hold for acetate catalysis of methylacetylacetone. The possibility that there is a water bridge in the transition state is unlikely due to the inhibiting steric effects found for the base catalysis of α-substituted pyridines on simple ketones and nitroalkanes.[35] Kresge[36] has been reconsidering the idea that Brønsted exponents α or β are in fact a measure of the degree of proton transfer. In studies of vinyl ether hydrolysis he has noted that certain side groups on the substrate may interact with the acid H_3O^+ in the transition state causing deviations in the value of α. If the maximum value for a primary isotope occurs when $z = 0.5$, where z is the true degree of proton transfer, then α is too large by 0.1 to 0.15. Such a contribution to α need occur only from an interaction of 400–500 cal/mol. It has been shown that structure-making and structure-breaking groups held closely together by chemical bonds or in concentrated solutions cause an overlap in the spheres of structural influence, greatly reducing the magnitude of the $\Delta\bar{H}_t°$'s as compared with the isolated groups in water.[27] Such changes may or may not be accompanied by canceling changes in $\Delta S_t°$. It is, however, obvious that such interactions in dilute solutions would only occur in the transition state due to the fact that the various reactants (as well as the various products) are separated from each other and are close together only when exchanging the proton in the transition state. These solvent structure interactions could explain why the degenerate activity coefficient is not necessarily between those of the reactants and products.

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Acknowledgment. We thank Professor C. Wilcox for the use of his gas–liquid chromatography unit and G. Hammes for the use of his stopped-flow apparatus.

Structural Chemistry of Cholinergic Neural Transmission Systems. II. A Quantum Theoretical Study of the Molecular Electronic Structure of Muscarine, Nicotine, Acetyl-α-methylcholine, Acetyl-β-methylcholine, Acetyl-α,β-dimethylcholine, and Further Studies on Acetylcholine

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Abstract: The results of INDO molecular orbital calculations on the conformational energies and electronic structures of muscarine, nicotine, acetyl-α-methylcholine, acetyl-β-methylcholine, and acetyl-α,β-dimethylcholine are presented and discussed in terms of recently completed INDO studies on acetylcholine. The calculated potential energy surfaces and electronic charge distributions for each compound are systematically considered in terms of crystallographic, spectroscopic, and bioassay data.

This series of papers describes the calculated conformational energy and molecular electronic structure of a group of molecules relevant to cholinergic neural transmission using self-consistent field (SCF) molecular orbital (MO) theory. Our aim is to characterize the structural chemistry of these molecules as fully as possible from a quantum theoretical view point and consider the relationship between the calculated results, experimentally observed structural data from crystallographic and resonance spectral studies, and structures believed relevant to cholinergic processes at a molecular level. Paper I of this series[2] described a quantum theoretical study of acetylcholine (Ach) using valence electron SCF–MO methods at the level of intermediate neglect of differential overlap (INDO). We report herein updated results on Ach and analogous studies of a series of compounds structurally related to Ach and important in the muscarinic and nicotinic subclassification of cholinergic receptors.

I. Background

Acetylcholine, as the endogenous synaptic chemical transmitting agent in cholinergic neural systems, is active at parasympathetic postganglionic sites, voluntary neuromuscular junctions, and autonomic ganglia.[3] Cholinergic receptors are operationally divided into two practically nonoverlapping subclassifications based on the observed activities of muscarine, nicotine, and related compounds.[4] Parasympathetic postganglionic sites are "muscarinic" since Ach action is

(1) (a) Department of Chemistry, Hunter College, and the Department of Neurosurgery, Mount Sinai School of Medicine; (b) Department of Chemistry, Hunter College; (c) Department of Neurology, Mount Sinai School of Medicine.
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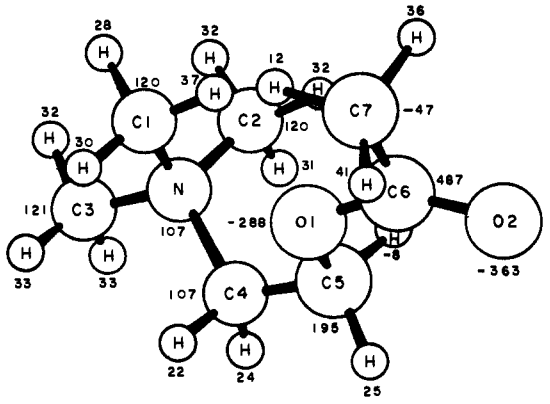

Figure 1. The molecular structure, atomic numbering system, and INDO calculated net atomic charges ($\times 10^3$) for the {60°,180°} conformer of acetylcholine. The calculated dipole moment is 9.08 D.

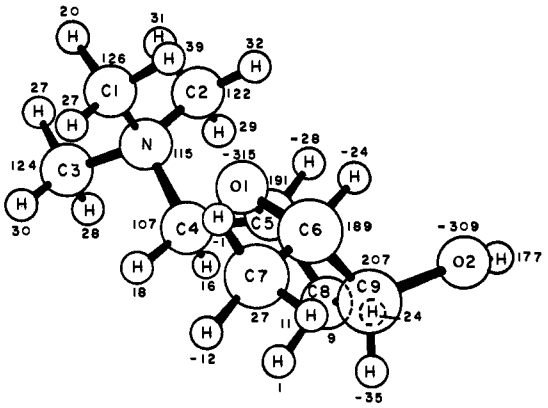

Figure 2. The molecular structure and INDO calculated net atomic charges ($\times 10^3$) for the crystal geometry of C6(*S*),C9(*R*),C5(*S*)-muscarine, {60°,144°}. The calculated dipole moment is 8.05 D.

mimicked by muscarine and related compounds and inhibited by atropine alkaloids. The neuromuscular junction and ganglia are considered "nicotinic" in that the action of Ach is mimicked by nicotine and blocked by curare and methonium compounds. Further classification is no doubt possible since among other things nicotinic receptors in motor endplates and ganglia are preferentially blocked by different methonium compounds,[4a] and some receptors considered nicotinic are not necessarily activated by nicotine.[5] The recent evidence with regard to polyfunctional active sites complicates matters still further.[6] Nevertheless, the muscarinic/nicotinic terminology is in wide clinical use, and the molecular structural basis of this differentiation and of the enzymatic hydrolysis of cholinergic substances by the acetylcholinesterases is of considerable importance in molecular pharmacology.

For future reference the molecular geometry of acetylcholine can be specified in terms of the four dihedral angles τ(C5–C4–N–C3), τ(O1–C5–C4–N), τ(C6–O1–C5–C4), and τ(O2–C6–O1–C5), defined with respect to the numbering system given in Figure 1. In the crystal structures of a large number of Ach analogs, the coordinates τ(C5–C4–N–C3) and τ(O2–C6–O1–C5) are observed to be antiplanar and synplanar, respectively.[7,8] Interesting variations are found in τ(O1–C5–C4–N), which positions the ester oxygen with respect to the trimethylammonium cationic head, and in τ(C6–O1–C5–C4), which positions the acetate moiety with respect to the choline group. The crystal structure Ach^+Br^- determined by Canepa, Pauling, and Sörum[9] shows τ(O1–C5–C4–N) = 77° and τ(C6–O1–C5–C4) = 79°, hereafter abbreviated as {77°,79°}. The structure of Ach^+Cl^- was recently reported by Herdklotz and Sass,[10] and corresponds to {85°,−167°}. Nuclear magnetic resonance studies of Ach^+ in D_2O have been reported by Culvenor and Ham[11] and Cushley and Mautner[12] and point to a {sc,ap} geometry in aqueous solution. Theoretical studies on the conformational energy of Ach have been carried out using a wide variety of methods ranging from semiempirical potential functions[13,14] to approximate molecular orbital theory[2,15,16] and perturbative configuration interaction methods.[17,18] Details of the previously published theoretical studies relevant to this investigation will be discussed in subsequent sections.

Muscarine and nicotine each have recognizable analogs of τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) with asymetrically substituted atoms imparting a possible degree of stereospecificity. The active form of muscarine was determined by Waser[19] to be the C6(*S*),C9(*R*),C5(*S*) isomer shown in Figure 2. The crystal geometry for this isomer as determined by Jellinek[20] corresponds to {74°,144°}. For nicotine, the naturally occurring 2(*S*) isomer has been demonstrated by Barlow and Hamilton[21] to be more active than the 2(*R*) form, but the difference in activity depended somewhat on the preparation. The crystal structure of 1(*R*),2(*S*)-nicotine dihydriodide as determined by Koo and Kim,[22] and given in Figure 3, corresponds to the {−60°,180°} geometry. Theoretical calculations on muscarine have been described by Liquori[23] using semiempirical classical energy calculations and for muscarine and nicotine by Kier[24] and Pullman[18] using molecular quantum mechanics.

