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SHORT COMMUNICATION

Conformational Analysis of *N*-(β-Acetoxyethyl)Pyridinium Ion: Comparisons with Acetylcholine

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SUMMARY

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A conformational study for a pyridinium analogue of acetylcholine has been performed by a semiempirical approach using the PCILO method. The results have been compared with those of previous PCILO studies on cholinergic molecules. Our analysis shows that the *N*-(β-acetoxyethyl)pyridinium ion (PyACh) has an absolute minimum for a gauche-gauche conformation. The gauche conformation characteristic of acetylcholine (ACh) remains a local minimum. The more crowded conformation of PyACh is related to intramolecular interactions between the anionic and cationic heads, which are more effective than in ACh due to the reduced spherical screening around the nitrogen atom. This characteristic and the consequently low conformational flexibility of PyACh could explain the reduced biological activity of pyridinium analogues of ACh.

INTRODUCTION

The fundamental synaptic neurotransmitter acetylcholine (ACh) and its analogues, active in both muscarinic and nicotinic sites, have stimulated several experimental and theoretical studies (see, e.g., Refs. 1-8 and reference therein). Several correlations have been attempted between biological activity and molecular properties (e.g., conformational flexibility (2, 9), charge distribution (10), and shape of the molecule (11)).

Recently synthesized pyridinium derivatives of ACh have been shown to have some muscarinic activity and effectively block the action of acetylcholinesterase (AChE) (12).

In an attempt to correlate these findings to structural modifications with respect to ACh, we have undertaken a theoretical study of *N*-(β-acetoxyethyl)pyridinium (PyACh), chosen as a model compound. The PyACh, in fact, has a limited muscarinic activity and is a weak inhibitor of AChE.

The results will be discussed with special reference to previous PCILO computations on cholinergic molecules.

NOTATION, STRUCTURE, AND METHOD

Due to the complete absence of experimental data on the geometry of PyACh, we have used the structural parameters determined by X-ray technique for ACh (13), but for the pyridinium ring for which we have used a structure based on microwave measurements on pyridine (14).

The energies have been computed by the PCILO method (15 and references therein) optimizing the bond polarities for each conformation. The PCILO method has been chosen in view of the good agreement with both experimental and *ab initio* results for molecular conformations as a function of torsional angles. In particular it has been shown that in the cases of conjugated systems (16) and of secondary or tertiary amines (or onium ions) (3, 17), the PCILO method works better than other semiempirical methods. The acetylcholine results (3, 17) may be considered as a positive check of such a statement.

As is well known, the PCILO method is a perturbative treatment using as zero-order approximation a completely localized description of the molecule (15). In the case of substituted benzenes, the two Kekulé formulas are not equivalent: As a consequence, the results depend on the choice of the zero-order localized wave function, the differences being, in several cases, of the same order as the conformational energy differences (18). In our computations, we have followed the practical criterion suggested by Diner *et al.* (19), i.e., to choose as the starting wave function the one having the best energy.

As for charge distributions, it has been shown that the PCILO method is not particularly suitable, especially for orbitals describing lone pairs, which are not so well

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defined as bonding electron pairs (20). Even though it has been recently shown that the introduction of the INDO approximation (in place of the original CNDO one) and of carefully chosen hybridization coefficients for lone pairs can overcome this difficulty (21, 22), in the present paper we have preferred to compute charge distributions by the CNDO/2 method (23). This choice has been made to allow a comparison between PyACh and other cholinergic molecules, whose CNDO/2 charges have already been published. Furthermore, it was found that CNDO/2 charges are in agreement with both *ab initio* ones (at least in trends) and with experimental parameters such as ^{13}C chemical shifts (21, 23, and references therein).

As for definition of the conformations, following the standard convention, the torsion angle τ of the bonded atoms A–B–C–D is defined as the angle between the planes ABC and BCD. Viewed from the direction of A, this angle is positive for clockwise rotations around B–C and negative for counterclockwise rotations.

RESULTS AND DISCUSSION

The PyACh molecule has four torsional degrees of freedom (the methyl group is considered frozen in a staggered conformation), expressed here by means of the dihedral angles τ_0, τ_1, τ_2, τ_3 (see Fig. 1). As in previous studies on cholinergic molecules (e.g., 4, 5), τ_0 is fixed at 180° following standard stereochemical considerations and several experimental evidences. On the other hand, in the case of PyACh, it is not possible to choose a fixed value of τ_3 in analogy with ACh, due to the different symmetry of the cationic head (C_{3v} for ACh and C_{2v} for PyACh). As a consequence, the conformational analysis has been carried out as a function of the three dihedral angles τ_1, τ_2, and τ_3. The computations have been performed in steps of 20° for τ_1 and τ_2 and of 15° (between 0 and 90°) for τ_3.

