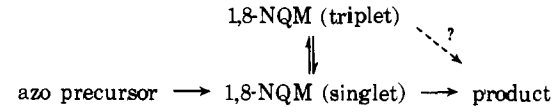
of **1** and **2**, this does not imply that the final products isolated from these reactions necessarily arise from these triplet states. On the contrary, all the present and previous[1] results are consistent with the singlet states of **1** and **2** isomerizing to the observed products as illustrated below.[14,15]



One of the outstanding questions remaining in this research is the actual shape of the triplet and singlet states of **1** and **2**. A match up of the absorption spectra of **1** and **2** with those calculated by PPP-CI may indicate the geometry of these species. Experiments along these lines are currently being explored.

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(1) C. R. Watson, Jr., R. M. Pagni, J. R. Dodd, and J. E. Bloor, *J. Am. Chem. Soc.*, **98**, 2551 (1976), and references cited therein.
(2) **1** was found to be conformationally mobile.[1] Since the diyl carbons are joined together by a $-CH_2CH_2-$ bridge, their motion must of necessity be synchronized. The diyl carbons of **2**, in addition to being unsubstituted, are not forced to rotate in a synchronous manner.
(3) L. A. Carpino, *J. Am. Chem. Soc.*, **85**, 2144 (1963).
(4) Control experiments demonstrated that the isomerization of **3** into **5** is photochemical and not thermal.
(5) M. Gisin and J. Wirz, *Helv. Chim. Acta*, **59**, 2273 (1976).
(6) **5** is not the percursor of **6** for photolysis of **5** under conditions where **3** led to **6** resulted in no reaction. **4** does yield **6** on π,π^* excitation.[5]
(7) Carpino has observed[3] the thermal conversion of **3** into **6**. 1,8-NQM is likely an intermediate in this reaction.
(8) An entirely different triplet was observed in THF–DME at 77 K (D = 0.0061 cm^{-1} and E = 0.000 43 cm^{-1}). We have no conclusive proof as to the identity of this triplet.
(9) Control experiments demonstrated that **5** was not a precursor to the triplet described in the text or in ref 8.
(10) C_6F_6 matrices are not completely transparent and we could not perform preparative irradiations of **3** in this medium at 77 K. Irradiation of **3** in ethanol at 77 K does yield **6**, however.[5]
(11) Power saturation can be a problem at these temperatures. Only those line intensities which did not show any power saturation were used in the Curie law study.
(12) R. Breslow, H. W. Chang, R. Hill, and E. Wasserman, *J. Am. Chem. Soc.*, **89**, 1112 (1967).
(13) Direct and benzophenone-sensitized photolysis of **3** in $CDCl_3$ in the probe of an NMR spectrometer afforded acenaphthene (**6**) but no CIDNP was observed in either case. This suggests that **2** has a singlet ground state with a triplet excited state greater in energy by at least a fraction of a calorie per mole. Attempts to observe CIDNP in the direct and benzophenone-sensitized photolysis of the azo precursor to **1** were also negative. S. Buchwalter and G. Closs, unpublished results.
(14) Although we do not know whether the triplet state or singlet state of **1** and **2** yields the observed products, the use of known photochemical data and group additive relations suggest that the conversion of **1** and **2** into the lowest triplet states of the products is endothermic.
(15) Our diyls behave quite differently than the bridged trimethylene methane observed by Berson and co-workers. See J. A. Berson, C. D. Duncan, G. C. O'Connell, and M. Platz, *J. Am. Chem. Soc.*, **98**, 2358 (1976), and earlier papers in the series. Our results and those of Berson suggest that the chemical behavior of diyls will be quite varied and will depend on whether the singlet or triplet is the ground state in addition to the energy gap between the two states.

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Selenation of *Escherichia coli* Mixed Transfer Ribonucleic Acids[1]

Sir:

It has been recently demonstrated that when *E. coli* is grown in a medium containing ^{75}Se as selenite or selenosulfate, part of the Se is metabolized to form 4-selenouridine in tRNA.[2,3] Wise and Townsend[4] observed that synthetic 4-selenouridine is very labile, and predicted that, if proper modifications are made in the current isolation procedures, one should be able to isolate selenopyrimidine nucleosides from certain tRNA species. Presumably, due to the lability of 4-selenouridine and the toxicity of Se to *E. coli*, it has been difficult to isolate selenated tRNA in quantity, and the identification of 4-selenouridine in the *E. coli* tRNA hydrolysate is based on the cochromatography of ^{75}Se radioactivity with authentic 4-selenouridine. In order to prepare selenated tRNA in quantity for biological studies, we decided to try to transform the 4-thiouridine in tRNA to 4-selenouridine.