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(24) L. B. Kier, *Mol. Pharmacol.*, **4**, 70 (1968).

Many other cholinergic substances bear obvious structural similarities to Ach and usually have clearly defined analogs of τ(O1–C5–C4–N) and τ(C6–O1–C5–C4). Recent papers dealing with the structural basis of cholinergic action are due to Kier,[25] Pauling, Chothia, and coworkers,[26,27] and Beers and Reich;[28] the area has recently been reviewed by Shefter.[29] The proposed theories differ mainly in relative emphasis on functional groups and molecular conformation. The trimethylammonium group is generally accepted as a primary effector in both muscarinic and nicotinic action. The ideas that the ester oxygen of Ach (or its equivalent in a structural analog) figures in muscarinic activity whereas the carbonyl oxygen of Ach or its equivalent is pertinent to nicotinic action are evident in early work in this area.[3] Kier[24,25] studied the role of conformation by comparing the molecular structure of Ach with muscarine, muscarone, and nicotine. He proposed that the geometry of Ach at nicotinic receptors differs from that at muscarinic receptors and appears to implicate the Ach carbonyl oxygen in muscarinic activity. From Figure 4 of ref 25 his muscarinic Ach conformer appears to be {−sc,ap} with τ(O2–C6–O1–C5) = 0°, and his nicotinic Ach conformer, Figure 12 of ref 25, is {−sc,ap} with τ(O2–C6–O1–C5) = −90°.

Chothia and Pauling[27] correlated the crystal structure analyses of six nicotinic agonists and proposed the Ach geometry implicated in nicotinic action to be {75°,180°}. Baker, Chothia, Pauling, and Petcher[26] considered ten muscarinic agonists of varying potency and concluded that the Ach geometry complementary to muscarinic receptors was {85°,150°}. The acetate methyl group but not the carbonyl oxygen was implicated in the muscarinic pharmacophore. The resonance energy stabilizing a synplanar orientation of τ(O2–C6–O1–C5) was presented as 24 kcal/mol, which would preclude the geometry proposed by Kier for the nicotinic pharmacophore. Earlier Chothia,[30] reasoning from the observation that the muscarinic and nicotinic Ach geometries were nearly the same, identified the side of Ach having the cationic head, acetate methyl, and ester oxygen on the periphery with muscarinic action and the side with the cationic head and carbonyl oxygen on the periphery with nicotinic activity. The basis for these conclusions rested on the blocking actions of C4 and C5 substituents, which presumably interfere with the agonist–receptor interaction. Related studies by Chothia and Pauling[31] describe collected evidence that a {150°,180°} geometry is optimal for the hydrolysis of acetylcholine by acetylcholinesterase, consistent with the model proposed by Krupka and Laidler.[32]

Beers and Reich[28] presented structural correlations of 12 muscarinics and 8 nicotinics in support of the role of the cationic head and ester oxygen in muscarinic action, and the cationic head and carbonyl oxygen in nicotinic action. The presence of the acetate methyl group or its equivalent was not necessary for muscarinic action, but when present seemed to potentiate the effect. No role for a carbonyl oxygen in muscarinic action was indicated. The correlations were based on possible conformations deduced from molecular models, rather than consideration of experimentally observed or calculated preferred structures.

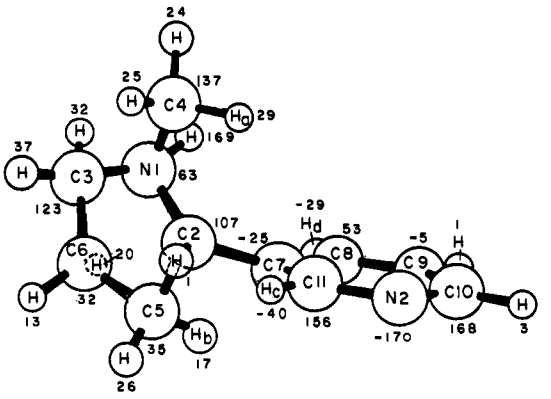

Figure 3. The molecular structure and INDO calculated net atomic charges ($\times 10^3$) for monoprotonated nicotinium in the crystal geometry of 1(*R*),2(*S*)-nicotine dihydriodide, {−60°,180°}. The calculated dipole moment is 8.24 D. Nicotine's pyridine nitrogen is not protonated at physiologic pH since the pK_2 of dissociation of the conjugate base is 10.85.

Of all the cholinergic substances considered in the correlations described above, the compounds most closely related to Ach but with more specific activities are the α- and β-methyl and dimethyl Ach derivatives. Considerable structural information is available on each compound. In addition, this set of compounds includes significant possible exceptions to each of the theories previously described. The crystal structure analysis of acetyl-α(*R*)-methylcholine by Chothia and Pauling[33] revealed two polymorphic forms, {90°,170°} and {212°,176°}. In Chothia's theory[31] the α(*R*) substituent blocks the methyl (muscarinic) side, leaving the nicotinic side available. Acetyl-α(*R*)-methylcholine is indeed a nicotinic agonist, but according to Lesser[5] so is acetyl-α(*S*)-methylcholine, wherein the carbonyl (nicotinic) side of Ach would be at least partially blocked. The active enantiomer of the β-methyl derivative as determined by Ellenbroek and van Rossum[34] and Beckett, Harper, and Clitherow[35] is acetyl-β(*S*)-methylcholine, which Chothia and Pauling[36] have found to be in the {85°,213°} conformation in the crystalline solid. Beckett, *et al.*,[37] observed that the β(*R*) derivative is not hydrolyzed by acetylcholinesterase and inferred that a positive orientation of τ(O1–C5–C4–N) is complementary to the cholinesterase receptor, not inconsistent with the (+)-antiperiplanar form proposed by Chothia and Pauling. Casy, Hassan, and Wu[38] presented nuclear

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magnetic resonance data showing a {sc,ap} conformation of acetyl-β-methylcholine in solution, while a lack of conformational preference was reported for τ(O1–C5–C4–N) in acetyl-α-methylcholine.

The pharmacological properties of erythro and threo dimethylacetylcholine have been studied by Smissman, Nelson, LaPidus, and Day.[39] The erythro (±) derivative had 10% the muscarinic activity of acetylcholine and was negligibly hydrolyzed by cholinesterase, whereas the threo (±) derivative showed negligible muscarinic activity and 10% the hydrolysis rate of Ach. The crystallography of *erythro*-α(*S*),β(*R*)- and *threo*-α(*R*),β(*R*)-dimethylacetylcholine was determined to be {284°,155°} and {217°,95°}, respectively.[40] Shefter[29] tested the Chothia theory with these compounds and pointed out that whereas the muscarinic side would be predicted to be blocked in *erythro*-α(*R*),β(*S*) and open in *threo*-α(*S*),β(*S*), the erythro isomer is observed to be a more potent muscarinic agonist. All of the methyl and dimethyl derivatives possess the functional groups necessary for dual muscarinic and nicotinic action along the lines described by Beers and Reich;[28] the observed specificity indicates that these conditions can be necessary but not sufficient for agonist action.

While the methyl and dimethyl derivatives of Ach thus figure significantly in current structural theories of cholinergic action, the actual role of the α- and β-methyl groups is not yet clearly established. The methyl groups may interact directly with a receptor, sterically restrict an agonist to geometries compatible or incompatible with a certain type of receptor, or otherwise indirectly influence the position of energetically preferred conformations. Molecular models can give leading evidence with regard to steric restrictions, while crystal structure data reveal one, or sometimes two energetically preferred conformations. Theoretical calculations of molecular energies are in principle capable of a more complete enumeration of energetically preferred conformations, but in practice this approach is limited in accuracy by the approximations necessary to treat many-electron systems.

