ically favored cyclobutane isomers;[1,13] heptafulvalene formation rather than its dimethylenecyclobutane isomer,[4] etc.), these calculations indicate that aromatic carbenes as small as the seven-membered ring system might well be in equilibrium with allene forms. Although it must be recognized that the position of such equilibria would certainly be affected by differences in solvation due to the large difference in dipole moments of the two species, an explanation for the anomalous chemistry of the intermediate in eq 2 would simply require an equilibrium lying far enough toward the allene to allow reactions to dominate those of the carbene.

It is also interesting to compare the effect of the location of the annelating benzene ring on the relative stabilities of the carbene and the allene forms of seven-membered ring systems. As would be expected from

classical resonance forms, monoannelation at the 2,3 or 4,5 positions stabilizes the carbene form relative to its allene isomer. Conversely, annelation in the 3,4 position has a pronounced stabilizing effect on the allene form. As would be expected, these trends are even more pronounced in the diannelated cases. Thus, it would appear from these calculations that the 3,4-diannelated isomer should be a good model system for a study of the chemistry of cycloheptatetraene whereas 2,3- or 4,5-annelated isomers would better represent the carbene.

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A Theoretical Study of Solvent Effects on the Conformational Stability of Acetylcholine

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Abstract: The conformational stability of the chemical neurotransmitter acetylcholine in water and in chloroform is discussed based on quantum theoretical studies of the isolated molecular ion and solvation energies computed from the calculated solute properties and physicochemical properties of the solvent. The solvation energy is partitioned into electrostatic, dispersion, and cavity terms. A comparison of theory and experiment and a discussion of the biological significance of the results are presented.

The development of theoretical methods based on quantum mechanics for treating the electronic structure of large polyatomic molecules has provided widely used techniques for treating conformational stability problems in chemistry and biochemistry. The theoretical calculations are customarily carried out in the "free-space approximation," treating the molecule or molecular ion under consideration as an isolated, independent entity. There has generally been considerable accord between theoretically calculated energetically preferred geometries and the structures observed in the crystalline solid using X-ray or neutron diffraction methods and in the gas phase using microwave spectroscopy. Nevertheless, a number of systems wherein the geometry of a molecule adopted in the solid or gas phase differs significantly from that observed in solution have been experimentally characterized, and it is clear that a general theoretical account of the conformational stability of a molecule in solution requires explicit consideration of environmental effects.

Such problems are clearly evident in structural studies of acetylcholine (Ach), where a detailed knowledge of the structural chemistry is considered a vital prerequisite to the understanding of cholinergic neural transmission systems at a molecular level.[1] Experi-

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mental data are available on Ach in solid[2,3] and solution,[4-8] and theoretical studies have been reported for Ach using a variety of models and methods.[1,9-17] The relevance of the calculated results to the conformational

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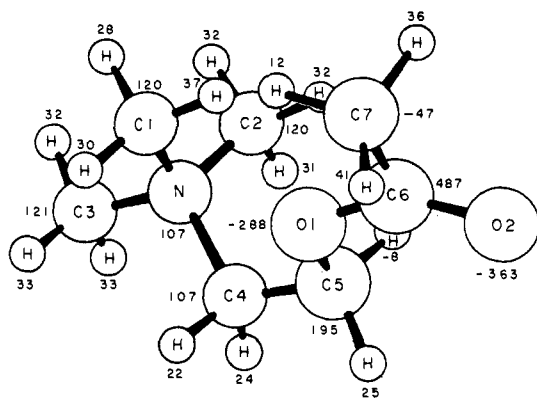(17) G. N. J. Port and A. Pullman, J. Amer. Chem. Soc., 95, 4059 (1973).

Figure 1. Molecular structure and atomic numbering system for acetylcholine. The conformation illustrated is that found in the Ach⁺Cl⁻ crystal structure, τ(O1–C5–C4–N) = 85° and τ(C6–O1–C5–C4) = −167°. Dihedral angles are defined such that, e.g., τ(O1–C5–C4–N) refers to the angle between the planes defined by O1–C5–C4 and C5–C4–N.

stability of the system in solution has not previously been considered on a quantitative basis.

We present herein a theoretical study of the conformational stability of Ach in solution based on conformational energies computed for the isolated molecule from self-consistent field (SCF) molecular methods at the level of intermediate neglect of differential overlap (INDO)[18] and solvation energies estimated from properties computed from the SCF–INDO molecular wave functions, structural parameters, and physicochemical properties of Ach and the solvent. The solvent methodology described is an adaptation of "solvent effect theory" developed by Sinanoglu[19–22] and applicable to a general solute–solvent system.

I. Background

The molecular geometry of Ach 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 in Figure 1. In Ach and a large number of analogous compounds, the coordinates τ(C5–C4–N–C3) and τ(O2–C6–O1–C5) are observed to be antiperiplanar (ap) and synplanar (sp), respectively. Thus a specification of τ(O1–C5–C4–N), which positions the ester oxygen with respect to the trimethylammonium cationic head, and τ(C6–O1–C5–C4), which positions the acetate group with respect to the choline moiety, is sufficient to specify the three-dimensional configuration of the molecule.

The crystal structure of Ach⁺Br⁻ as determined by Canepa, Pauling, and Sorum[2] has τ(O1–C6–C5–C4) = 77° and τ(C6–O1–C5–C4) = 79°, hereafter referred to as {77°, 79°}. The structure of Ach⁺Cl⁻ in the crystalline solid was recently reported by Herdklotz and Sass[3] to be {85°, −167°}. The structure of Ach⁺ in D₂O solution has been investigated using nmr spec-

troscopy by Culvenor and Ham,[4] Cushley and Mautner,[5] Casy, Hassan, and Wu,[6] Inch, Chittenden, and Dean,[7] and Partington, Feeney, and Burgen.[8] Spin coupling analyses and comparisons with model compounds point to a synclinal value for τ(O1–C5–C4–N) and an antiplanar value for τ(C6–O1–C5–C4), i.e., a {sc, ap} geometry. Casy, et al., report nmr studies for several other solvents, chloroform being the least polar, and the results indicate that in all cases τ(O1–C5–C4–N) is synclinal. They concluded that the conformation about τ(O1–C5–C4–N) is little influenced by solvent and is governed by intramolecular interactions. The dipole moment of Ach⁺ in chloroform has been reported by Maurel and Galzinga[23] as 2.65 D. A number of infrared spectra studies have produced interesting results and some controversy (see ref 1) but have not specifically resolved three-dimensional conformational effects.

