocarbons (13–16) and recently of heteroatom-containing molecules (17–19). A recent theoretical study of EHT calcu-

lations (20) has revealed that the method indicates preferred conformations comparable to conformations predicted from ab initio calculations, indicating that EHT is more fundamentally sound than originally conceived.

Another recent study by Adam, Grimison, and Rodriguez (21) has dealt with the parameterization of a quaternized nitrogen. They have confirmed that parameters used by us in a previous study (18) and in the present study have yielded reasonable charge densities on adjacent C—H bonds, which correlate well with NMR chemical shifts, thus lending some justification for the use of the nitrogen parameters. Their work has tended to dispel somewhat the concern we expressed before (18) about the use of these parameters.

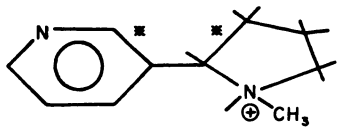
In our previous technique to study medicinal chemical problems associated with conformation (18), calculations of acetylcholine and muscarine gave geometrics very close to those found by X-ray analysis of their crystals. This coincidence, coupled with the facts that the two molecules possessed identical biological activity (muscarinics) and that calculations showed that the heteroatoms were similarly disposed in the two molecules, led us to the conclusion that EHT calculations might be applied to molecules in biological media. While there has been no definite relationship shown among crystal structure, calculated preferred conformation, and conformation in solution, the possibility of its existence nevertheless is intriguing and deserving of considerable attention and testing. It is reasonable that, if a calculated barrier to rotation about a bond is quite high, solution effects would not be sufficient to overcome it and that the calculated preferred conformation would remain unchanged in solution. Similarly, perturbing influences of receptor surface features would be inadequate to alter preferred conformation in solution.

One shortcoming of the EHT method is its exaggeration of energy barrier heights. Only by comparison with bonds of comparable composition and with known barriers can an approximation of true values be realized (18). A second shortcoming of the EHT method comprises the extreme values obtained for the charge densities. Although usually of correct sign and internal relative values, they are quantitatively exaggerated.

In view of these successful applications and with their limitations in mind, we sought to apply EHT calculations to the problem of the nicotinic receptor. In this study we considered the nicotinium ion, since it is the archetype of nicotinic activity and it presents the least complicated conformational possibilities of the nicotinic-acting molecules discussed. Also, findings from a recent NMR study dealing with conformation of nicotine derivatives were available for comparison (22).

CALCULATIONS

We employed the same parameters and techniques described in our previous studies (17–18). The calculation was made on the nicotinium ion (V) since this univalent



(V)

cation is likely to be the active molecule *in vivo* (23). We assumed that the pyridyl ring and the N-methyl group were trans, based on chemical evidence (24). The bond lengths and bond angles used in the calculations were those commonly assigned to bonds of comparable order (25). For these calculations, a planar ring model was used for the pyrrolidine ring. This is not a true picture of the ring; however, it is likely that the pyrrolidine ring is a free or slightly restricted pseudorotor (26), i.e., a ring for which no single, simple, nonplanar structure will accurately represent the molecule. The pseudorotor concept was first proposed by Kilpatrick, Pitzer, and Spitzer (27). With free pseudorotation, the assumption of ring planarity would represent an averaging of all nonplanar possibilities, and thus not seriously alter

the calculated barriers to rotation of the pyridyl ring. For convenience, total-energy calculations were made for every 60 degrees of rotation of the pyridyl ring through a full cycle. Population analyses were performed on conformations at the minima. For comparison and as an adjunct to the study, the population analysis was calculated on the basis of the preferred conformation of acetylcholine (18).

RESULTS

The total energy calculated by EHT is plotted against the angle of rotation of the pyridyl ring, the zero angle taken as the conformation in which the two starred bonds in (V) are eclipsed (see Fig. 1).

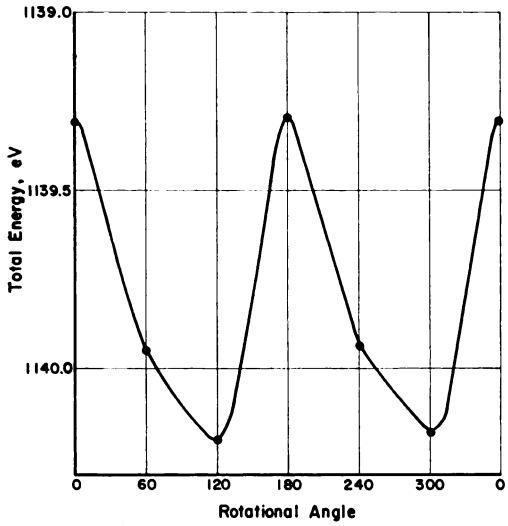


FIG. 1. *Angle of pyridyl ring rotation and calculated total energies for the nicotinium ion*

It is evident from the relationship of rotation to total energy that two virtually identical minima exist, i.e., there are two equally preferred conformations, at 120 and at 300 degrees. The conformer at 120 degrees is preferred by only 0.013 eV. The two calculated preferred conformations correspond to the two pyridyl-ring rotamers in which the pyridyl and pyrrolidine ring planes are perpendicular. The calculated barriers are of virtually the same magnitude, about 0.9 eV.

