isomerizations of **1** and **2** must involve cyclopropane ring opened species. Since the thermal reaction undoubtedly occurs with conservation of spin, the thermally opened singlet intermediate[8] is likely to be **5** rather than **5a**, which presumably would be of considerably higher energy. It is significant that no quinone methides are produced thermally, which suggests that ground-state singlet 1,3 diradicals such as **5** may close but do not rearrange.[9] From the observation that neither quinone methide **3** nor **4** are formed during the addition of triplet carbene **6** to *cis*-2-butene,[10] it is obvious that triplet **5** also closes but does not rearrange. Since neither 350-mμ light-induced nor photosensitized[3] *cis–trans* isomerization of **1** or **2** affords quinone methides, triplet **5** could be an intermediate in either or both of these reactions. However, the possibility that the species thus obtained is **5a** rather than **5** cannot be presently excluded. While species **5a** could suffice for *cis–trans* isomerization, it is not a likely precursor of methyl or hydrogen migration.

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[8] Whether this intermediate should be considered as diradical or as dipolar is debatable, although the former certainly seems preferable in terms of energetics and observed chemistry.

[9] There are numerous instances in the literature where singlet 1,3 diradicals, generated either thermally or photochemically, have been thought to rearrange to afford propenes. For example, see D. I. Schuster and I. Krull, *J. Mol. Photochem.*, in press; E. T. McBee, J. A. Bosoms, and C. J. Morton, *J. Org. Chem.*, 31, 768 (1966); R. J. Crawford and A. Mishra, *J. Amer. Chem. Soc.*, **88**, 3963 (1966), and references therein.

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W. H. Pirkle, S. G. Smith, G. F. Koser
Department of Chemistry and Chemical Engineering
University of Illinois, Urbana, Illinois 61801

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Oligonucleotide Studies. VII. Optical Rotatory Dispersion of Adenylyl-(3′-5′)-4-thiouridine and Guanylyl-(3′-5′)-4-thiouridine[1]

Sir:

Some new findings on the optical properties of the two 4-thiouridine-containing dinucleoside monophosphates, adenylyl-(3′-5′)-4-thiouridine (ApTU) and guanylyl-(3′-5′)-4-thiouridine (GpTU), are presented. These results are believed to be useful for the sequence analysis of 4-thiouridine-containing oligonucleotides obtainable from the pancreatic ribonuclease (RNase IA; EC 2.7.7.16) treatment of *E. coli* transfer ribonucleic acids (tRNA) as well as for understanding the conformation of such oligonucleotide segments in those tRNA molecules.

A study of these two particular dinucleoside monophosphates of eight possible 4-thiouridine-containing dimers made up of 4-thiouridine and the four major nucleotides is now warranted by the recurrence of a - - - - PuTU - - - - and/or - - - - PuPuTU - - - - sequence in *E. coli* tRNA as recently found by Lipsett.[2] Furthermore, the current problem of determining the nucleotide sequence of some 4-thiouridine-containing species of tRNA, which is being actively pursued in many laboratories, prompts us to report our results on the ultraviolet absorption and optical rotatory dispersion of ApTU and GpTU.