The theoretical calculations reported for Ach using semiempirical potential functions, molecular orbital theory, and perturbative configuration interaction methods have been reviewed in previous papers from this laboratory[1,12,24] and in recent articles by Pullman and coworkers.[11,25] Most relevant to this study, SCF–INDO molecular orbital calculations have been used to compute the conformational energy of Ach as a function of τ(O1–C5–C4–N) and τ(C6–O1–C5–C4), and all experimentally observed or implicated geometries can be identified with one or another of the calculated energy minima on the conformational energy map.[1] Results from other methods are in substantial accord. Based on this observation we speculated that environmental effects on Ach in a given solvent tended to preferentially stabilize an energy minima intrinsic to the molecule as produced by calculations in the free space approximation. An extended study including muscarine, nicotine, and several methyl and dimethyl derivatives of Ach indicate that the structure implicated in cholinergic processes such as muscarinic and nicotinic activity and enzymatic hydrolysis correspond to sterically permitted but not necessarily energetically preferred geometrically preferred geometries.[12]

The explicit treatment of solvent effects in theoretical calculations is of course a formidable problem for statistical mechanics, and the theoretical description of the thermodynamic properties of solutions remains an area of active research interest. Differential solvation effects on physical properties have proved somewhat amenable to approximate methods, and the problem of solvent effects on conformational stability is tractable since even qualitative or semiquanititative results are sufficient for a useful theoretical description of the system. In representative studies from the recent literature, Daudel, Chalvet, and Peradejordi[26] estimated solvation effects on the base strength of azaaromatic compounds and derivatives using an electrostatic model due to Born,[27] further developed by Hush and Black-

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ledge[28] and elaborated in the form of solvaton theory by Klopman.[29] The role of especially solvent water on biomolecular conformational stability has received considerable attention in the extensive theoretical studies of the conformation of polypeptides by Scheraga and coworkers,[30] both in the modification of electrostatic interactions by dielectric effects and in an explicit statistical mechanical study. These approaches have been recently reviewed by Nemethy.[31]

The quantum mechanical procedures used for the calculation of conformational energies of the isolated solute molecules can be generalized to include solvent molecules explicity, and progress in this approach has been reported for systems of solvated protons and hydroxide ions.[32] In conformational studies of large molecules, this approach suffers from an arbitrariness in positioning solvent molecules in the absence of extensive geometrical optimizations. A single water molecule was included in a quantum mechanical study of choline by Beveridge, Radna, and Guth[33] with little effect on the conformational stability. Extensive studies on specific sites of solvation in biological molecules including acetylcholine are being carried out by Pullman and Port,[34] using *ab initio* SCF–MO methods to compute an electric multipolar representation of solute and solvent molecules and calculating the interaction energy electrostatically as a function of the position of solvent molecules.

An alternative approach to solvent effects involves the calculation of the interaction energy of a solute molecule with its entire environment, using a continuum model for the solvent. Recent studies of some thermodynamic properties of liquids from this viewpoint have been reported by Huron and Claverie.[35] Earlier Sinanoglu[19] used this approach in the solvent effect theory for a series of chemical and biochemical problems, partitioning the solvation energy into three contributions: electrostatic solute–solvent interaction, dispersion solute–solvent interaction, and the energy required for cavity formation. The measured physicochemical properties of the solvent are used together with specific solute properties to estimate each term. The solute properties involved are the molecular dipole moments, molecular volume, and ionization energy. Applications reported by Sinanoglu and coworkers include an extensive investigation of the role of solvent in the conformational stability of DNA,[20] thymine photodimerization,[21] and the cis–trans isomerization of azobenzene.[22] Aspects of this method have been involved in the experimental work of Cassidy and Moser[36] on the *p*-benzoquinone–hydroquinone association. The continuum model is of course a greatly simplified representation of the environment, but the contributions have clearly ascribed physical significance and the methodology can be implemented with a minimum of

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parameters. It is thus worthwhile to characterize the capabilities and limitations of this model on a wide range of systems.

The point of departure for our studies is the recognition that quantum mechanical methods and solvent effect theory can be used in a complementary sense in fashioning an extended theoretical approach to a study of environmental effects on conformational stability. For a given conformation, the calculated quantum mechanical molecular wave function can be used to calculate a solute conformational energy in the free-space approximation and a value for the solute dipole and other parameters to be used in the electrostatic and van der Waals terms of the solvation energy. The solute volume can be estimated from the set of Cartesian coordinates used as input to the wave function calculation. Computation of the wave function as a function of crucial internal coordinates permits computation of all required variables in the study as a function of conformation and the estimation of differential solvation energy.

This paper describes the theory generalized for molecular ions and applied to a study of acetylcholine in water and in chloroform. The theory and methodology is given in detail in the next section, followed by a presentation and discussion of results on Ach and a comparison of theory and experiment.

II. Theory

The total energy of a molecule in a given geometry under the influence of solvent effects can be approximately written as

$$E_{\text{total}} = E_{\text{solute}} + E_{\text{solvation}} \tag{1}$$

where E_{solute} is the total energy of a solute molecule in the free space approximation and $E_{\text{solvation}}$ is the energy of solute–solvent interaction. We consider the solvation energy as partitioned into three contributions

$$E_{\text{solvation}} = E_{\text{es}} + E_{\text{dis}} + E_{\text{cav}} \tag{2}$$

Here E_{es} represents the electrostatic solute–solvent binding energy arising from the interaction of the permanent and induced electric moments of the solute with the solvent and E_{dis} is the interaction energy due to dispersion forces. The terms E_{es} and E_{dis} are negative and E_{cav} is positive. Each of the terms depend parametrically upon quantities which vary as a function of the molecular geometry. The specific evaluation of each term is described in the following paragraphs.

E_{solute}. The calculation of the conformational energy of the isolated solute is based on approximate self-consistent field theory[18] with the closed shell $2n$ valence electron wave function Ψ considered as a Slater determinant of molecular spin orbitals ψ_i

$$\Psi = |\psi_1(1)\bar{\psi}_1(2)\psi_2(3)\bar{\psi}_2(4)\ldots\psi_n(2n-1)\bar{\psi}_n(2n)| \tag{3}$$

The molecular orbitals are expanded as linear combinations of atomic orbitals ϕ_μ centered on each of the atoms in the molecule

$$\psi_i = \sum_\mu c_{\mu i}\phi_\mu \tag{4}$$

where the $c_{\mu i}$ are linear expansion coefficients. The calculation of the wave function reduces to the determination of the $c_{\mu i}$ by matrix Hartree–Fock procedures.