A population analysis is reproduced, in part, in Fig. 2 for the preferred rotamers

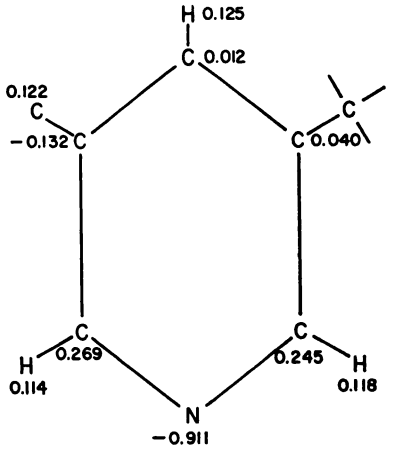


FIG. 2. *Population analysis for the pyridyl ring of the nicotinium ion*

Charges are net charge densities ($\sigma + \pi$).

of the pyridyl ring of the nicotinium ion. The charges and values shown are net charge densities ($\sigma + \pi$ electrons). For comparison, the charges calculated by Adam and Grimison for unsubstituted pyridine are reproduced in Fig. 3 (28).

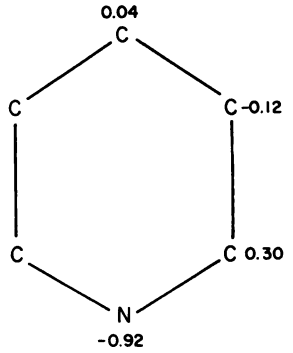


FIG. 3. *Population analyses for first-row atoms of pyridine*

Charges are net charge densities ($\sigma + \pi$). From Adam and Grimison (28).

Key interatomic distances calculated by the EHT method are shown in Fig. 4 for the 120 degrees rotamer and Fig. 5 for the 300 degrees rotamer. The population analysis for acetylcholine in its preferred conformation is shown, in part, in Fig. 6. It should be emphasized for these electron density calculations on both nicotine and acetylcholine that the numerical values

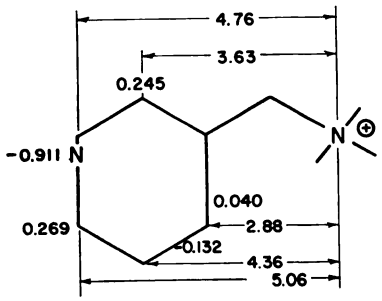


FIG. 4. *Interatomic distances (A) and net total charges for the 120-degree nicotinium ion conformer*

represent the net charge over both σ and π electron orbitals in the molecules. This is a truer representation of the atom's charge and its subsequent coulombic interaction

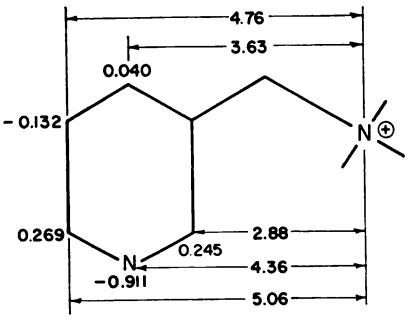
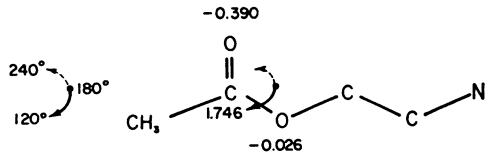


FIG. 5. *Interatomic distances (A) and net total charges for the 300-degree nicotinium ion conformers*

role than just π charge densities which have been customarily calculated. As has been shown (18), rotation of the acyl portion of acetylcholine through 120 degrees



Acyl Group Rotation Angle, degree	⊕ N to =O Distance, Å	⊕ N to >C= Distance, Å	⊕ N to −O− Distance, Å
120	4.93	4.52	3.33
180	5.07	4.52	3.33
240	5.40	4.52	3.33

FIG. 6. *Interatomic distances (A) for preferred conformations of acetylcholine*

Acyl group charges are net charges ($\sigma + \pi$).

produces a constant minimum. Therefore, the N to carbonyl-oxygen distances are reproduced for every 60 degrees of rotation of this group in Fig. 6.