From an analysis of the results, it emerges that lower energies in the whole range of τ_1 and τ_2 are obtained for $\tau_3 = 45°$. As a consequence, we report in Fig. 2 only the section E (τ_1, τ_2) at $\tau_3 = 45°$ of the multidimensional energy surface. Contrary to other cholinergic molecules, the conformational map of PyACh does not have an inversion conter at $\tau_1 = 180°$, $\tau_2 = 180°$ due to the value of τ_3.

The absolute minimum is found for a gauche–gauche

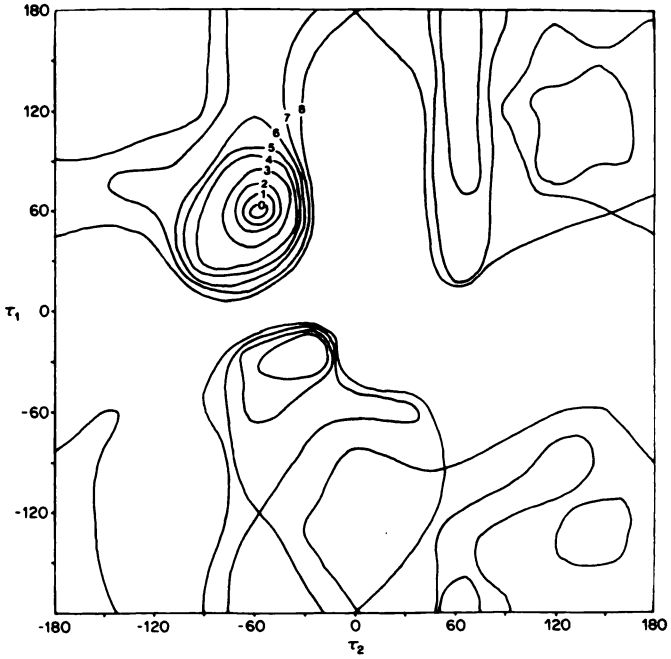

FIG. 2. *Conformational energy map for PyACh (PCILO method) as a function of the dihedral angles τ_1 and τ_2 for the optimum value of τ_3 (45°)*

Isoenergetic curves are spaced 1 kcal/mol.

conformation ($\tau_1 = 60°$, $\tau_2 = -60°$). A perspective drawing of PyACh in this conformation is reported in Fig. 3. In a range of energy values relevant for biological activity (4, 8), only another local minimum is found for PyACh. It corresponds to $\tau_1 = -30°$ and $\tau_2 = -30°$ and is 4.5 kcal/mol higher than the absolute minimum, and the interconversion energy barrier is about 7 kcal/mol.

The gauche conformation characteristic of ACh ($\tau_1 \simeq 180°$, $\tau_2 \simeq 60°$) according to PCILO computations is, in the present case, a local minimum of 5.5 kcal/mol above the global one and with an interconversion barrier of about 8 kcal/mol. The fully extended conformation ($\tau_1 \simeq 180°$, $\tau_2 \simeq 180°$), which corresponds to a local minimum in ACh, is now about 8 kcal/mol above the absolute minimum. This energy corresponds to an almost free internal rotation of PyACh around both τ_1 and τ_2 degrees of freedom.

As a whole, a comparison between our Fig. 2 and Fig.