The energy of the solute at a certain geometry is given by

$$E_{\text{solute}} = \sum_{\mu\nu}P_{\mu\nu}(H_{\mu\nu} + G_{\mu\nu}) + \sum_{A<B}Z_AZ_BR_{AB}^{-1} \quad (5)$$

where the sums over Greek and Latin letters refer to orbitals and atoms, respectively. The first term of the right-hand side of eq 5 is the electronic energy and involves $H_{\mu\nu}$, $G_{\mu\nu}$, and $P_{\mu\nu}$. The $H_{\mu\nu}$ are one-electron atomic integrals over kinetic energy and nuclear attraction operators, and the $G_{\mu\nu}$ are atomic integrals over electron repulsion operators. The density matrix elements $P_{\mu\nu}$ are defined in terms of the LCAO coefficients as

$$P_{\mu\nu} = 2\sum_{i}c_{\mu i}c_{\nu i} \quad (6)$$

where the sum is over all occupied orbitals. The second term in eq 5 accounts for intercore repulsions and involves the core charges Z_A and Z_B and the intercore separation between atoms A and B, R_{AB}. The reduction in intramolecular interactions due to the presence of the solvent (E_{red} in Sinanoglu's development[19]) is neglected in this study; however, a method for treating this is proposed in section III.

The atomic integrals involved in computation are evaluated at the level of intermediate neglect of differential overlap (INDO).[18] The INDO method is one of a series of approximate SCF–MO methods developed by Pople and coworkers specifically for studies of the structure and properties of large molecules.[37] All valence electrons are treated explicitly, with inner shell electrons considered as part of a nonpolarizable core. The suitability of this method for conformational problems has been discussed in detail in several recent papers.[38,39]

In the specific consideration of the acetylcholine system, the bond lengths, bond angles, and dihedral angles not explicitly considered variable for input to the wave function calculations were taken from the Ach geometry observed in the bromide crystal.[2] The consequences of this assumption will be discussed below. The geometrical input for a given conformation also was used to calculate the molecular volume, a parameter required in each of the contributions to $E_{\text{solvation}}$. The molecular volume at a given geometry is assumed to the volume V of the corresponding rectangular solid delineated by the extrema of x, y, and z values of the atomic Cartesian coordinates plus 2 Å in each direction as a minimum molecular width. The origin of the coordinate system is placed at the center of gravity of the molecule and the orientation of the coordinate system is coincident with the principle axes of the moment of inertia tensor. An effective molecular solute cavity radius a is defined by equating V and the volume of a sphere so that

$$a = (3V/4\pi)^{1/3} = (V/4.189)^{1/3} \quad (7)$$

The electric dipole moment required in the evaluation of E_{es} described below is computed directly from the molecular wave function. Only the first nonzero moment of a charge distribution is independent of the coordinate system, so for the ionic species the calculated

dipole moments were referred to the center of positive charge.

E_{es}. For the calculation of the electrostatic solute–solvent binding energy, the solute is treated as a sphere of radius a with a point dipolar ion of charge Q and total dipole moment $\mathbf{m}$ positioned at the center. The solute sphere is imbedded in a cavity of identical size in a homogeneous dielectric continuum representing the solvent. Following the development due to Onsager,[40] the electric multipoles of the solute induce effects in the medium which produce an induced electrical potential and electric field within the cavity, referred to as the reaction potential Φ_R and reaction field $\mathbf{E}_R$. Assuming retardation effects are negligible or included in an averaged sense, the electrostatic solute–solvent interaction energy is

$$E_{\text{es}} = Q\Phi_R - \mathbf{m}\cdot\mathbf{E}_R \quad (8)$$

and is considered to include effectively both orientation and induction solute–solvent van der Waals forces.

The reaction potential Φ_R and the monopolar contribution to E_{es} are readily evaluated, and the energy term varies as Q^2/a. In most molecular ions including Ach, the ionic charge is localized in a functional group on the periphery of the molecule. This group is likely to be heavily solvated in a manner independent of the remainder of the molecule, and a conformational dependence in the ionic energy term may be spurious. In preliminary studies of Ach this appeared to be the case, and thus we assume that the differential solvation energy of the cationic head is negligible and the ionic contribution to E_{es} is zero. More detail on this point is presented in the discussion of results in section III. The reaction field due to a point dipole was derived by Onsager[40] as

$$\mathbf{E}_R = \frac{2(\epsilon - 1)}{2\epsilon + 1}\frac{\mathbf{m}}{a^3} \quad (9)$$

where ϵ is the dielectric constant of the solvent. The total electric dipole moment $\mathbf{m}$ is a sum of a permanent moment μ and induced moment $\alpha\mathbf{E}_R$

$$\mathbf{m} = \mu + \alpha\mathbf{E}_R \quad (10)$$

where α is the polarizability of the sphere, related to the refractive index n and radius a in the ideal case by

$$\alpha = \frac{n^2 - 1}{n^2 + 1}a^3 \cong {}^1/_3a^3 \quad (11)$$

since n^2 for a wide range of molecules is ~ 2.5. Introducing eq 10 and 11 into eq 9

$$\mathbf{E}_R = \frac{3(\epsilon - 1)}{2\epsilon + 2.5}\frac{\mu}{a^3} = D\frac{\mu}{a^3} \quad (12)$$

where the rightmost equation serves to define D.

The final expression for E_{es} used in this investigation is thus

$$E_{\text{es}} = -\mathbf{m}\cdot\mathbf{E}_R = -(D\mu^2/a^3)(1 + D/3) \quad (13)$$

With μ in debyes and a in ångstroms, eq 3 becomes

$$E_{\text{es}} = -14.39(D\mu^2/a^3)(1 + D/3) \text{ kcal/mol} \quad (14)$$

The basic advantage of the reaction field model for electrostatic solute–solvent binding energies is descrip-

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tiveness and broad physical meaning with a minimum number of parameters. The role of solute parameters and their corresponding conformational dependence in eq 13 and 14 follows chemical intuition closely, with large solute dipole moments leading to increased stabilization in polar solvents and large solute volumes contributing a destabilizing effect. Reaction field theory has been used with some success in studies of solvent effects on electronic excitation energies[41] and nuclear magnetic resonance spin coupling constants[42] and chemical shifts[43] as well as studies of molecular and bimolecular structure.[19-22] An extensive review of applications and limitations of reaction field theory of intermolecular forces has been given by Linder.[44] The principal limitations of the reaction field model are the neglect of quadrupole and higher terms and the lack of explicit consideration of hydrogen bonding. Also the use of the macroscopic solvent dielectric constant ϵ is not entirely appropriate due to saturation effects in solvent regions proximal to the solute. These problems will also be discussed in the context of our results in section III.