Molecular orbital theory with atomic integrals evaluated at the INDO level of approximation[41] provides a quantum mechanical computational vantage point tractable for extensive calculation of conformational energy maps of molecules of the size considered herein. INDO molecular orbital calculations have been reported for a wide range of organic and small inorganic molecules, and the capabilities and limitations of the method are well documented.[42,43] Electronic charge distributions (as evidenced by electric dipole moments) and molecular geometries are reasonably well accommodated, but ionization and excitation energies are unreliable. Most relevant to this study, dihedral angles have been found to be in relatively good agreement with experiment in a number of cases, with some notable failures.[44] The method can produce low-energy regions even where (charged) nonbonded atoms come slightly within van der Waals contact distances of one another, but generally the INDO results have been in good agreement with those produced by more rigorous molecular orbital calculations[45] and by semiempirical classical calculations.[14]

In paper I of this series, we observed that each of the geometries reported for Ach on the basis of physiochemical techniques or inferred from measurements of the cholinergic activity of structural analogs could be identified with one or another of the minima on the INDO molecular energy surface calculated as a function of the crucial dihedral angles τ(O1–C5–C4–N) and τ(C6–O1–C5–C4). We felt that this result was of interest since a preferred conformation computed in the free space approximation of quantum mechanics need not bear a direct relation to the geometry the molecule may adopt in pure solution, biological fluids, or at a neutral receptor site. One could tentatively infer that environmental effects in this case tend to preferentially stabilize one or another of the geometries corresponding to local energy minima intrinsic to the isolated molecule. To the extent that this holds true, the similarities in energetically preferred conformations throughout a series of structural analogs as determined from quantum mechanical studies can provide relevant information for more detailed considerations of the structural basis of the various aspects of cholinergic activity.

This paper describes our studies of the conformational energy and electronic structure of muscarine, nicotine, acetyl-α-methylcholine, acetyl-β-methylcholine, and acetyl-α,β-dimethylcholine and updates our previous work on acetylcholine. The results on individual molecules are presented and compared with available experimental and theoretical structure data in the next section, followed in section III by a consideration of the significance of the results in the context of cholinergic action.

II. Results

The results of SCF–INDO calculations on each of the molecules to be considered herein are described in the following paragraphs. Details of the theory and methodology have been presented in preceding papers.[2,46] The molecular geometries used as input data for the computations are taken as much as possible from crystal structure data. A computer program was specifically developed to transform crystalline coordinates to a geometrical representation in which individual dihedral angles can be conveniently varied and made available to the wave function programs. Standard values for bond lengths and bond angles are assumed where no experimental data are available, with a C–H internuclear distance taken as 1.09 Å.

The results are presented in the form of conformational energy contour maps of the calculated energy, computer generated drawings of the relevant molecular structures[47] labeled with calculated net electrical charges, and the electric dipole moments. The contour maps are each based on 324 calculated grid points, a 20° interval in each variable. The calculated charge distributions are essentially independent of geometry ex-

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(46) D. L. Beveridge, R. J. Radna, and Elias Guth in ref 17.
(47) Oak Ridge Thermal-Ellipsoid Plot Program, Carroll K. Johnson, Oak Ridge National Laboratory, Oak Ridge, Tenn.

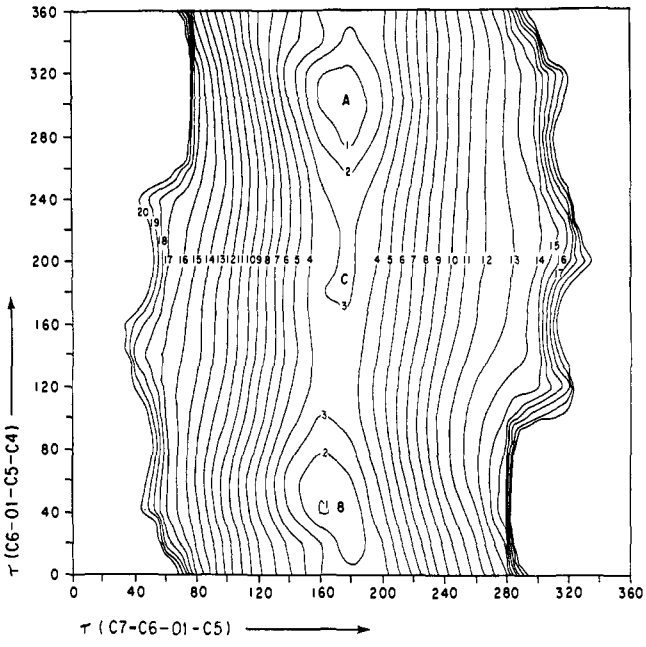

Figure 4. INDO calculated energy *vs.* τ(O2–C6–O1–C5) and τ(C6–O1–C5–C4) for acetylcholine.

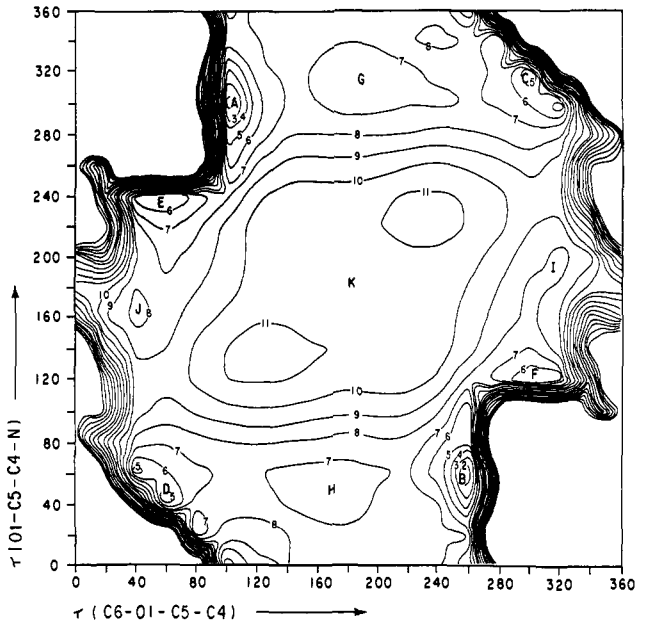

Figure 5. INDO calculated energy *vs.* τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) for acetylcholine.

cept where noted otherwise, and the range of calculated dipole moments over the different conformations is reported for each compound. For the cationic species only the first nonzero moment is independent of the coordinate system, so the calculated dipole moments are referred to the center of positive charge.

In comparing theory and experiment, an account of relative conformational energies is influenced by approximations in the form of the molecular wave function (a molecular orbital product function), methodology and parameterization in atomic orbital integral evaluation, and in the geometry assumed for those molecular coordinates not explicitly considered variable. Thus the structures of energetically preferred conformations can be enumerated in terms of local minima, but we attribute no special significance to a global minimum separated by only a few kilocalories per mole from a local energy minimum since a consideration of conformational entropy could alter the relative (free) energies significantly.[48] Each conformational map presents isoenergy contour lines of the lower 20 kcal/mol of the calculated potential energy surface. This is somewhat extended beyond the 3 kcal/mol range most significant from an equilibrium Boltzmann viewpoint. However, for agonist–receptor interactions including environmental effects, interactions may easily be several tens of kilocalories per mole. Structural reorganization beyond the 3-kcal/mol level should be feasible, and it is thus relevant to consider a more extensive range of energies. Calculated local minima for all compounds are listed in Table I.

Acetylcholine. The structural studies on Ach up to mid-1970 were reviewed in paper I.[2] Since that time, several additional theoretical and experimental papers have appeared,[14,16,18,46] and additional relevant results are available. In this laboratory we have calculated two new Ach conformational maps. The first of these tests our assumption that τ(C7–C6–O1–C5) is antiplanar for all variations in τ(C6–O1–C5–C4). The INDO calculated potential surface of Ach as a function of τ (C6–O1–C5–C4) and τ(C7–C6–O1–C5) with τ(O1–C5–C4–N) held antiplanar is given in Figure 4. There are three minima on the surface, all with τ(C7–C6–O1–C5) = 180°. Rotation of this coordinate out of the antiplanar configuration is accompanied by an increase in energy to 20 kcal/mol, consistent with the expected double bond character of the C6–O1 bond as discussed by Baker, *et al.*[26] This coordinate is maintained antiplanar in all subsequent studies.

In order to obtain more detail, we have recalculated the SCF–INDO conformational map for Ach as a function of τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) with a closer grid on the full range of 360° in both variables. The results are relevant to the succeeding discussion and are given in Figure 5. The global minimum on the surface is in the {−sc,sc} region, and local minima D and H can be identified with the bromide and chloride crystal geometries, respectively. The qualitative features are in accord with chemical intuition as described in paper I. On a more detailed level the SCF–INDO calculations place the synclinal minima of both coordinates somewhat inside the experimentally observed values, with in some cases charged nonbonded atoms coming within van der Waals contact distances.[14] Using the wave functions generated in the latter calculation, the dipole moment of Ach was calculated to be 1.5–9.8 D, depending upon the conformation. An experimentally observed value for Ach in chloroform was estimated to be 2.65 D.[49] While a number of assumptions on structure and polarizabilities are involved in reduction of the experimental data, this relatively low value suggests that structure of Ach in chloroform may resemble the crystalline bromide geometry rather than the crystalline chloride geometry observed for Ach in D_2O.

