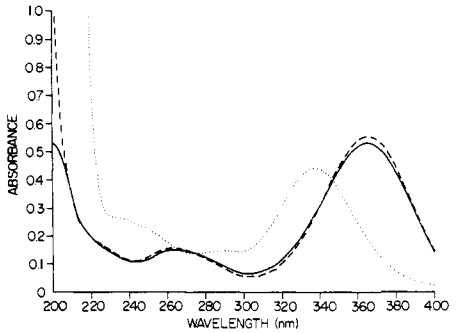


Figure 2. Ultraviolet absorption spectrum of synthetic 4-selenouridine in water (—), at pH 2(- - -), and at pH 12 (• • •).

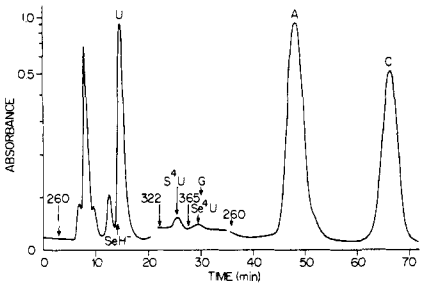
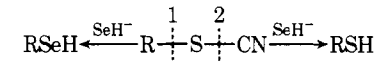


Figure 3. Chromatography of a hydrolysate of selenated tRNA on a column of Bio-Rad Aminex A-6 (cation exchanger), 17.5 × 0.63 cm. A 30-μL sample (≡2 A_{260} units) was injected into the column, maintained at 50 °C, and eluted with 0.4 M $HCOONH_4$–0.005 M S_2threitol, pH 4.5, at 0.28 mL/min (80 psi pressure). 4SUrd and 4SeUrd were monitored at 322 and 365 nm, respectively. The rest of the chromatogram was monitored at 260 nm. The elution positions of Guo and SeH^- are marked on the chromatogram. Since Guo does not have any absorbance at 365 nm, it does not interfere with the monitoring of 4 SeUrd at 365 nm. The peak at 8 min is partly due to the enzymes used in the hydrolysis of tRNA, but most of it is probably due to unhydrolyzed tRNA or its digestion products which have not reached the nucleoside level. The amount of material represented by this peak is about 6% of the total UV-absorbing material represented in the chromatogram in terms of A_{260} units.

1 M ethanolic CNBr. The reaction mixture was allowed to stand at 24 °C for 15 min and then evaporated at 24 °C under high vacuum using a rotary evaporator. The residue was dissolved in 1 mL of oxygen-free water and treated with 10 μL of methanolic 1 M NaHSe[8] in a nitrogen atmosphere and allowed to stand 1 h at 24 °C, then overnight at 4 °C. The tRNA was then dialyzed against 0.005 M aqueous dithiothreitol (S_2 threitol) to remove excess reagent and phosphate.

In order to demonstrate that 4-thiouridine moiety in tRNA has been converted into 4-selenouridine, we hydrolyzed the selenated tRNA to the nucleoside level in two steps, by incubating the tRNA with RNase T_1 at 37 °C at pH 7.2 for 30 min followed by incubation at 48 °C for 3 h with phosphatase and venom phosphodiesterase at pH 8.8. The hydrolyzed tRNA was then fractionated on a Bio-Rad A6 column (17.5 × 0.63 cm) using 0.4 M $HCOONH_4$ buffer, pH 4.5, containing 0.005 M S_2threitol. The column effluent was monitored with a Beckman DB spectrophotometer as described by Uziel et al.[9] We found that for good recovery of 4-selenouridine, use of S_2 threitol in eluting buffer is essential. In another experiment, synthetic 4-selenouridine was incubated with the same enzymes used for tRNA hydrolysis and similarly analyzed on the column. The recovery was about 77%. Recovery of synthetic 4-thiouridine, on the other hand, was essentially quantitative. This ammonium formate buffer system does separate HSe^-, 4-thiouridine, and 4-selenouridine. Based on the integration of the peak areas in the chromatogram, the conversion of 4-thiouridine to 4-selenouridine was calculated to be about 60% (Figure 3). Partial conversion of 4-thiouridine to 4-selenouridine is not unexpected. As indicated earlier,[5] reduction of the 4-thiocyanatopyrimidine by SeH^- could follow two pathways, one by scission of the pyrimidine ring C–S bond, and the other by scission of the exocyclic C–S bond. The former pathway will lead to 4-selenouridine and the latter will lead to the regeneration of 4-thiouridine.



Partial conversion of 4-thiouridine to 4-selenouridine in tRNA has been achieved and such selenated tRNAs can now be easily prepared for biological studies.