E_{dis}. The solute–solvent interaction energy due to dispersion forces is estimated as

$$E_{dis} = \frac{\rho}{2}\int_0^\infty v_{ab}^{eff}(r)g^{(2)}(r)4\pi r^2\,dr \qquad (15)$$

where ρ is the number density of the solvent, $v_{ab}^{eff}(r)$ is an effective pair potential approximation to the interaction energy between solute a and solvent b at a separation r, and $g^{(2)}(r)$ is the radial distribution function for the solvent molecules about a central solute molecule. The use of this expression in estimating solvent effects has been described by Sinanoglu,[19,45,46] and his procedures are adopted with only slight modification herein. Specifically, $g^{(2)}(r)$ is taken as zero for $r < a$ and unity for $r \geqslant a$. The form for $v_{ab}^{eff}(r)$ suitable for use in liquids is

$$v_{ab}^{eff}(r) = v_{ab}(r)B_{ab}'(r) \qquad (16)$$

where $v_{ab}(r)$ is an effective pairwise interaction potential for molecules in the gas phase and $B_{ab}'(r)$ is a liquid phase correction factor.[48]

The function $v_{ab}(r)$ is taken in the form of a Kihara potential,[47] considering each molecule as a spherical core of diameter l. The potential function is

$$v_{ab} = C_{ab}\left[\frac{\sigma_{ab}^6}{\rho_{ab}^{12}} - \frac{1}{\rho_{ab}^8}\right] \qquad (17)$$

where C_{ab} is the coefficient described below, ρ_{ab} is the intercore distance, and σ_{ab} is the collision diameter. The intercore distance is calculated as

$$\rho_{ab} = r_{ab} - l_{ab} \qquad (18)$$

where l_{ab} is the contact distance of Kihara cores, defined in terms of the core diameters as

$$l_{ab} = \frac{1}{2}(l_a + l_b) \qquad (19)$$

The collision diameter σ_{ab} is the point of zero energy on the repulsive branch of the curve, and is defined analogous to l_{ab} as

$$\sigma_{ab} = \frac{1}{2}(\sigma_a + \sigma_b) \qquad (20)$$

The Kihara core size parameters appropriate for thermodynamic properties of fluids were found to be considerably smaller than the polarizable electron cloud for the molecule, and are given by Sinanoglu[45] as

$$l_a = \frac{a}{\beta}\left(\frac{1}{3.24 + 7\omega_a}\right) \qquad (21)$$

$$\sigma_a = \frac{a}{\beta}\left(\frac{2.24 + \omega_a}{3.24 + \omega_a}\right) \qquad (22)$$

where ω_a is Pitzer's "acentric factor,"[48] intended to correct for interactions between noncentral portions of the molecules, β is a constant taken as 1.15, and a is the effective solute cavity radius defined in eq 7.

The liquid phase correction factor for the gas phase potential function is given as[45]

$$B_{ab}' = 1 - \frac{1}{2}\frac{\Delta_{ab}'D_b'L_{ab}'}{1 - [\sigma_{ab}/(r - l_{ab})]} \qquad (23)$$

where Δ_{ab}' is defined in terms of ionization energies I_a and I_b as

$$\Delta_{ab}' = (I_a + 2I_b)/[2(I_a + I_b)] \qquad (24)$$

and

$$D_b' = D_b/(1 + D_b) \qquad (25)$$

with

$$D_b = (n_b^2 - 1)/(n_b^2 + 1) \qquad (26)$$

The quantity L_{ab} is a dimensionless function of r and the relative sizes of solute and solvent molecules, and a full expression is given in ref 46.

The London dispersion coefficient C_{ab}^{dis} is based on the classic form

$$C_{ab} = -\frac{3}{2}\alpha_a\alpha_b\frac{I_aI_b}{I_a + I_b} \qquad (27)$$

Using eq 7 and 11 this can be transformed to the expression given by Halicioglu and Sinanoglu[22]

$$C_{ab} = \frac{27}{32\pi^2}\Delta_{ab}D_aD_bV_aV_b \qquad (28)$$

where

$$\Delta_{ab} = 1.35I_aI_b/(I_a + I_b) \qquad (29)$$

The ionization energies can be expected to be relatively independent of geometry and are taken as constants. With $I_a = I_b = 10$ eV and $n^2 = 2.5$, $\Delta_{ab} = 6.25$ and $D_a = D_b = 3/7$. Then

$$C_{ab} = 6.45V_aV_b \text{ kcal mol}^{-1}\text{ Å}^6 \qquad (30)$$

In our calculations the solute and solvent volumes V_a and V_b are taken from the computed molecular geometry and the number density ρ is calculated from the observed solvent density. In the actual calculations the ionization energy of the solute was taken as 10 eV and those of the solvents were taken from reported experimental values. The integration indicated in eq 15

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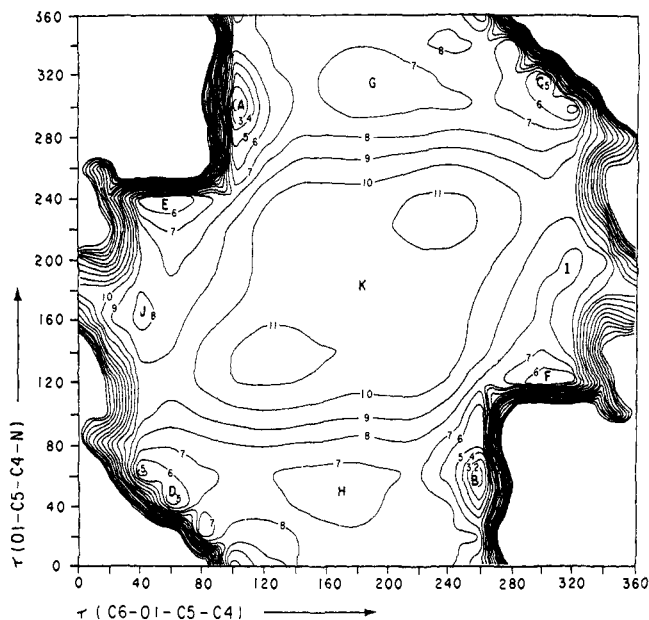
Figure 2. Conformational energy map of the isolated solute energy, E_{solute} (kcal/mol).

Table I. Physicochemical Parameters for Solvents Water and Chloroform[a]

Parameter	Water	Chloroform
ϵ	78.54	4.81
ρ	29.9133	133.1203
ω	-0.1790	-0.2967
μ_b	1.85	1.01
$\kappa(V_b/V_a)$	1.277	0.620
$\partial \ln \gamma/\partial \ln T$	0.157	0.112
α	0.257	1.27
I_b	12.60	11.42

[a] Values taken from Table 1 of ref 22 and Lange's "Handbook of Chemistry," Handbook Publishers, Inc., Sandusky, Ohio, 1956.

was carried out by numerical methods based on Simpson's rule.[49]