Since the publication of our SCF–INDO conformational map for Ach, analogous calculations have been

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(49) P. Maurel and L. Galzigna, *Biophys. J.*, 11, 550 (1971).

Table I. Summary of Biological and Conformational Data

Compound	Muscarinic activity[a]	Nicotinic activity[a]	Susceptibility to hydrolysis[a]	Calculated conformation energies[b] (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Acetylcholine	100	100	100	{40°,60°} D, 4.14	{40°,180°} H, 6.42	{60°,260°} B, 0.74	{160°,40°} J, 7.62	{180°,180°} K, 10.20	{120°,320°} F, 4.97	{200°,320°} I, 7.61	{240°,60°} E, 4.73	{300°,100°} A, 0.0	{320°,180°} G, 6.11	{320°,300°} C, 3.97
Acetyl-α(*R*)-methylcholine	3.6,[c] 7.7[d]	100[e,f]	78[g]	{80°,80°} C, 1.87	y	{80°,240°} A, 0.0	{180°,40°} F, 2.28	{180°,180°} K, 5.09	{120°,260°} D, 2.06	{180°,260°} J, 4.11	{220°,60°} E, 2.68	{300°,120°} B, 0.45	{300°,200°} H, 3.25	{300°,320°} G, 3.20
Acetyl-α(*S*)-methylcholine	0.4,[c] 0.7[d]	100[e,f]	97[g]	{60°,40°} G, 3.20	{60°,160°} H, 3.25	{60°,240°} B, 0.45	{180°,100°} J, 4.11	{180°,180°} K, 5.09	{140°,300°} E, 2.68	{180°,320°} F, 2.28	{240°,100°} D, 2.06	{280°,120°} A, 0.0	y	{280°,280°} C, 1,87
Acetyl-β(*R*)-methylcholine	0.4,[c] 0.5[d]	0[e,f]	Weak[g] inhibitor	x	x	x	x	x	x	x	{240°,60°} C, 5.15	{300°,120°} B, 4.77	y	{300°,330°} A, 0.0
Acetyl-β-(*S*)-methylcholine	99,[c] 133[d]	0[e,f]	54[g]	{60°,30°} A, 0.0	y	{60°,240°} B, 4.77	x	x	{120°,300°} C, 5.15	x	x	x	x	x
erythro-Acetyl-α(*R*),-β(*S*)-dimethylcholine	14[h]		0[i]	{80°,80°} C, 3.34	y	{80°,240°} A, 0.0	x	y	{120°,280°} B, 1.91	x	x	{340°,120°} E, 8.40	{340°,180°} D, 6.36	x
erythro-Acetyl-α(*S*),-β(*R*)-dimethylcholine	14[h]		0[i]	x	{20°,180°} D, 6.36	{20°,240°} E, 8.40	x	y	x	x	{240°,80°} B, 1.91	{280°,120°} A, 0.0	y	{280°,280°} C, 3.34
threo-Acetyl-α(*R*),-β(*R*)-dimethylcholine	0.04[h]		9[i]	x	{0°,160°} D, 8.06	x	x	y	x	x	{220°,60°} B, 0.30	{280°,140°} A, 0.0	y	{280°,300°} C, 2.13
threo-Acetyl-α(*S*),-β(*S*)-dimethylcholine	0.04[h]		9[i]	{80°,60°} C, 2.13	y	{80°,220°} A, 0.0	x	y	{140°,300°} B, 0.30	x	x x	x	{360°,200°} D, 8.06	x
C6(*S*),C9(*R*),C5(*S*)-Muscarine	270[j]	<15,[k] <25[l]		x	{60°,144°} A, 0.0	x	x	{160°,144°} B, 6.9	x	x	x	x	x	x
C6(*S*),C9(*S*),C5(*S*)-Epimuscarine	0.3[j]	<2,[k] <2.5[l]		x	{60°,144°} A, 0.0	x	x	{160°,144°} B, 6.9	x	x	x	x	x	x
C6(*S*),C9(*R*),C5(*R*)-Allomuscarine	<0.5[j]	<1.5[k], <2.5[l]		x	{60°,144°} A, 0.0	x	x	{160°,144°} B, 6.9	x	x	x	x	x	x
C6(*S*),C9(*S*),C5(*R*)-Epiallomuscarine	0.8[j]	<30,[k] <50[l]		x	{60°,144°} A, 0.0	x	x	{160°,144°} B, 6.9	x	x	x	x	x	x
1(*R*),2(*S*)-Nicotine	0[m]	100[n]		x	{100°,180°}	x	y	y	x	x	x	x	y	x

[a] Given as percentages relative to acetylcholine. [b] Sterically forbidden conformations: x; sterically allowed conformations: y; other entries correspond to local minima on Figures 5, 6, 7, 8, 10, 12, and 14 and are listed with the {τ(O1–C5–C4–N),τ(C6–O1–C5–C4)} designation, and energy above the global minimum in kcal/mol. [c] Guinea pig ileum, A. H. Beckett, N. J. Harper, J. W. Clitherow, and E. Lesser, *Nature (London)*, **139**, 671 (1961). [d] Cat blood pressure.[g] [e] Frog rectus muscle, ref 39 and M. Wurzel, *Experientia*, **15**, 430 (1959). [f] Blood pressure rise with atropine, and cat gastrocnemius stimulation, A. Simonart, *J. Pharm. Exp. Ther.*, **46**, 157 (1932). [g] Hydrolysis by a standard bovine erythrocyte acetylcholinesterase homogenate, ref 35. [h] Guinea pig ileum, ref 39. [i] Hydrolysis by true acetylcholinesterase from the electric organ of the eel, ref 39. [j] Cat blood pressure, ref 19. [k] Blockage of cat superior cervical ganglion, ref 19. [l] Curariform action on cat gastrocnemius muscle, ref 19. [m] Guinea pig ileum during anoxia, H. Day and J. R. Vane, *Brit. J. Pharmacol. Chemother.*, **20**, 150 (1963). [n] Cat superior cervical ganglion, ref 4a.

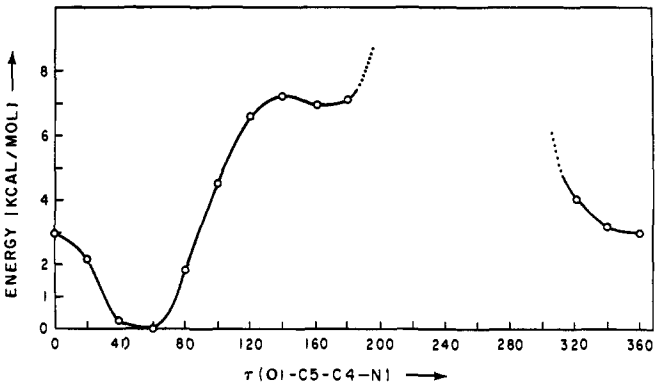

Figure 6. INDO calculated energy *vs.* τ(O1–C5–C4–N) for muscarine.

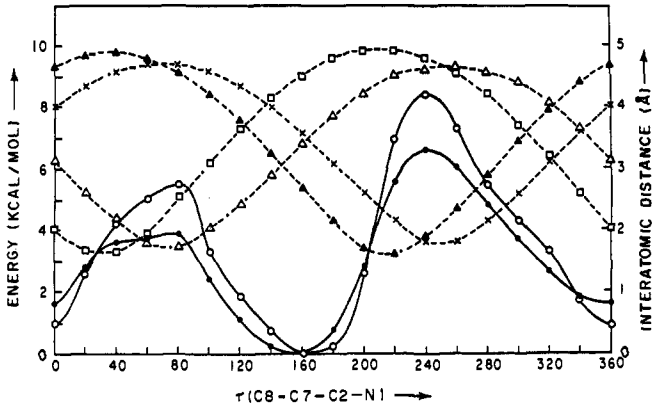

Figure 7. INDO calculated energy (solid lines) and selected interatomic distances (dotted lines) for nicotine. Energy plots: (○) standard geometry, (●) hydrogens modified. Distance plots: (□) H_a–H_d distance, (△) H_b–H_c distance, (×) H_b–H_d distance, and (▲) H_a–H_c distance.