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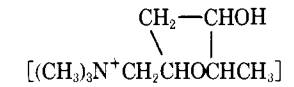
On the Conformational Changes of Acetylcholine and Some of Its Analogues

Sir:

Lately numerous efforts have been made to elucidate the conformation of acetylcholine and related compounds. X-ray diffraction studies and quantum mechanical calculations contributed structural details in the solid state. NMR spectroscopy provided interesting information in solutions. Nevertheless the complexity of the problem prevented the accumulation of sufficient knowledge on the structural and conformational possibilities of these molecules. Observations in solid state and in aqueous solutions with the same method seem quite desirable. Therefore we used Raman spectrometry complemented by infrared to study the vibrational properties of acetylcholine (Ach), $[(CH_3)_3N^+CH_2CH_2OCO\,CH_3]$,[1] and nicotine (Ni), $[CH{:}CHCH{:}NCH{:}CCH(CH_2)_3NCH_3]$.[2] Although it was not possible to obtain a transferable force field, qualitative attributions enabled us to deduce the conformational changes when passing from solid state to aqueous solutions.

Here we present the spectra of β-methylacetylcholine (β-MeAch) $[(CH_3)_3N^+CH_2(CH_3)CHOCOCH_3]$ and muscarine (Mu),



two potent muscarinic agonists of Ach with a known crystal structure,[3,4] and we discuss them in comparison with the

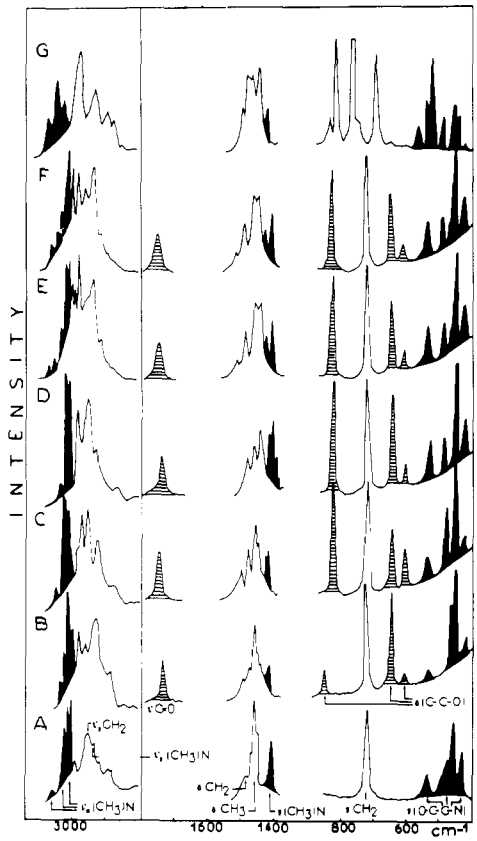


Figure 1. Solid state Raman spectra of: A, ChI; B, AchCl; C, AchBr; D, AchI; E, β-MeAchCl; F, β-MeAchBr; G, MuCl.

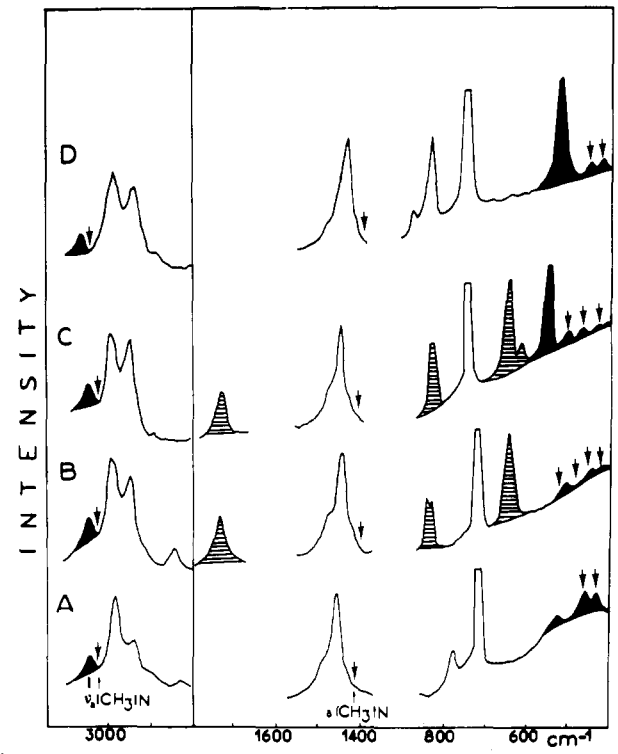


Figure 2. Aqueous solution Raman spectra of: A, ChI; B, AchI; C, β-MeAchCl; D, MuCl.

spectra of Ach and Ni. Figures 1 and 2 display the Ch, Ach, β-MeAch, and Mu spectra respectively for solid state and aqueous solutions. Using ChI spectra we distinguished the vibrations of the choline part from those of the acetyl part in the molecules.