E_{cav}. The energy required to create a solvent cavity of sufficient size to accommodate a spherical solute of volume V can be estimated as γA, where γ is the surface tension of the solvent and A is the area of the surface of the cavity, related to V by the expression

$$A = 4\pi a^2 = 4.835 V^{2/3} \tag{31}$$

A consideration of the factors which carry γA to microscopic dimensions leads to the expression[19]

$$E_{cav} =$$

$$4.835\gamma V_a^{2/3}\kappa_b(V_b/V_a)\left[1 - \frac{\partial \ln \gamma_1}{\partial \ln T} - \frac{2}{3}\alpha T\right] \tag{32}$$

where κ_b is a constant dependent upon the volume fraction (V_b/V_a) that adjusts the planar surface area to curved microscopic dimensions, given as

$$\kappa_b(V_b/V_a) = 1 + (V_b/V_a)^{2/3}[\kappa_b - 1] \tag{33}$$

Here κ_b is the microscopic cavity factor for pure solvent, tabulated in ref 22. The remaining quantities in eq 32 are α and T, representing the thermal expansion coefficient of solvent and absolute temperature of the system. With γ in dynes per centimeter and V in ångstroms cubed, the energy of cavity formation is

$$E_{cav} = 6.96 \times 10^{-3}\gamma V^{2/3}\kappa_b\left(\frac{V_a}{V_b}\right) \times$$

$$\left[1 - \frac{\partial \ln \gamma}{\partial \ln T} - \frac{2}{3}\alpha T\right] \text{kcal mol}^{-1} \tag{34}$$

The individual values for physicochemical properties used in E_{es}, E_{dis}, and E_{cav} are listed in Table I.

III. Results and Discussion

Calculations were carried out for the molecular wave function, quantum mechanical energy, and each of the

components of the solvation energy of Ach in water and chloroform as a function of $\tau(O1-C5-C4-N)$ and $\tau(C6-O1-C5-C4)$. The results are presented as isoenergy contour maps, each of which was drawn from a plot generated by digital computer and incremental plotter from 324 calculated grid points (a 20° interval in each variable). The contour on each map is 1 kcal/mol and covers the lower 25 kcal/mol of the surface except as specifically noted for the dispersion energy contributions.

The quantum mechanically calculated conformational energy map for Ach in the free space approximation is given in Figure 2 using the SCF–INDO method and based on the crystalline bromide geometry. A number of energy minima are in evidence, certain of which correspond to structures observed in physicochemical studies of Ach or implicated in cholinergic processes by a consideration of the relative biological activity of Ach and a number of structural analogs. Details on this have been discussed in preceding papers,[1,23] and it suffices for present purposes to note that the Ach geometry observed in the bromide crystal can be identified with the minimum labelled D and the geometry observed in the chloride crystal and deduced for aqueous solution can be identified with the minimum H, 2 kcal/mol above D. This geometry figures significantly in current theories of muscarinic and nicotinic cholinergic neural activity.[24] The broad region K, 3 kcal/mol above H, corresponds to a geometry implicated in a proposed mechanism of hydrolysis of Ach by acetylcholinesterase.[50,51] The variation in solute energy in sterically allowed regions of the conformational map is $\sim$12 kcal/mol. The Ach dipole moment and molecular volume were computed as a function of conformation in the course of the quantum mechanical calculations. The calculated dipole moment ranged from 1.5 to 9.8 D. Both dipole moment and molecular volume are largest for the fully extended conformations of the molecule, where $\tau(O1-C5-C4-N)$ and $\tau(C6-O1-C5-C4)$ are both antiperiplanar (central region of maps) and smallest for the structures with both these dihedral angles synperiplanar or synclinal (edges of maps). The magnitude of the Ach dipole moment is especially sensitive to the orientation of the acetate group, $\tau(C6-O1-C5-C4)$.

A conformational map of the electrostatic term E_{es} for Ach in water is given in Figure 3. The significant features of the plot are the paired global minima at

(49) K. J. Nielson, "Methods in Numerical Analysis," Macmillan, New York, N. Y., 1956.

(50) R. M. Krupka and K. J. Laidler, *J. Amer. Chem. Soc.*, **83**, 1445 (1961).

(51) C. H. Chothia and P. J. Pauling, *Nature (London)*, **223**, 919 (1969).
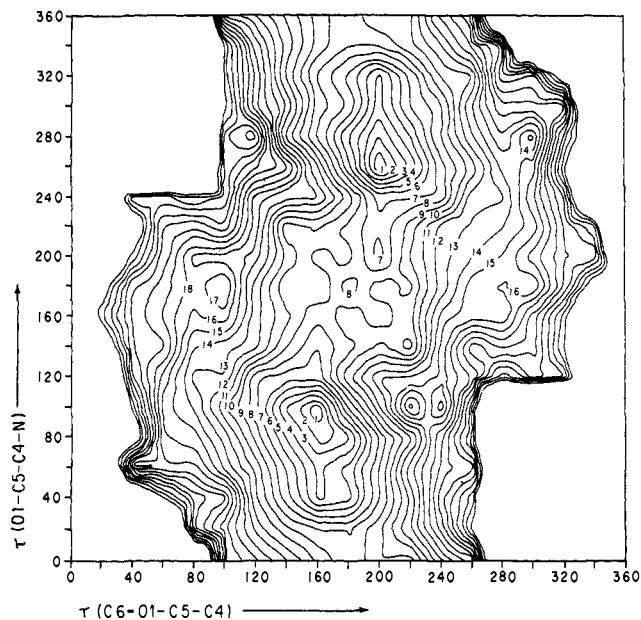
Figure 3. Conformational energy map of the electrostatic solute-solvent interaction energy, E_{es}, for Ach–water (kcal/mol).
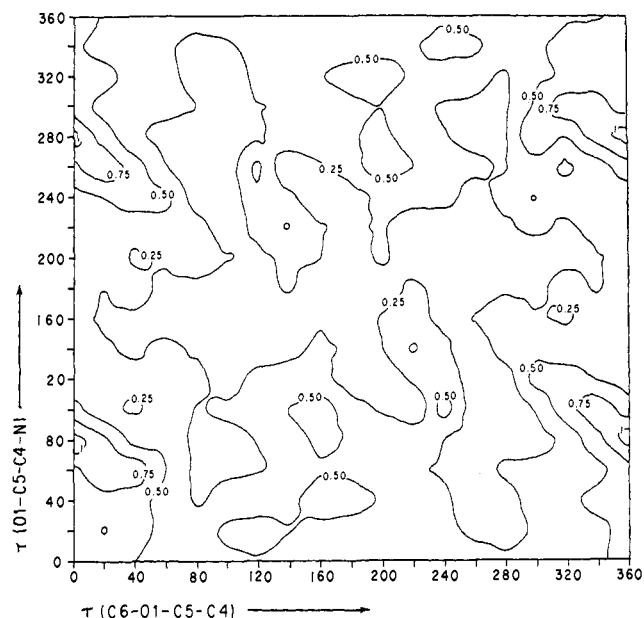

Figure 5. Conformational energy map of the cavity term, E_{cav}, for Ach–water (kcal/mol).