reported by Pullman, Courriere, and Coubeils[18] using the PCILO method with atomic integrals evaluated in the CNDO approximation. An SCF–CNDO calculation should give results essentially the same as our SCF–INDO results, since this is the ground state of a closed shell system. The PCILO method includes a degree of electron correlation, and comparison of the PCILO–CNDO and SCF–INDO results show some differences. Overall the PCILO surface is somewhat flatter than the SCF, a probable consequence of configuration interaction depressing higher energy points more than lower energy points. A further comparison between the two methods is described in a recent paper by Pullman and Courriere.[50]

Muscarine. Muscarine has been studied from a theoretical viewpoint by a variety of methods[13,15,18] with no substantive disagreement, and we report our SCF–INDO results only for reference with other systems computed at the same level of approximation. A conformational study of muscarine reduces to a one-parameter problem, with τ(C6–O1–C5–C4) constrained to 144° by the intramolecular five-membered ring. Calculations were carried out on the C6(*S*),C9(*R*),C5(*S*) isomer, shown in Figure 2, and for the related compounds C6(*S*),C9(*S*),C5(*S*) epimuscarine, C6(*R*),C9(*S*),-C5(*S*) allomuscarine and C6(*R*),C9(*R*),C5(*S*) epiallomuscarine. The SCF–INDO conformational energy of muscarine is plotted as a function of τ(O1–C5–C4–N) in Figure 6. The calculations for muscarine show the global minimum at 60° in accord with the observed crystal geometry. A local energy minimum appears at 160° in τ(O1–C5–C4–N) at an energy 7 kcal/mol above the local minimum. The results for epimuscarine, allomuscarine, and epiallomuscarine are essentially identical; these compounds differ from muscarine only in the absolute configuration of the methylated and hydroxylated ring carbon atoms and in their biological activities to be discussed in the next section. The calculated net atomic charges for muscarine in the {60°,144°} conformation are given in Figure 2. The calculated electric dipole moment is 8.05 D.

Nicotine. The crystal geometry for nicotine as determined by Koo and Kim[22] is shown in Figure 3. Here τ(C8–C7–C2–N1) is analogous to τ(O1–C5–C4–N) in Ach, and τ(C9–C8–C7–C2) is analogous to τ(C6–O1–C5–C4). With the latter coordinate constrained antiplanar by the pyridine ring, the crystal geometry can be represented as {−60°,180°}. Rotation of the pyridine ring by 180° places N2 in a position roughly analogous to the carbonyl oxygen of Ach in the {60°,180°} conformation. The pyrrolidine ring deviates somewhat from planarity, with τ(C5–C2–N1–C3) = 17° and τ(N1–C3–C6–C5) = 14°. Simple steric considerations indicate that interaction between pyridine hydrogens and hydrogens attached to the pyrrolidine ring and *N*-methyl group may strongly influence the conformational energy about τ(C8–C7–C2–N1). While rapid inversion of the absolute configuration around nicotine's pyrrolidine nitrogen is likely in solution, the 1(*R*),2(*S*) configuration of nicotine is studied here since crystal data are available for this epimer.

In our initial SCF–INDO calculations on nicotine, a conformational energy map was generated as a function of τ(C8–C7–C2–N1) and τ(Ha–C4–N1–C3), the latter coordinate specifying the orientation of the *N*-methyl hydrogens. Two energy minima were found, differing in energy by less than 1 kcal/mol and both corresponding to τ(Ha–C4–N1–C3) = 80°. The calculated energy *vs.* τ(C8–C7–C2–N1) with τ(Ha–C4–N1–C3) = 80° is shown in Figure 7. The energy minima corresponds to τ(C8–C7–C2–N1) = 0 and 160°, both somewhat removed from the observed value of −60°. In an attempt to understand the basis of the discrepancy between the calculated and observed values, we explored the effect of deviations in hydrogen positions on the calculated result. The coordinates specifying the hydrogen positions were adjusted slightly in the direction expected to reduce steric effects and calculation of conformational energy *vs.* τ(C8–C7–C2–N1) was repeated. The resulting plot is also presented in Figure 7. The barrier heights were reduced by 1.5 to 2 kcal/mol, but the positions of the minima were essentially unchanged. Superimposed on the same plot are the interatomic distances of hydrogen atoms coming within van der Waals contact distance of one another. At τ(C8–C7–C2–N1) = 40 to 80°, the H_a–H_d and the H_b–H_c distances are very low resulting in an increased conformational energy. At τ(C8–C7–C2–N1) = 220 to 260°, the H_a–H_c and H_b–H_d distances are close, also destabilizing the system. Our calculated minima correspond roughly to regions where all these interatomic distances are simultaneously maximal. Thus in spite of the lack of

(50) B. Pullman and Ph. Courriere, *Mol. Pharm.*, 8, 612 (1972).

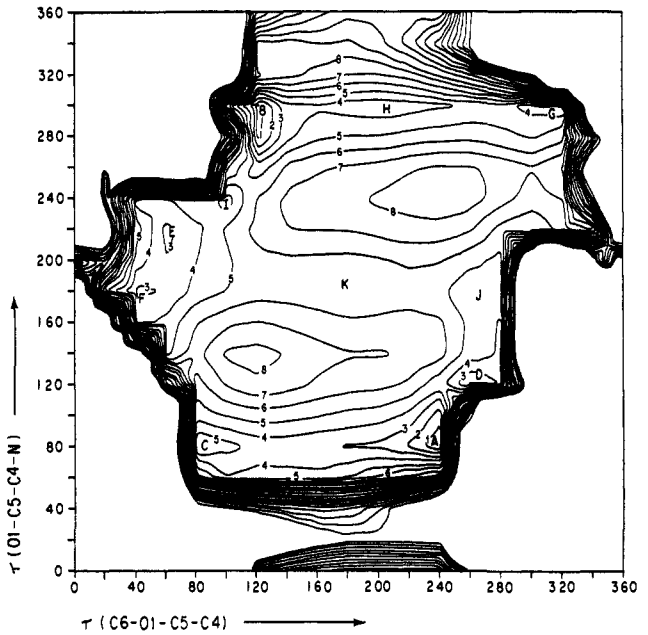

Figure 8. INDO calculated energy *vs.* τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) for acetyl-$\alpha(R)$-methylcholine.

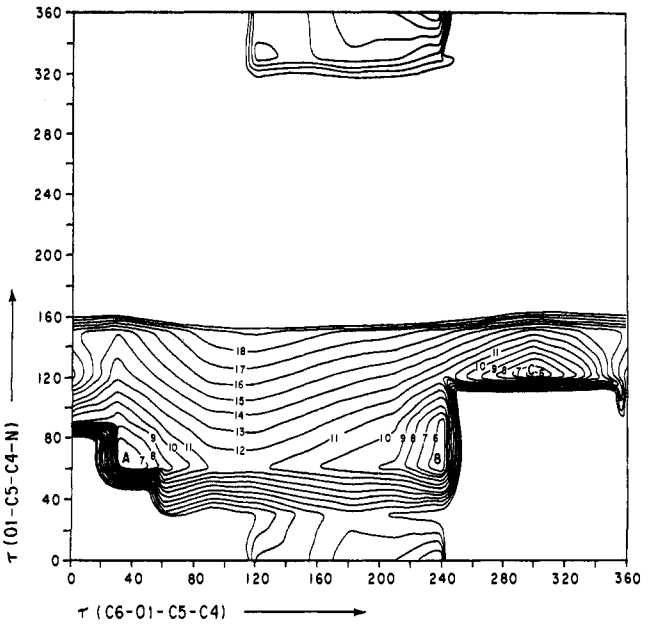

Figure 10. INDO calculated energy *vs.* τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) for acetyl-$\beta(S)$-methylcholine.

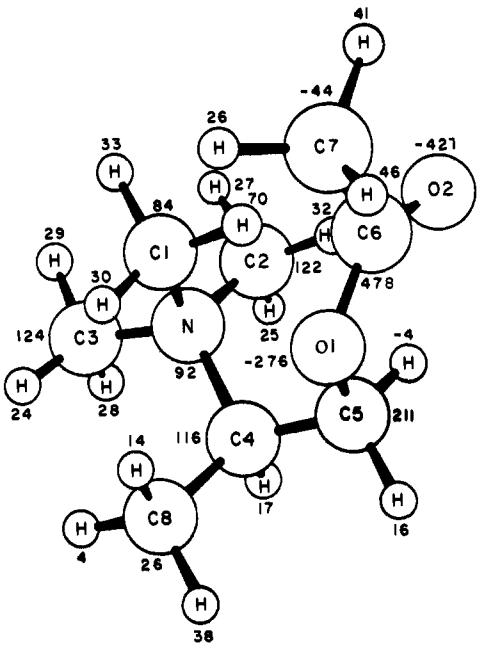

Figure 9. The molecular structure and INDO calculated net atomic charges for acetyl-α-(R)-methylcholine in the {80°,240°} geometry. The calculated dipole moment is 6.20 D.

a calculated energy minima in the region of the observed geometry, our results are understandable and represent the degree of agreement to be achieved assuming the crystal geometry for nonhydrogen atoms. Conformations of nicotine with τ(C8–C7–C2–N1) from 100 to 200° and from −40 to 20° are energetically removed from the absolute minimum by less than 2.5 kcal/mol. The calculated charge distribution for nicotine at the crystal geometry is recorded in Figure 3, and the calculated dipole moment ranged from 7.4 to 8.9 D.