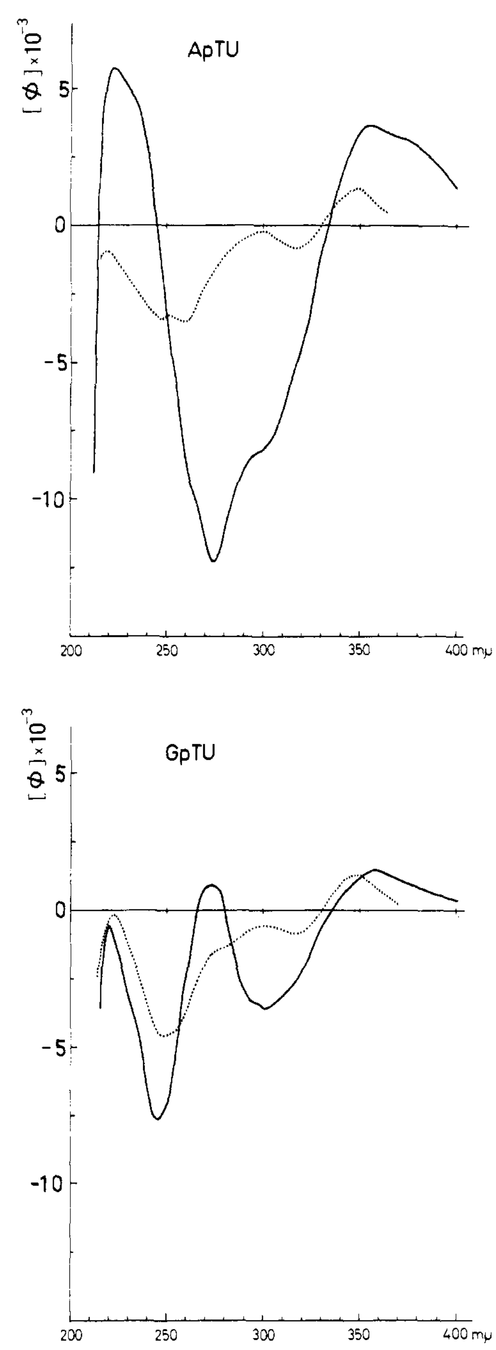

Figure 1. The ORD spectra of ApTU and GpTU at pH 5.8, 25°, and ionic strength 0.1 (—), and of their corresponding components, 5′-AMP + TU and 5′-GMP + TU (......). [ϕ] is molecular rotation per residue, defined as [ϕ] = [M]/2.

ApTU and GpTU were respectively prepared with the aid of a synthetic reaction[3] by RNase U$_2$ from *Ustilago sphaerogena* and RNase N$_1$ from *Neurospora crassa*, both of which were discovered in our laboratories.[4,5]

[1] Part VI in this series is by S. Aoyagi and Y. Inoue, *J. Biochem.* (Tokyo), 64, 603 (1968).

[2] M. N. Lipsett, Abstracts, Symposium on Transfer of Genetic Information in Protein Synthesis, New York, N. Y., June 7–8, 1968.

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Table I. Per Cent Hypochromicity at 260 and 330 mμ and $|[M]_{dimer} - \Sigma[M]_{monomer}|_{max}$ at pH 5.8 and Ionic Strength 0.1 (25°)

Dimer	$\lvert[M]_{dimer} - \Sigma[M]_{monomer}\rvert_{max}$[a]	h, %[b] 260 mμ	h, %[b] 330 mμ
ApTU	2.14×10^4 (at 276 mμ)	11.4	14.0
GpTU	0.66×10^4 (at 242 mμ)	3.6	14.3
	0.54×10^4 (at 270 mμ)		

[a] $|[M]_{dimer} - \Sigma[M]_{monomer}|_{max} = |[M]_{XpTU} - ([M]_{5'\text{-}XMP} + [M]_{TU})|_{max}$. [b] $h = 100[(A_{XMP} + A_{TU} - A_{XpTU}/(A_{XMP} + A_{TU})]$; the extinction coefficients used are ε_{260} 1.54×10^4, 1.16×10^4, and 3.7×10^3 for AMP, GMP, and 4-thiouridine, respectively, and ε_{330} 2.06×10^4 for 4-thiouridine.

Table II. Ultraviolet Absorption Characteristics of ApTU, GpTU, and Their Disulfides[a]

Compd	pH	λ_{max}, mμ ($\varepsilon \times 10^{-3}$)	λ_{min}, mμ ($\varepsilon \times 10^{-3}$)	λ_{max}, mμ ($\varepsilon \times 10^{-3}$)
ApTU	5.8	259 (16.9)	289 (3.4)	333 (17.9)
	10.0	261 (17.7)	287 (7.0)	318 (17.7)
$(ApTU\text{-}S\text{-})_2$	6.2	261 (17.3)	290 (5.0)	318 (16.8)
GpTU	6.0	252 (17.3)	297 (8.4)	333 (17.9)
	10.0	255 (16.1)	294 (9.9)	318 (18.0)
$(GpTU\text{-}S\text{-})_2$	6.0		299 (7.7)	317 (19.1)
4-Thiouridine (TU)	6.0	245 (4.6)	280 (2.3)	330 (20.6)
$(TU\text{-}S\text{-})_2$	6.0	261 (12.3)	280 (10.0)	310 (19.7)