Figure 4. Conformational energy map of the dispersion solute-solvent interaction energy, E_{dis}, for Ach–water (kcal/mol).
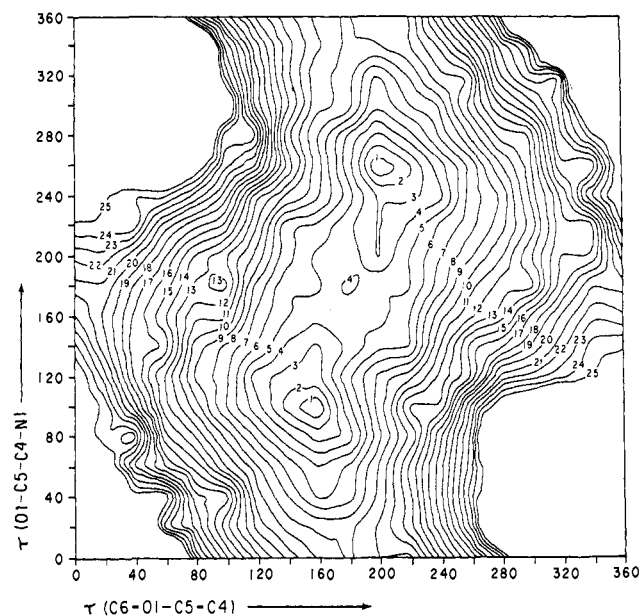
Figure 6. Conformational energy map of the total energy for Ach–water (kcal/mol).

$\{100°, 160°\}$ and $\{260°, 200°\}$ and the well-defined energy corridor for antiperiplanar extended forms of τ(C6–O1–C5–C4). The minima are developed as a consequence of opposing trends in the reaction field parameters. Extended conformations are stabilized by large dipole moments and destabilized by the correspondingly large cavity size and while geometries with smaller volumes are destabilized by smaller dipole moments. The minima found correspond to conformations with a compromise of relatively large dipole moment and relatively small volume, with large solute dipoles specifically related to antiplanar values of τ(C6–O1–C5–C4). The variation in the electrostatic term in sterically allowed regions of the map is $\sim$15 kcal/mol.
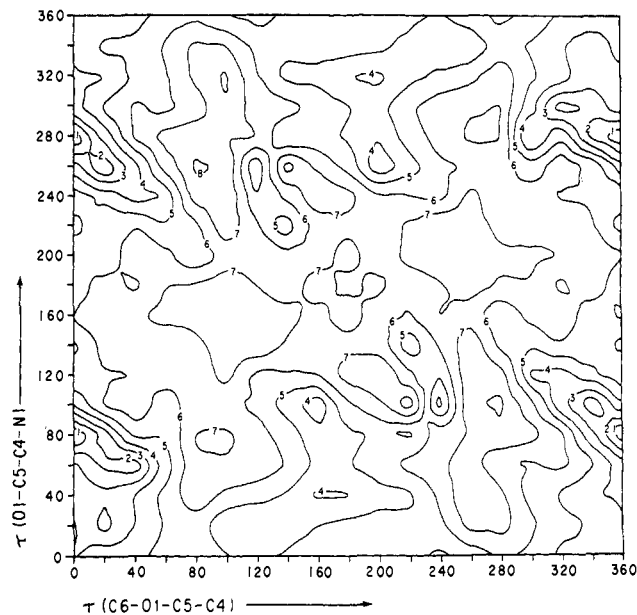
The conformational map for the dispersion energy E_{dis} for Ach in water is shown in Figure 4. The contour interval is 0.25 kcal/mol. The dispersion energy depends essentially on solute volume and thus central regions of the plot are preferentially stabilized. The variation in dispersion energies is $\sim$1 kcal/mol, and compared to E_{solute} and E_{es} the variation in the dispersion contributions with conformation is relatively small.

A conformational map of the cavity term E_{cav} for Ach in water is shown in Figure 5. The low-energy regions of this plot correspond to small volumes and are developed along the edges of the plot, with the central regions corresponding to large volumes destabilized. The variation in cavity energy in sterically allowed regions is 3 kcal/mol.
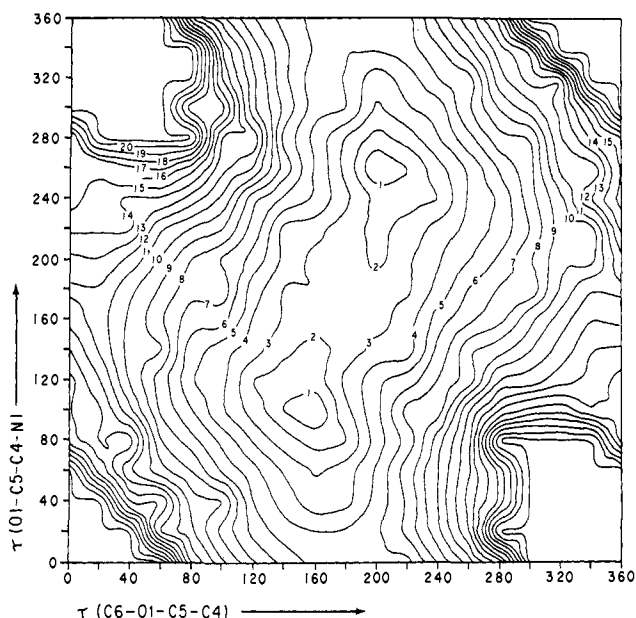
Figure 7. Conformational energy map of the electrostatic solute–solvent interaction energy, E_{es}, for Ach–chloroform (kcal/mol).

Figure 8. Conformational energy map of the dispersion solute–solvent interaction energy, E_{ind}, for Ach–chloroform (kcal/mol).

The total conformational energy map for the Ach–water system is the sum of the contributions shown in Figures 2–5 and is given in Figure 6. The appearance of the map is significantly different from that of the isolated solute, with solvation effects tending to destabilized the various synclinal minima in both geometrical variables. The electrostatic term dominates the solvation energy, so that the destabilization of the synclinal minima in aqueous solution is due to the relatively small solute dipole moments and thus small electrostatic solute–solvent interaction energies at these geometries. The global minima in the total energy map are clearly established at $\{100°, 160°\}$ and $\{260°, 200°\}$ and are identified with the geometry deduced for Ach in D_2O solution from nmr studies and implicated in cholinergic mechanisms. A local minimum is developed at $\{180°, 180°\}$ and can be identified with the geometry implicated for Ach in current theories of enzymatic hydrolysis.[51]

With these results in mind it is possible to discuss the effect of the decision on the monopole contribution to E_{es} in concrete terms. As mentioned previously, this term varies simply as the inverse of cavity radius, and produces an electrostatic conformational map (monopole plus dipole terms) with a well defined valley for $\tau(\text{O1–C5–C4–N}) = 180° \pm 20°$ and $\tau(\text{C6–O1–C5–C4})$ variable. These results would be inconsistent with geometry determined in the nmr studies. This leads us to conclude that the cationic head of Ach will be solvated in a manner independent of the conformation about $\tau(\text{O1–C5–C4–N})$ and $\tau(\text{C6–O1–C5–C4})$ and justify maintaining the monopolar contribution independent of geometry. The reasonable agreement between theory and experiment evidenced by Figure 6 implies that the approximations invoked are reasonable and specific solute–solvent hydrogen bonding effects (neglected in this theory) may not change significantly with conformation.