The calculations on nicotine reported by other investigators leave the relation between calculated and observed geometries incompletely resolved. The study reported by Kier[24] using extended Hückel theory gave good agreement with experiment but assumed a planar pyrrolidine ring and computed energies on a 60° grid. INDO calculations performed on 1(R),2(S)-nicotine with the pyrrolidine ring planar yield minima in τ(C8–C7–C2–N1) at −40 and 140°. However, it is difficult to justify comparison of this result with that of that crystallographic study. Pullman, *et al.*,[18] using the PCILO method and a 1(R),2(S) standard geometry obtained minima at τ(C8–C7–C2–N1) = −40° and 120°, in improved agreement with the crystal geometry.

Acetyl-α-methylcholine. The SCF–INDO conformational energy map of $\alpha(R)$-methylacetylcholine is presented in Figure 8. The conformational map for $\alpha(S)$-methylacetylcholine, the enantiomer, can be generated through the transformation $\{x,y\} \rightarrow \{-x,-y\}$. The global minimum for the $\alpha(R)$ derivative occurs in the vicinity of {80°,240°}. The nine other local minima are listed in Table I. The crystal geometry at {90°,170°} is calculated to be 4 kcal/mol above the absolute minimum, and the {212°,176°}[33] crystal result can be identified with a local minimum at {180°,180°}. In the latter case, with τ(O1–C5–C4–N) = 180° the shape of the surface indicates considerable lability in τ(C6–O1–C5–C4) from 80 to 240°. Steric interference of the $\alpha(R)$-methyl group with the acetate carbonyl group precludes conformations with τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) simultaneously in the range of 40 to 200° and −40 to 60°, respectively. The calculated magnitudes of angles of the {sc,sc} minima are increased over that calculated for Ach, but comparison of Figure 8 and Figure 5 shows that the overall effect of α-methyl substitution on the conformational energy map is relatively minor. None of the local minima on the Ach potential surface are energetically precluded for the α-methyl derivative. The α-methyl surface is slightly flatter than that of Ach, consistent with the results indicated in nmr studies.

The calculated net atomic charges for the {80°,240°} conformation of α-(R)-methylacetylcholine are presented in Figure 9. Comparison of Figure 9 with the

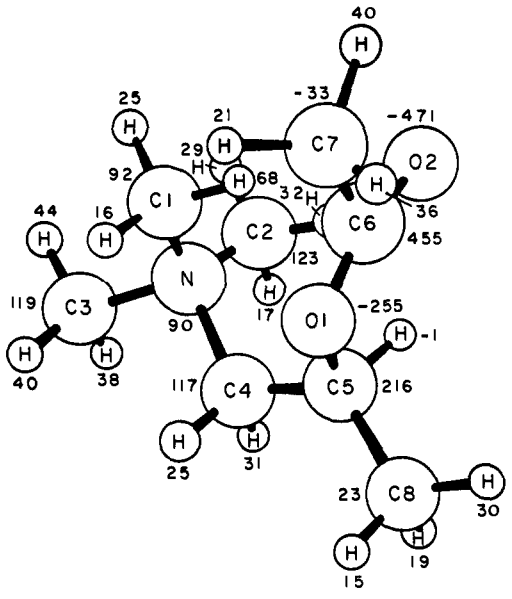

Figure 11. The molecular and INDO calculated net atomic charges for acetyl-$\beta(S)$-methylcholine in the {80°,240°} geometry. The calculated dipole moment is 7.75 D.

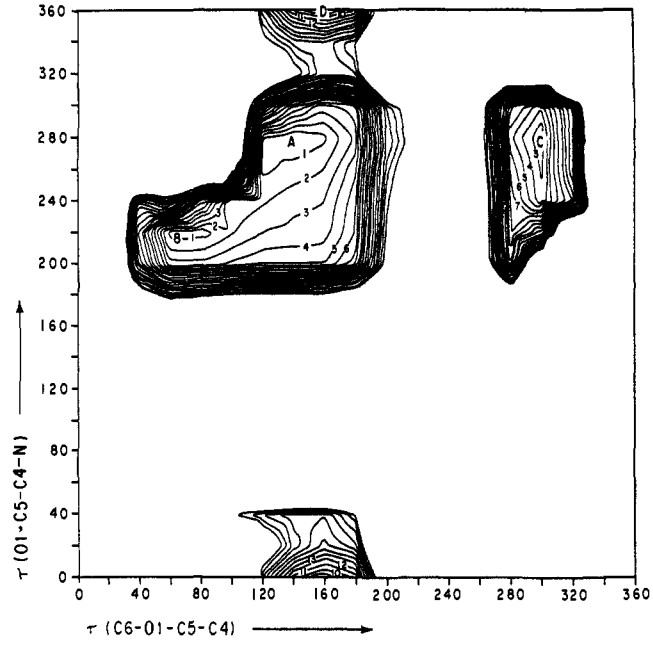

Figure 12. INDO calculated energy *vs.* τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) for *threo*-$\alpha(R),\beta(R)$-dimethylacetylcholine.

charge distribution reported for acetylcholine in Figure 1 shows that there is a charge redistribution induced by α-methyl substitution. There is a decrease in positive charge on N, Cl, and C6 and a decrease and increase in negative charge on O1 and O2, respectively. The calculated dipole moment ranges from 0.5 to 9.1 D.

Acetyl-β-methylcholine. The conformational energy map computed for $\beta(S)$-methylacetylcholine using the INDO method is given in Figure 10. The surface may be viewed in marked contrast to that of Ach, with 50% of the conformational space sterically excluded. The primary source of the steric effect is the interaction between the β-methyl group and the trimethylammonium methyl groups, important for all values of τ(O1–C5–C4–N) above 180°. The global minimum on the surface is located at {60°,30°}, with a local minimum at {60°,240°} computed to be about 4.8 kcal/mol higher in energy. The latter local minimum may be identified with the crystal geometry at {85°,203°}.[36]

The calculated charge distribution for the {60°,240°} conformer of acetylcholine is given in Figure 11. A comparison with the calculated charge distribution for Ach shows that the effect of β-methyl substitution is to decrease positive charge on N, Cl, and C6 and to decrease and increase negative charge on O1 and O2, respectively. These charge redistributions are directly analogous to those which occur on α-methyl substitution. The calculated dipole ranges from 1.4 to 11.4 D in the sterically allowed region of space.

Acetyl-α,β-dimethylcholine. The SCF–INDO conformational map calculated for *threo*-$\alpha(R),\beta(R)$-dimethylacetylcholine is presented in Figure 12. Here 87% of the surface is sterically unfavored. All conformers with τ(O1–C5–C4–N) between 40 and 200° are excluded due to interactions between β-methyl and trimethylammonium hydrogens, and conformers with τ(C6–O1–C5–C4) between 200 and 260° have unfavorable interactions between the β-methyl group and the carbonyl oxygen. The global minimum on the surface is at {280°,140°}, with a local minimum at about the same energy at {220°,60°}, separated by a barrier computed to be less than 2 kcal/mol. A second local minimum is present at {280°,300°}, separated from the first by a barrier of some 25 kcal/mol. The experimentally observed crystal geometry for the threo $\alpha(R),\beta(R)$ derivative is {217°,95°}. Figure 13 presents the computed net atomic charges for the {80°,-220°} conformer of *threo*-$\alpha(S),\beta(S)$-dimethylacetylcholine. In comparison with the calculated net atomic charge densities for acetylcholine, there is a decrease in positive charge on N, an increase on C6, and an increase in negative charge on O1. Charges on other atoms are essentially unchanged from those of acetylcholine. The calculated electric dipole moment ranges from 0.9 to 8.8 D.