DISCUSSION

To establish an important point before considering the nicotinium ion results, it is instructive to examine the population analysis of acetylcholine in Fig. 6. The net total charge on the ether oxygen atom is clearly not positive; in fact, it is slightly negative. A valence-bond positive charge has been assigned to this atom traditionally due to consideration of the π electron system only (III). When considering the forces involved in the reversible interaction of a drug molecule with a receptor, it is quite probable that the total valence electron composition of the atom must be considered in a point-charge model. Based on molecular-orbital calculations, it is incorrect to consider this as a partially positively charged atom. Previous comparisons in test molecules and acetylcholine must now be reexamined in the light of these considerations.

The calculated preferred conformations of the nicotinium ion are in agreement with a recently reported study by Simpson, Craig, and Kumler (22). These authors determined NMR chemical shifts of appropriate hydrogen atoms of nicotine and several quaternary derivatives of the pyrrolidine ring. They conclude that the "extreme conformations" (energetically least preferred) were those identical to our 0 and 180 degree rotamers. It is interesting that neither the NMR evidence nor our calculations indicate that the least preferred pair of conformers would be at 60 and 240 degrees. An examination of wire models reveals no apparent difference between the 0, 60, 180, or 240 degree rotamers as far as the interaction of the ortho hydrogens on the pyridine ring and the adjacent cis hydrogens on the pyrrolidinium ring. The Thompson, Craig, and Kumler study only speculated that the preferred conformation lay somewhere between their two "extreme conformations."

Because of the virtually identical total

energy for the 120 and 300 degree rotamers, it is impossible to specify the preferred conformation. Indeed, it presents the interesting possibility that two demonstrable isomers of the nicotinium ion exist.

This same possibility was suggested by Barlow and Hamilton, who noted the considerable restriction to pyridyl ring rotation in models of quaternary nicotine analogs (9). No such experimental observation has been made to date. This may possibly be due to a relatively low barrier to interconversion of the two rotamers. The quantitation of this barrier is not possible from EHT calculations, due to their known exaggeration of energy barrier heights. However, recent work has suggested that the barrier to rotation about an sp^2-sp^3 carbon-carbon is fairly high (29). An answer to the intriguing possibility of there being two isomers is being sought in our laboratory. As a working hypothesis in the present study, we must consider both rotamers as likely conformations of the molecule.

The population analysis of the substituted pyridine ring (Fig. 2) reveals a charge-density pattern somewhat different than that of unsubstituted pyridine (Fig. 3). The major change occurs at the carbon atom involved in the interring bond. This atom acquires a slight positive character. The only two atoms with a total negative charge are the nitrogen atom, with a high negative charge, and the carbon atom meta to it, with a modest negative charge.

In an effort to shed light on the original question, viz. the electronic character of the secondary binding site remote from the quaternary nitrogen in the nicotinium ion, we have portrayed the two rotamers calculated as possibilities in Figs. 4 and 5. The net charges and key interatomic distances are included. An obvious molecule for comparison is acetylcholine, a potent nicotinic agent that was subjected to conformational and charge-density calculations in our previous EHT study (18) (Fig. 6). We have sought in this comparison to find an atom in each molecule with a similar electronic charge and of closely comparable distance from the quaternary nitrogen atom. These conditions are found in the 120 degree rotamer of the nicotinium ion in Fig. 4. The pyridine nitrogen atom has a substantial negative charge and is 4.76 A from the quaternary nitrogen atom. This is very close to the situation in acetylcholine (Fig. 6), in which the negatively charged carbonyl oxygen atom is 4.93 A from the quarternary nitrogen atom when the acyl group is in a 120 degree orientation to the ether oxygen-carbon bond. This possibility is energetically permitted since, according to our calculations (18), the preferred conformation extends over 120 degrees of aycl group rotation. No other set of circumstances, viz. comparable charge and comparable distance, is to be found in either preferred conformation of the nicotinium ion.

It must be borne in mind that it is not justifiable to equate high biological activity with binding alone for any drug which is an agonist. Two factors are involved, affinity and efficacy. It is likely in this work that we are dealing with a composite of both phenomena. A clearer insight into this may evolve from further studies on other nicotinic agents, particularly those whose activity is greater than nicotine.

The conclusion to be drawn from these observations is that the secondary site of binding in the nicotinium ion is a negatively charged atom and that its receptor involvement is comparable to that of the carbonyl-oxygen atom of acetylcholine. Both of these atoms are of comparable distance (4.76–4.93 A) from their respective quaternary nitrogen atoms. This suggested relationship is portrayed in Fig. 7, which

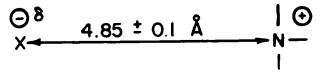


FIG. 7. *Key features of a proposed nicotinic-acting molecule*

shows graphically the key features of the nicotinic-acting molecule. This does not preclude the possibility of additional secondary points of binding, but evidence for them is not apparent from this study. Future work on other nicotinic agents should extend these findings.

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