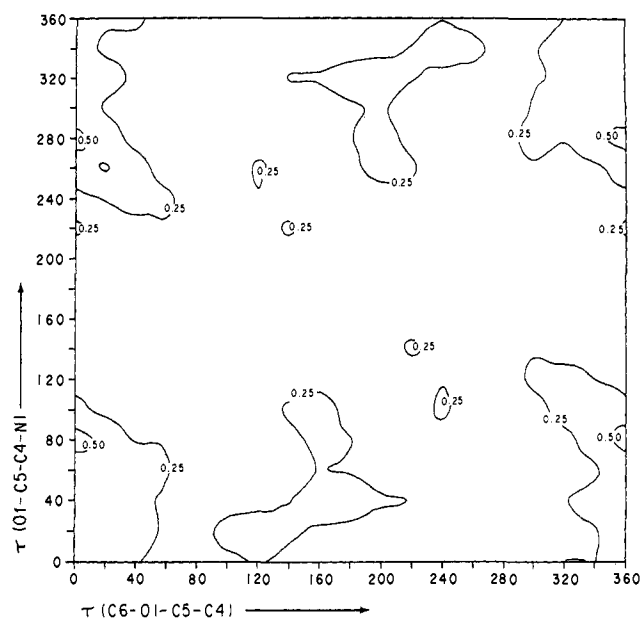A strictly parallel study was carried out for the Ach–chloroform system, keeping in mind the possibly significant effects of the counterion since ion pairing is expected in this solvent. The results for the four contributions to the solvation energy are given in Figures 7–10. The variation of the electrostatic term for chloroform is significantly less than for water and due directly to the lower dielectric constant for $CHCl_3$. The position of the minima is relatively unchanged. The dispersion energy maps is less affected and still relatively flat as a function of conformation. The variation in the cavity term for Ach in chloroform is also relatively less than for Ach in water, due to decreased surface tension. The physical basis for the low-energy regions of each map are, however, analogous to those described for water.

The total conformational energy plot for the Ach–chloroform system is given in Figure 10. The over-all appearance of the map is similar to that of water, but the over-all variation in energy is somewhat less. The paired global minima on the map are somewhat less. The paired global minima on the map are somewhat broader, corresponding to $\tau(\text{O1–C5–C4–N}) = 40$ to $100°$ with $\tau(\text{C6–O1–C5–C4}) = 160°$, and $\tau(\text{O1–C5–C4–N}) = 240$ to $320°$, with $\tau(\text{C6–O1–C5–C4}) = 200°$. The former minimum can be identified with the geometry discussed for Ach in chloroform by Casy, Hassan, and Wu.[6] In chloroform as in water, we find the electrostatic term contributing significantly if not totally dominating the conformational preference. Since the water and chloroform represent more or less the extremes in solvent polarity, and the results indicate that Ach has similar energetically preferred conformation in both solvents, it follows that results for solvents of intermediate polarity and probably even the relevant biological fluid would be similar to those explicitly considered herein. The results indicate that the equilibrium geometry of Ach in solution is not especially labile but confined to the $\{sc, ap\}$ structure and its mirror image.

The results so described and subject to all of the assumptions and approximations enumerated in sections
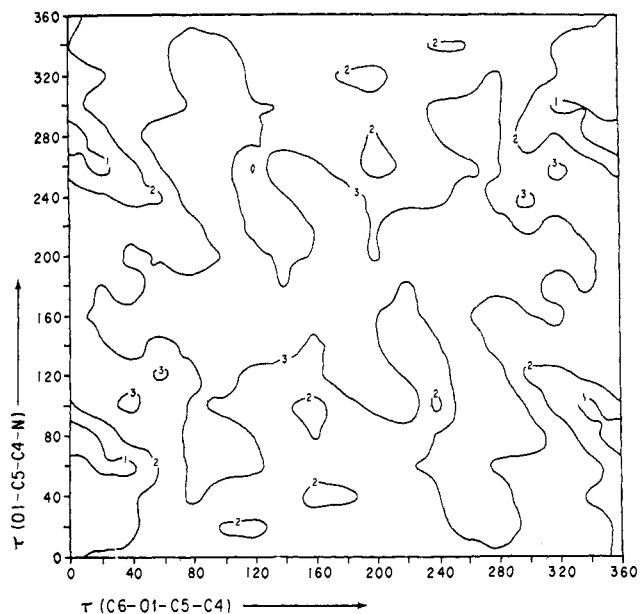
Figure 9. Conformational energy map of the cavity term, E_{cav}, for Ach–chloroform (kcal/mol).

Figure 10. Conformational energy map of the total energy for Ach–chloroform (kcal/mol).

I and II indicate that the dominant factor in establishing the {sc, ap} conformational preference of Ach in water is the electrostatic solute–solvent intermolecular interaction. It is true as previously observed that the isolated molecule has an energetically preferred geometry in this region, but the electrostatic solvation energy term indicates the solvent–solute interaction heavily favors this structure as well. One should note in this regard that the calculations of the isolated molecule were based on the Ach^+Br^- crystal geometry and were carried out before the Ach^+Cl^- crystal structure was published. Pullman, et al.,[11] noted that quantum mechanical calculations using the PCILO method and the bromide geometry produce a conformational map quite similar to the INDO results in Figure 2. The PCILO calculations based on the chloride geometry are significantly different, with global minima at {±sc, ap} geometries and a local minimum at {ap, ap} lying 3 kcal/mol above the global minima. It is clear from this result that had we started our study using the Ach^+Cl^- geometry the results on solvent effects would have been substantially the same as we have described and particularly still dominated by the intermolecular electrostatic term.