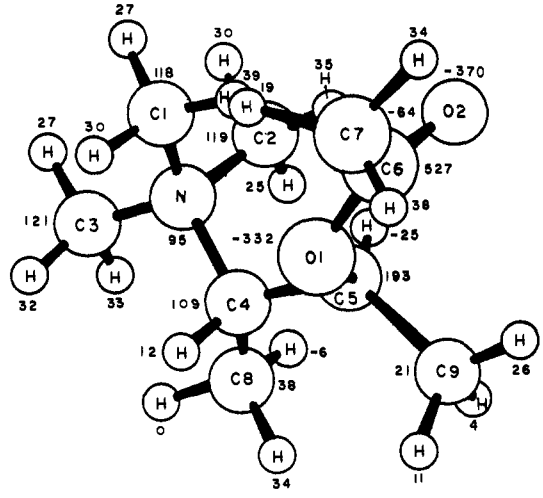

Figure 13. The molecular structure and INDO calculated net atomic charges for *threo*-$\alpha(S),\beta(S)$-dimethylacetylcholine in the {80°,220°} geometry. The calculated dipole moment is 7.16 D.

The conformational energy map for *erythro*-$\alpha(S),\beta(R)$-dimethylacetylcholine is given in Figure 14. Steric interactions analogous to those described above

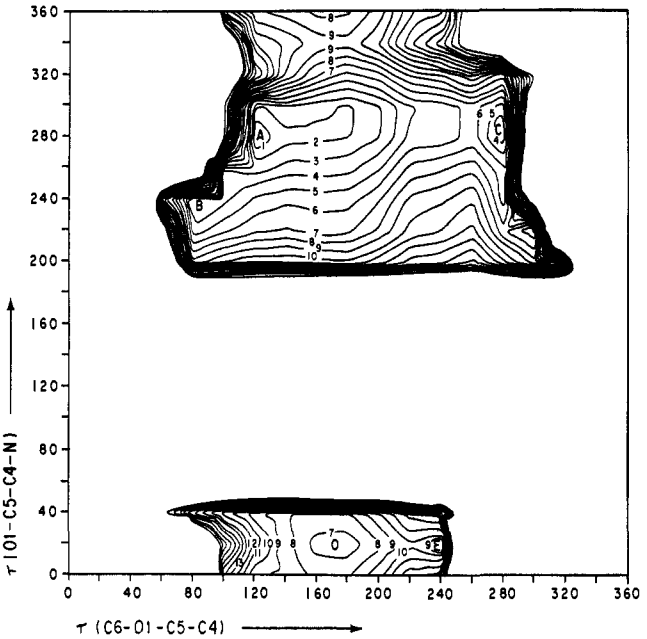

Figure 14. INDO calculated energy *vs.* τ(O1–C5–C4–N) and τ(C6–O1–C5–C4) for *erythro*-$\alpha(S),\beta(R)$-dimethylacetylcholine.

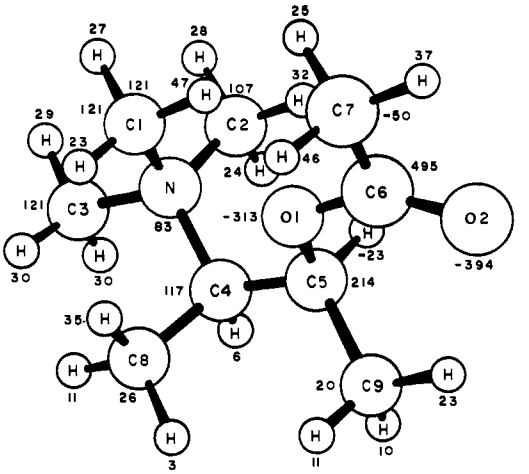

Figure 15. The molecular structure and INDO calculated net atomic charges for *erythro*-$\alpha(R),\beta(S)$-dimethylacetylcholine, in the {80°,240°} geometry. The calculated dipole moment is 6.70 D.

exclude about 86% of the conformers on this surface. In the remaining area, the global minimum is located at {280°,120°}, with a local minimum at {240°,80°} and {280°,280°} at relative energies of 1.91 and 3.34 kcal/mol, respectively. The latter two minima are separated by a computed barrier of 6 kcal/mol. The crystal geometry is near {280°,160°}, a point computed to be 1.7 kcal/mol above the global minimum. The charge distribution for *erythro*-$\alpha(R),\beta(R)$-dimethylacetylcholine, as presented in Figure 15, is similar to that presented for the threo derivative except that C6 does not evidence an increase in positive charge over the analogous atom in Ach. The calculated dipole ranges from 0.9 to 9.0 D.

III. Discussion

The results of the preceding section can be used together with the biological activity data to approach a deeper understanding of the role of α- and β-methyl substituents in cholinergic action and to gain perspective on general aspects of the structural chemistry of cholinergic transmission. Since the methyl derivatives of Ach have all of the functional groups necessary for either muscarinic or nicotinic activity, the present data may be used to examine the role of molecular conformation *vis-à-vis* the direct interaction of the methyl groups with the cholinergic receptor. To the extent molecular conformation is a dominant factor, compounds in this series with a particular type of activity should have the appropriate functional groups disposed in three dimensional space in a common pattern. In terms of structural parameters, this disposition is specified by sterically permitted or energetically preferred values of τ(O1–C5–C4–N) and τ(C6–O1–C5–C4), and any common pattern should be revealed by a comparison of allowed regions or energy minima in the {τ(O1–C5–C4–N),τ(C6–O1–C5–C4)} conformational maps.

The conformational data presented in the preceding section have been reduced to tabular form and collected with the biological activity data in Table I. Each compound considered is represented by a row of the table, containing from left to right its muscarinic activity, nicotinic activity, and enzymatic hydrolysis rate, each given as percentages with respect to available data on Ach. The biological data represent results from a variety of preparations and experimental conditions, and the relative activities listed are thus of qualitative or semiquantitative significance at best. Further to the right of Table I, the positions of energy minima calculated for the substance are enumerated. The columns of the table in this region are organized with respect to the calculated energy minima for Ach given in the top row; entries in the same column down the table indicate corresponding energetically preferred conformations in different substances. All preferred conformations energetically within 20 kcal/mol of the global minimum of each compound are included.

In examination of the data for compounds with significant muscarinic acitivity, it is again evident that the geometry common to muscarinic agonists is {sc,ac–ap}, consistent with the ideas proposed by Pauling and coworkers. For Ach and muscarine this geometry (column 2 in the conformational energies of Table I) corresponds to a calculated energy minimum and is thus energetically preferred. For the active $\beta(S)$ methyl derivative and the *erythro*-$\alpha(R),\beta(S)$-dimethyl derivative (probably the active *erythro*-dimethyl enantiomer), this geometry does not correspond to local energy minima but is not sterically excluded. Beyond this, it appears that $\beta(S)$-methyl or methylenic substitution actually enhances the muscarinic activity of an agonist. Bioassay data (see Table I) for muscarine, acetyl-$\beta(S)$-methylcholine, and *erythro*-$\alpha(R),\beta(S)$-dimethylacetylcholine show these compounds to have 270, 133, and 14% of the muscarinic activity of Ach, respectively. The muscarinic activities of *erythro*-$\alpha(R),\beta(S)$-dimethylacetylcholine and $\alpha(R)$-methylacetylcholine are 14 and 0.6% relative to Ach. Examination of the conformational maps and calculated electronic charge distributions for these molecules shows no outstanding detail which could explain the enhancement of muscarinic activity for a $\beta(S)$ substituent simply in terms of molecular conformation and other molecular properties. Therefore it appears that the variation in the

level of muscarinic activity in these compounds may involve a direct interaction of the methyl substituents with the muscarinic receptor.

Table I also lists a number of compounds which have calculated energetically preferred conformations in the vicinity of {sc,−ac} yet have low muscarinic activity. Both $\alpha(R)$- and $\alpha(S)$-methyl derivatives fall into this category, with $\alpha(S)$-methyl substitution diminishing muscarinic activity by a factor of 10 more than $\alpha(R)$-methyl substitution. Since no significant portion of the Ach conformational map is sterically precluded for the $\alpha(R)$- or $\alpha(S)$-methyl derivative, simple conformational effects cannot be responsible. A direct interaction of the α-methyl substituents with the receptor contributing destructively to agonist activity can explain the observed reduction in muscarinic potency. The loss of muscarinic activity upon α-methyl substitution is also seen with the dimethyl derivatives. *erythro*-$\alpha(R),\beta(S)$-Dimethylacetylcholine has an energetically preferred conformation at {sc,−ac} and a muscarinic activity in the racemate of 14% relative to Ach, compared to 133% for $\beta(S)$-methylacetylcholine. The greater reduction in muscarinic activity for $\alpha(S)$-methyl substitution as compared with $\alpha(R)$ substitution is evidenced again by the lack of activity in *threo*-$\alpha(S),\beta(S)$-dimethylacetylcholine whereas the *erythro*-$\alpha(R),\beta(S)$ derivative elicits small but significant muscarinic activity.