Three compounds of Ach have been studied: AchCl, AchBr, and AchI. Their frequency attributions and the observed differences have been discussed in detail.[1] Here we stress only the fact that the solid state conformations of all three compounds are different (Figure 1B, C, D), with the AchI one being quite close to that of AchBr. This is in agreement with crystallographic data for AchCl and AchBr.[5,6] In aqueous solution all three compounds show obviously the same spectra (Figure 2B). The following phenomena seem significant for the transition of Ach from solid state to aqueous solution: the conformation of the acetyl part (CH_3OCO) practically does not change, while the spectral differences for the choline part [$(CH_3)_3N^+CH_2CH_2O$] are considerable. Certain vibrations disappear (arrows in Figure 2B), others increase their frequency and change intensity.[1] This implies a conformation in aqueous solution quite different from that in solid state.

Two compounds of β-methylacetylcholine were studied: β-MeAchCl and β-MeAchBr. Their solid state spectra (Figure 1E, F) are identical in the frequency range 400–1800 cm^{-1}. The differences in 2800–3100 cm^{-1} concern only the methyl group stretching vibrations. Comparison with the solid state spectra of Ach indicates that the conformations of the choline part O–C–C–N and acetyl part C–C–O skeletons of β-MeAch are close to those in AchBr and AchI, but the conformation of methyl groups exhibits differences. The β-MeAch vibrations in aqueous solution differ only slightly in frequency from the solid state. Its conformation in solution should be close to that in the crystal. This is in agreement with the relative rigidity of its skeleton. The variations observed in the transition to aqueous solution concern the O–C–C–N vibrations and the vibrations of the methyl groups bonded to nitrogen, $\nu_a((CH_3)_3N)$ and $\delta((CH_3)_3N)$. The skeleton O–C–C–N variations differ from those of Ach, but the disappearence of the vibrations of the methyl groups is identical with the Ach (arrows in Figure 2B, C).

The muscarine was studied as MuCl compound (Figure 1G and Figure 2D). The vibrations of the O–C–C–N skeleton and the methyl groups bonded to nitrogen are different in solid state from those of Ach and β-MeAch. Like β-MeAch, Mu retains its conformation in solution close to that in the crystal. In the spectra of aqueous solution the skeleton O–C–C–N vibration variations are similar to these of β-MeAch. The disappearance of the vibrations of the methyl groups bonded to nitrogen is the same as in Ach and β-MeAch spectra.

The β-MeAch and Mu interact with the muscarinic receptors. Their activities and stereospecificities are basically very similar.[7,8] The synclinal conformation of the skeleton of β-MeAch iodide crystal is determined by the interaction between the carbonyl oxygen atom and the β-methyl group.[3] The position of the latter is similar to that of a ring carbon atom in the Mu iodide crystal which exhibits the same conformation.[4] This analogy is preserved in solution as shown by the Raman spectra of both molecules (Figure 2C and D). There is a strong band at ~550 cm^{-1} attributed to a bending vibration of the skeleton. This band disappears completely for Ach. The unsubstituted O–C–C–N chain of Ach is capable of relatively unhindered rotations around the O–C and C–C bonds while the β-methyl substitution of β-MeAch and the presence of the ring in Mu limit this possibility. We conclude that in aqueous solution the conformation of the skeleton O–C–C–N, similar for β-MeAch and Mu, is different for Ach. Due to the higher rigidity of the two analogues it can be considered that their conformation in solution is already closer to a structure which will fit with the muscarinic receptor. On the contrary Ach should take its "muscarinic" form only in the neighborhood of muscarinic receptors. Therefore the skeleton conformation of β-MeAch in solution could be more appropriate for comparison with models of muscarinic activity of Ach than that of Ach itself.

The conformation of methyl groups bonded to nitrogen,

different in solid state for the three molecules, appears identical in aqueous solutions.

We have previously shown[2] that the electronic distribution of the protonated nitrogens of Ach and monoprotonated Ni was closely the same. The similar values for the stretching vibrations of methyl groups bonded to nitrogen in the spectra of Ach, β-MeAch, Mu, and Ni indicate that in the neighborhood of the nitrogen atom the electronic density is closely the same for all these molecules.