We observed previously that 4-thiouridine in tRNA can be labeled with ^{35}S by the following sequence of reactions:

$$\text{tRNA(-SH)} \xrightarrow[\text{step 1}]{\text{CNBr}} \text{tRNA(-SCN)} \xrightarrow[\text{step 2}]{-^{35}\text{SH}} \text{tRNA(-}^{35}\text{SH)}$$

Study of the mechanism of reduction of 1-methyl-4-thiocyanatouracil by bisulfide revealed that the reaction proceeds to the extent of 70% by scission of the ring C–S bond and 30% by scission of the exocyclic C–S bond.[5] This finding led us to explore the possibility of transforming 4-thiouridine to selenouridine in tRNA following the same sequence of reactions, using -SeH instead of -SH in step 2. The feasibility of this reaction was first studied with the model compound 1-methyl-4-thiocyanatouracil synthesized by the method of Pal and Schmidt.[6] Conversion of 1-methyl-4-thiocyanatouracil to 1-methyl-4-selenouracil by NaHSe was followed spectrophotometrically in a cuvette purged with nitrogen. The cuvette was fitted with a rubber septum and air was replaced with nitrogen by syringe techniques.[7] The solution of NaHSe was introduced into the cuvette by a microliter syringe.

After NaHSe treatment, the 307.5-nm peak undergoes a bathochromic and hyperchromic shift. The pair of maximum and minimum 307.5- and 272-nm peaks is changed into 363 and 300 nm, respectively (Figure 1). This agrees with the spectrum of authentic 4-selenouridine (365 and 302 nm, respectively (Figure 2). 4-Selenouridine and 1-methyl-4-selenouracil are expected to have similar spectra. (Synthetic 4-selenouridine was a gift from Professor Leroy B. Townsend, University of Utah.)

Encouraged by these results we applied the sequence of reactions to *E. coli* mixed tRNA. One milliliter of aqueous (oxygen free) tRNA (A_{260} = 61, A_{340} = 1.15) was treated with 25 μL of 0.5 M phosphate buffer, pH 8, followed by 10 μL of

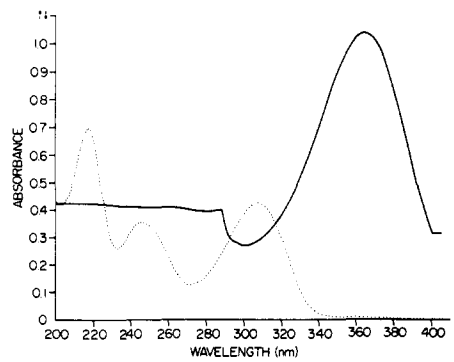


Figure 1. Ultraviolet absorption spectrum of 1-methyl-4-thiocyanatouracil before (···) and after treatment with NaHSe (—).

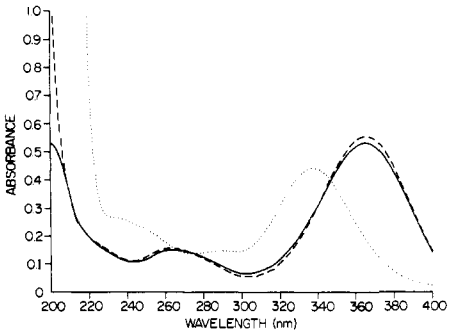


Figure 2. Ultraviolet absorption spectrum of synthetic 4-selenouridine in water (—), at pH 2(- - -), and at pH 12 (• • •).

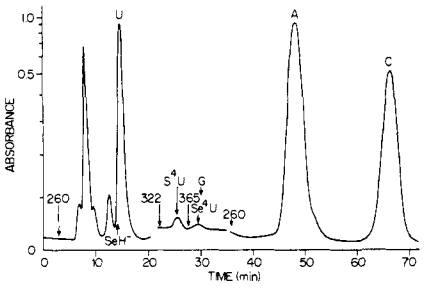
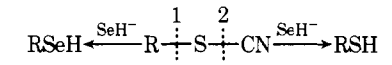


Figure 3. Chromatography of a hydrolysate of selenated tRNA on a column of Bio-Rad Aminex A-6 (cation exchanger), 17.5 × 0.63 cm. A 30-μL sample (≡2 A_{260} units) was injected into the column, maintained at 50 °C, and eluted with 0.4 M $HCOONH_4$–0.005 M S_2threitol, pH 4.5, at 0.28 mL/min (80 psi pressure). 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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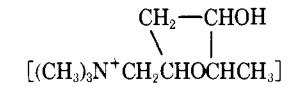
On the Conformational Changes of Acetylcholine and Some of Its Analogues

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