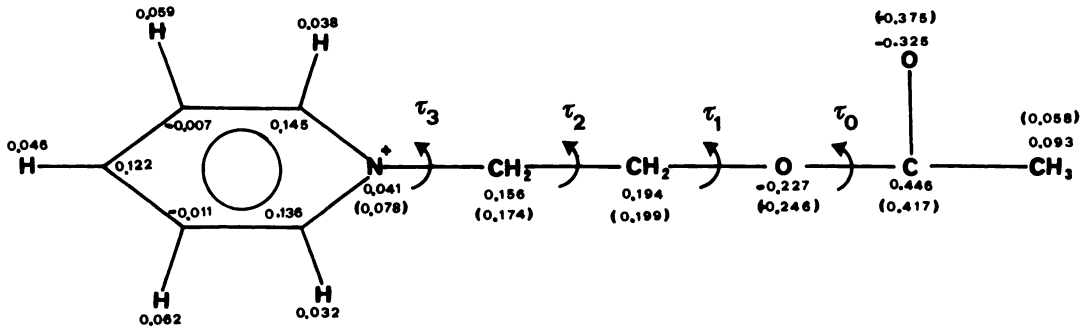

FIG. 1. *Definition of dihedral angles for PyACh*

All starting dihedral angles have been assumed in a *trans* orientation (values of 180°). The numbers over the main atoms are the CNDO/2 net charges for the most stable conformation of PyACh. The corresponding values for ACh (in its most stable conformation) are in parentheses.

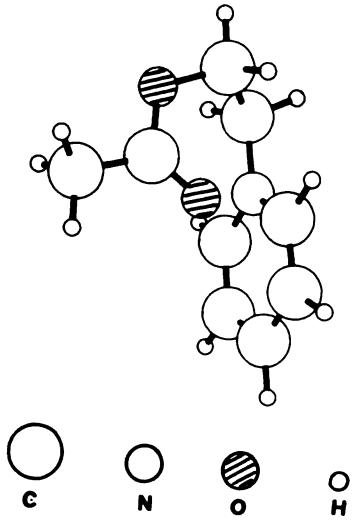

FIG. 3. *A perspective drawing of PyACh in its most stable conformation ($\tau_0 = 180°$, $\tau_1 = 60°$, $\tau_2 = -60°$, $\tau_3 = 45°$)*

2c of Ref. 5 clearly shows that the PCILO method forecasts that the replacement of the onium ion of ACh by a pyridinium ring strongly reduces the conformation freedom of the molecule.

The present results are quite similar to those obtained by the PCILO method for acetylethanolamine (AEt) (4), showing that each modification of the cationic head reducing the spherical screening around the nitrogen leads to more crowded conformations. This effect is connected with the formation of a strong coupling between the carbonyl group and the nitrogen atom when vicinal positions of the two groups are sterically allowed.

It is interesting to note that the large zone between the gauche and trans conformations ($\tau_1 \simeq 180°$, $\tau_2 = 60$–$180°$) becomes higher and higher in energy going from ACh to PyACh and AEt. Since a number of investigators place in this zone the conformations related to muscarinic and nicotinic activity (4), the PCILO results seem in line with the activities of the considered molecules.

The reported results must be considered particularly significant, as they confirm that the presence of large modifications on the onium ion does not inhibit ACh analogues to adopt crowded conformations. Similar results have been obtained by X-ray (24) and NMR (25) techniques for 2-*N*,*N*-diethyl-*N*-benzylammoniumethylcarbonate.

A Mulliken population analysis performed on the CNDO/2 wave function shows that the charges of PyACh are quite different from those of ACh. All the charges of polar groups are lower in PyACh than in ACh; this is particularly evident for the nitrogen and the carbonyl oxygen (see Fig. 1). Also, the total positive charge of the cationic head decreases from 0.773 for the $N(CH_3)_3$ group of ACh to 0.663 for the pyridinium ring of PyACh. This effect is connected with the strong coupling between the carbonyl group and the π system of the pyridinium ring. This leads to a not negligible intramolecular charge transfer from the whole anionic head (which undergoes a significant internal redistribution of charges due to inductive effects) to the pyridinium ring. Confirmations of this viewpoint can be found in the asymmetry of the net charges in the pyridinium ring (the portion of the ring near the carbonyl group bears fewer positive charges). On the contrary, no asymmetry is found in the case of a fully extended conformation. Moreover, the contribution to the PCILO energy by delocalization terms (15) from the carbonyl to the neighboring N–C π^* bond is strongly relevant.

In conclusion, a comparison of PyACh with ACh shows the combined effect of modifying the dimension of the cationic head, the preferred conformation of the whole molecule, the general morphology of important regions of the conformational map, and the charge distribution.

The present results are obtained by an approximate quantum-mechanical method, and even though they do not take into account some effects which might prove relevant (e.g., solute–solvent interactions (26–28) or structural flexibility (e.g., Ref. 6)), it is gratifying that all the quoted factors agree in forecasting a reduced biological activity of PyACh with respect to ACh.

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