Analogous conclusions can be drawn from a consideration of the calculated energy profiles of muscarine, given in Figure 6, as compared with those for epimuscarine, allomuscarine, and epiallomuscarine. The energy profiles are similar while the observed activities as listed in Table I are quite different. Neither conformational effects or a variation in electronic charge distribution can explain the variance in activity, and a direct interaction of functional groups with the receptor is indicated. The observed activities appear to implicate muscarine's hydroxyl oxygen and ring methyl group in positions analogous to the carbonyl oxygen and acetate methyl of Ach in muscarinic action.

Consideration of the data for compounds with significant nicotinic activity leads to additional information on the role of conformational effects in nicotinic action. As noted by others, nicotinic activity is clearly less stereospecific than muscarinic action, but a {sc,ac–ap} conformation has been implicated. Both $\alpha(R)$- and $\alpha(S)$-methyl derivatives are nearly as active at nicotinic sites as Ach. Methyl substitution at either $\beta(R)$ or $\beta(S)$ positions significantly reduces the nicotinic activity of an agonist. In consideration of the widely different effects of $\beta(R)$ and $\beta(S)$ substitution on conformational preferences and that the {sc,−ac} is permitted if not preferred in the $\beta(S)$ derivative, a direct interaction of methyl groups with the nicotinic receptor is indicated.

Consideration of the relative rates of cholinesterase mediated hydrolysis for the various compounds alongside the calculated results on structure and properties for methyl Ach derivatives points also toward some direct interaction of the methyl groups with the active site of the enzyme. Both $\alpha(R)$- and $\alpha(S)$-methylacetylcholine can achieve any point on the {τ(O1–C5–C4–N),τ(C6–O1–C5–C4)} conformational map accessible to Ach and have no significant differences in charge distributions. Thus the observed decreased hydrolysis rate of the $\alpha(R)$ derivative compared to the $\alpha(S)$ derivative of Ach does not appear to be dependent upon conformational effects, and may likely involve methyl groups directly. For the β-methyl derivatives, $\beta(R)$ substitution reduces the hydrolysis rate to zero whereas $\beta(S)$ substitution shows 54% the hydrolysis of Ach. This indicates positive synclinal and anticlinal values of τ(O1–C5–C4–N) lead to conformations complementary to the cholinesterase receptor. The calculated conformational map for $\beta(S)$-methylacetylcholine shows local minima for (+)-synclinal values of τ(O1–C5–C4–N) but none for ac to ap values. The {150°,180°} geometry implicated by Pauling, *et al.*, in enzymatic hydrolysis lies 12 kcal/mol above the global minimum.

For hydrolysis, the effects of α and β substitution may again be extrapolated to the α,β-dimethyl derivatives. The decreased hydrolysis rate for an $\alpha(R)$ derivative explains the negligible rate observed for *erythro*-$\alpha(R),\beta(S)$-dimethylacetylcholine. We predict the hydrolysis rate for *threo*-$\alpha(S),\beta(S)$-dimethylacetylcholine to be higher.

Overall, we can ascertain from these results that structures implicated in various aspects of cholinergic action can be identified with regions of conformational energy sterically permitted but not necessarily energetically preferred in the isolated molecule. This seems reasonable since a change in membrane permeability is probably a structural adaptation to agonist–receptor interaction, and a conformational effect on the agonist is certainly possible. Structural modifications in both enzyme and substrate are evident from spectral studies of enzymatic reactions. Still these results must be considered in the context of data on a larger set of representative compounds, including environmental effects and a consideration of conformational free energy as well as energy. Work is currently in progress on each of these points.[51]

IV. Conclusions

In this study we have calculated conformational energy maps and electronic charge distributions for muscarine, nicotine, and the α- and β-methyl and dimethyl derivatives of acetylcholine. The results have been considered in terms of physicochemical studies, the available bioassay data on cholinergic substances, and current theories of the structural chemistry of cholinergic transmission. Geometries observed from crystallographic and solution spectral studies for each compound may be identified with energetically preferred geometries (local minima) on the calculated conformational energy maps. General aspects of cholinergic activity are seen to be related to regions of conformational space sterically allowed but are not always directly associated with energetically preferred geometries. The variance in bioassay data for active compounds cannot be explained in terms of simple conformational effects and distributions of electronic charge, and direct interactions of methyl substituents with cholinergic receptors are indicated.

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Kinetics of Complexation of Macrocyclic Polyethers with Sodium Ions by Nuclear Magnetic Resonance Spectroscopy. II. Solvent Effects

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Abstract: The kinetics of complexation of sodium ions (or of its ion pairs) with dicyclohexyl-18-crown-6 (DCC) in methanol and with dibenzo-18-crown-6 (DBC) in DMF, methanol, and dimethoxyethane has been investigated using ^{23}Na nmr. The influence of substituents in the aromatic rings of DBC on the decomplexation rates was also studied. Kinetic data were derived from pulse nmr measurements of the longitudinal relaxation rates of solvated sodium in the presence of complexed species. Analysis of the results indicates that the dominant exchange mechanism involves, in all investigated systems, the decomplexation step $Na^+(X^-)$, crown $\rightleftarrows$ $Na^+(X^-)$ + crown. The energy of activation of the decomplexation of DBC and of its derivatives was found to be nearly constant (12.6 ± 1.0 kcal/mol) in all investigated solvents. The coincidence of this value with the energy of activation of a conformational rearrangement of the macrocyclic ring has been discussed. The relatively low energy of activation of decomplexation of DCC (8.3 kcal/mol) has been attributed to the flexibility of the macrocyclic ring in DCC. The values of the rate constants of complexation and of the rate constants of decomplexation at 25° were found, in all investigated systems, to be within the range of 2×10^7–3×10^8 M^{-1} sec^{-1} and of 10^4–2×10^5 sec^{-1}, respectively. The latter decrease and the former increase in the order of increasing stabilities of the complexes.

In the first part of the study,[1] the application of nmr spectroscopy of cations for the kinetic investigation of their complexation with macrocyclic polydentate ligands had been discussed. A detailed investigation of the complexation of a macrocyclic polyether dibenzo-18-crown-16 (DBC) with sodium ions in the *N,N*-dimethylformamide (DMF) solutions was described. The mechanism of the exchange reaction was elucidated and the rate constants of the decomplexation were calculated on the basis of a quantitative analysis of the line width of the ^{23}Na nmr signal at various temperatures and concentrations. The thermodynamic constants of the system were evaluated from conductometric measurements.

This work has now been extended and the complexation of DBC and of its derivatives was studied in DMF, methanol, and dimethoxyethane (DME). The kinetics and the thermodynamics of complexation of DBC with sodium ions could be thus compared in solvents characterized by different solvating power, and the effect of substituents on the complexing ability of DBC could be evaluated.

While in the previously reported study[1] relaxation rates were derived from the line width of the ^{23}Na absorption spectra, in the present investigation longitudinal relaxation rates directly determined by a pulse technique have been used for the kinetic analysis. Thus, accuracy of the results and the experimentally accessible concentration range could be greatly enhanced.

Experimental Section

Dibenzo-18-crown-6 (DBC) was prepared and purified as previously described.[1]

The two isomers of dicyclohexyl-18-crown-6 (DCC) (Aldrich, Practical) were separated chromatographically according to the procedure given by Frensdorff.[2] Isomer B [DCC (IB)], mp 65–67°, was used in our experiments.

cis-4,4′-Dinitrodibenzo-18-crown-6 (NDBC) was prepared by nitration of DBC. DBC (37 g) was dissolved in 800 ml of chloroform and 700 ml of acetic acid. Nitric acid (250 ml, 70%) was slowly added to this solution. The temperature was kept constant at 20 ± 5°. After 24 hr the reaction mixture was washed with water and aqueous Na_2CO_3. The residual light yellow solid obtained after evaporation of chloroform consisted of the two isomers of NDBC.[3] The crude product (10 g) was refluxed with 400 ml of ethylene glycol monomethyl ether, and the hot solution was filtered from the insoluble trans isomer and twice recrystallized from this solvent. The thus obtained cis isomer had a mp = 200–201°.

cis-4,4′-Diaminodibenzo-18-crown-6 (AmDBC) was prepared by reduction of the nitro derivative. Ethylene glycol monomethyl ether (300 ml) was mixed with 5.2 g of *cis*-NDBC. The reduction

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