As a final theoretical point, the one term in Sinanoglu's original formulation of the theory which is neglected in this study is the reduction of intramolecular solute interactions due to the presence of the solvent. A convenient means for estimating this contribution is to include the Onsager reaction field E_R as a perturbation in the Hartree–Fock Hamiltonian operator for the system and effectively solve the self-consistent field molecular orbital problem in the presence of the solvent. The formalism for this problem has been described by Kirkwood.[52] The difference in the E_{solute} computed this way and E_{es} as computed above gives Sinanoglu's E_{red}. Using this approach, the full intramolecular and intermolecular electrostatic interaction effects would be included in the quantum mechanically calculated solute energy. The computation in this form

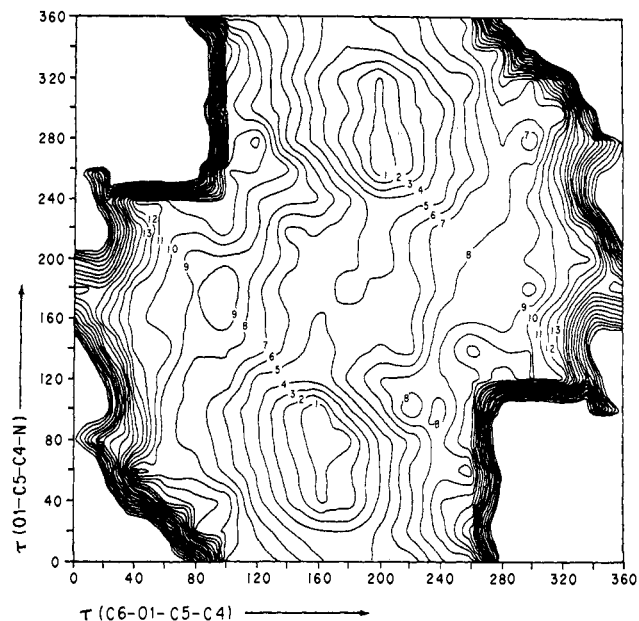(52) J. G. Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934).

is currently being carried out in this laboratory and will be reported in a later paper.

It is of some general interest to consider the conditions under which the geometrical preferences of a large molecule in solution will be established by intramolecular effects or by environmental effects. In the context of the theoretical description provided by the methods described herein, intramolecular effects will be important for solutes where the dipole moment and effective molecular volume are relatively independent of geometry. If the dipole moment or volume changes sharply with geometry, differential solvation effects will be large and environmental effects will be a significant factor in conformational stability.

IV. Summary and Conclusion

Solvation effects on the conformational stability of Ach in water and chloroform have been estimated using quantum mechanical calculations for intramolecular energies and a continuum model for the interaction of an Ach molecular ion with the solvent environment. The parameters entering the calculation were based on calculated solute properties and observed physico-chemical properties of the solvent; no disposable parameters (arbitrary parameters chosen for improved agreement between theory and experiment) are involved. The calculations give a reasonable account of conformational stability in the system as it is presently known on the basis of nmr conformational analysis. The dominant effect determining the solution geometry is the electrostatic solute–solvent interaction energy. The calculated preferred conformations are readily identified with geometries implicated in the structural chemistry of cholinergic neural transmission processes and the enzymatic hydrolysis of Ach. We are currently extending these studies to other cholinergic and adrenergic molecules in solution.

Analysis of Least Motion Paths for Molecular Deformations[1]

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Abstract: The fundamental basis of nuclear least motion correlations of reactivity order is demonstrated through analytical form comparisons with the theory of small vibrations and extensions thereof. The analytic motion equations are developed both for small deformations around arbitrary reactivity structures, including transition states, as well as for the gross changes between conventional (reactant and product) end-point states. A number of ramifications of the analysis are explored with particular emphasis on weighting of individual atom motions, directional effects of such motions, and on the choice of end points for optimal characterization of reactions. An example is made of a molecular system having modes of distortion which are simultaneously pertinent as regards reactivity and fully tractable in terms of the theory of small vibrations, preliminary to application of the principle of least motion to more complicated systems in an accompanying paper.

The current literature on analysis of reaction mechanisms contains an increasing number of references to paths of least motion and speculation as to whether these are favored paths for reactivity. With few exceptions, however, this aspect is usually only qualitatively and superficially considered and thereafter is essentially ignored. Three main reasons, to a degree interconnected, appear to be responsible for this strategy. Whereas least nuclear motion is recognized as complementary to least electronic motion, in that both are invoked in the Rice and Teller generalization which has come to be known as the principle of least motion (PLM),[2] it has not been formalized to the extent of the latter (*cf.* conservation of orbital symmetry rules[3]). Consequently, there is uncertainty in what constitutes a least motion path, especially for systems of any complexity, and confusion exists in how to quantitatively gauge motions along given paths. The third reason appears to be based on doubts that gross structural changes between reactants and products contain necessary and sufficient information of the dynamics of the reacting system.

Hine, subsequent to an examination of the history of PLM, has developed a quantitative procedure to test its validity on several reactions of resonance stabilized species[4] and later on problems of stereochemical control in elimination reactions.[5] Tee has presented details of a nonlinear least-squares technique for intramolecular invariant transformations,[6] generalizing Hines' harmonic distortion procedure, and has examined a number of other interesting reactivities, also principally involving stereochemical differentiation.[7] These studies while contributing information necessary to resolve the question of limits of applicability of PLM[8] do so entirely pragmatically and thereby cannot resolve the expressed uncertainties.

A preliminary analysis of these questions was presented at a recent meeting concerned with problems of chemical and biochemical reactivity.[9] Several generalizations were obtained from this analysis, relating to the potential enhancement of the correlational value of the PLM through consideration of structures of transition states and reactive intermediates, and differential distortions therefrom, rather than just of reactant and products and gross differences among them. In particular, it was shown that Hines coupled harmonic springs ensemble model for evaluating distortions in reaction systems is amenable to closed form analysis. The expressions obtained for systems of rather broad generality are sufficiently simple to allow recognition of individual atom or group contributions within the easily computed overall molecular least motion distortions. Application of the differential distortion relationships allows recognition of least motion, as distinguished from greater and most motion paths for reaction. Moreover, for geometry changes of any size, but particularly for differential changes, it appeared that these analytic terms could be compared with expressions and results obtainable from more firmly grounded theoretical methods.

In the present paper, development of these analytic formalisms will be detailed and continued. The major

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

(2) F. O. Rice and E. Teller, *J. Chem. Phys.*, **6**, 489 (1938); **7**, 199 (1939). As stated therein, "those elementary reactions will be favored that involve the least change in atomic position and electronic configuration."

(3) *Cf.* R. B. Woodward and R. Hoffmann, "The Conservation of Orbital Symmetry," Verlag Chemie, Weinheim/Bergstr., Germany, 1970.

(4) J. Hine, *J. Org. Chem.*, **31**, 1236 (1966).

(5) J. Hine, *J. Amer. Chem. Soc.*, **88**, 5525 (1966).

(6) O. S. Tee, *J. Amer. Chem. Soc.*, **91**, 7144 (1969).

(7) O. S. Tee and K. Yates, *J. Amer. Chem. Soc.*, **94**, 3074 (1972).

(8) See, *e.g.*, S. I. Miller, *Advan. Phys. Org. Chem.*, **6**, 185 (1968), who concludes from an examination of a number of stereoselection cases that the concept would be better labeled a hypothesis.

(9) S. Ehrenson, "Chemical and Biochemical Reactivity," Proceedings of the International Jersusalem Symposium, 6th, 1973, in press.