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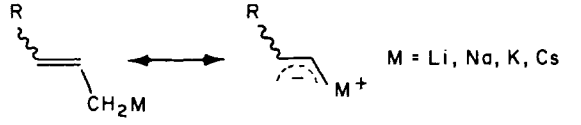
Gas Phase Acidities of the 2-Butenes. Regarding the Use of Organometallics as Models for Free Carbanions

Sir:

Interest has focused in recent years on the generation and characterization of allyl organometallics, I, in which M is an alkali metal. Perhaps the most startling feature of these sys-

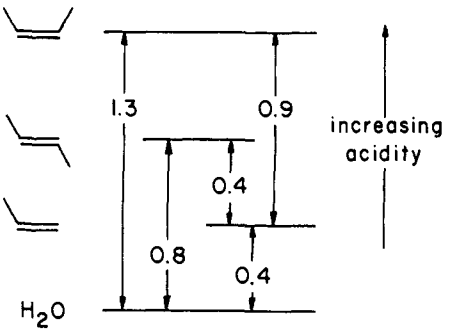


I

tems is their thermodynamic preference to adopt cis rather than trans skeletal geometries.[1-4] For example, Schlosser and Hartmann[1i] have recently reported that equilibrium mixtures of the 1-methylallyl compounds (I, R = CH_3) of lithium, sodium, potassium, and cesium are comprised of 67, 93, 96, and >99.9% of the cis isomers, respectively. These ratios correspond to free energy differences (favoring the cis compounds) of 0.4, 1.2, 1.4, and >3.1 kcal/mol for the four metals, respectively, which are to be compared with a cis-trans $\Delta G°$ (trans favored) of 0.7 kcal/mol for 2-butene itself.[6] Similar cis/trans ratios have been reported for equilibrium mixtures of more highly substituted alkenyl organometallics, which generally show enhanced preference for cis skeletal arrangements with increasing electropositivity of the metal.

The preferences noted for alkenyl organometallics to adopt cis skeletal geometries have often been ascribed to those of the underlying allyl carbanions. It has also recently been pointed out that "the properties of resonance-stabilized organometallic contact pairs approach closer and closer to those of true (free) carbanions as the metal is varied from the less electropositive (lithium) to the more electropositive (cesium)."[1i] In order to provide evidence for or against such notions as these, we have undertaken to measure the relative acidities of *cis*- and *trans*-2-butene in the gas phase, using pulsed ion cyclotron resonance (ICR) spectroscopy.[7] Our data, displayed in Chart I, indicates a $\Delta G°_{298}$ for the proton transfer reaction (eq 1),

Chart I




(1)

(obtained by independent measurements of the acidities of *cis*- and *trans*-2-butene relative to both water and propene), of -0.5 ± 0.2 kcal/mol (i.e., favoring of formation of the cis 1-methylallyl anion). It needs to be emphasized that the ICR experiment provides no direct information regarding the geometrical structures of ions in the gas phase. This is because only a species' charge to mass ratio is measured directly. Therefore, the interpretation of our data requires that three assumptions be made: (1) that on the time scale of the ICR experiment, the cis and trans isomers of the 1-methylallyl anion do not interconvert; (2) that deprotonation of the 2-butenes results in formation of allylic rather than vinylic anions; (3) that reprotonation of the cis and trans 1-methylallyl anions leads to the corresponding 2-butenes rather than to the thermodynamically less stable C_4H_8 isomer, 1-butene. Our first assumption is supported by the fact that, in solution, cis–trans interconversion barriers of alkenyl organometallics are sizable. Thus, rotation about the allylic bond in a variety of 1-phenylallyl organometallics requires between 15 and 20 kcal/mol,[8a,b] while degenerate isomerization in allyllithium occurs with a somewhat smaller (10.5 kcal/mol[8c]) barrier. In view of known preferences for charge localized cations to be more heavily solvated than delocalized species,[9] it seems likely that the barriers hindering cis–trans isomerization in the free anions will be even larger than those measured in solution. Although no experimental data are available, an ab initio molecular orbital calculation indicates a barrier of 25 kcal/mol for rotation in the allyl anion.[10] Our second assumption, regarding the site of deprotonation in the 2-butenes, seems quite secure, since proton detachment from ethylene (leading to the vinyl anion) is at least 15 kcal/mol more difficult than allylic deprotonation.[13] Our final assumption, that reprotonation of the cis and trans methylallyl anions leads back to their respective alkene precursors and not to 1-butene, will be satisfied on thermodynamic grounds (1-butene is 1.3 and 2.0 kcal/mol less stable than *cis*- of *trans*-2-butene, respectively) since the rates of the proton transfer reactions considered here are nearly diffusion controlled. It should be stressed that the breakdown of any of these three assumptions would result in an incorrect measure of the acidity of one or both of the isomeric 2-butenes.[14] In this instance we would not have expected the consistent results illustrated in Chart I, i.e., that the acidity of each alkene, independently determined relative to each of two standards (water and propene) would, within our experimental error, be the same.