[a] $(ApTU\text{-}S\text{-})_2$ = bis[adenylyl-(3'-5')-4-deoxy-4-uridinyl] disulfide; $(GpTU\text{-}S\text{-})_2$ = bis[guanylyl-(3'-5')-4-deoxy-4-uridinyl] disulfide; $(TU\text{-}S\text{-})_2$ = bis(4-deoxy-4-uridinyl) disulfide.

The extinction coefficients for ApTU and GpTU were determined by hydrolyzing them with RNase T_2 (EC 2.7.7.17) and evaluating the concentration, using the extinction coefficients of the component monomers. The hypochromicity measurements were made twice for each sample. The results are summarized in Table I together with the values of $|[M]_{dimer} - \sum[M]_{monomer}|_{max}$, which are taken as the interaction parameter for base–base stacking interactions present in dinucleoside monophosphates.[6-9]

The ultraviolet absorption spectra of ApTU and GpTU are not identical with those of the sum of their respective component monomers. The differences are not only the extinction coefficients of the dinucleoside monophosphates at their longest wavelength band but also a 3-mμ bathochromic shift, relative to the 4-thiouridine peak, which has been attributed to base–base interactions operating in each of the dimers. The absorption extrema are those listed in Table II. The presently found red shift of 3 mμ is coincident with a longer wavelength shift observed for a native *E. coli* tRNA,[10] which exhibits a maximum at 336 mμ; but, in urea solution, the peak is shifted to 333 mμ. Hypsochromic shifts previously observed at a monomer level[11-13] and at a polynucleotide level[14,15] were also

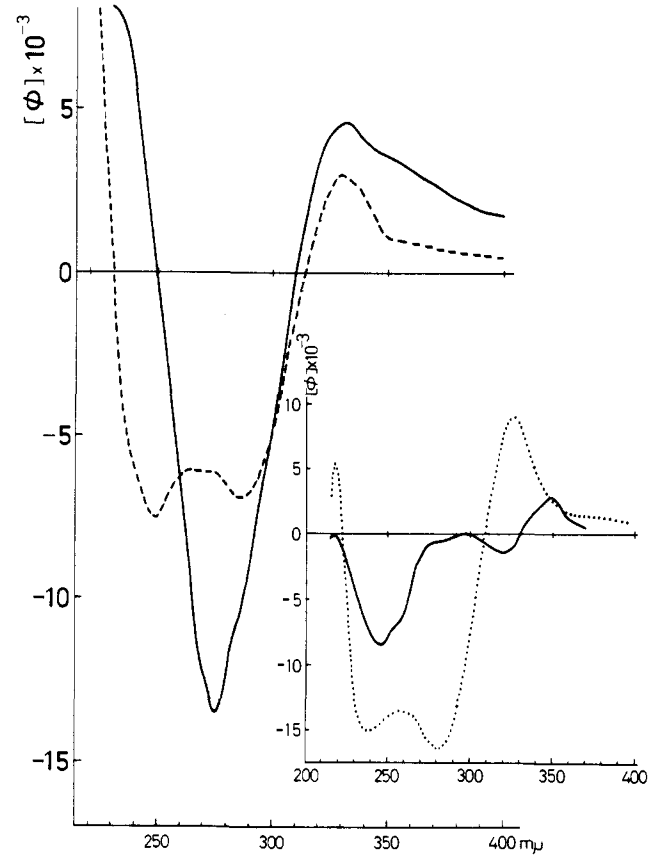

Figure 2. The ORD spectra of $[ApTU\text{-}S\text{-}]_2$ (—) and $[GpTU\text{-}S\text{-}]_2$ (------) at pH 7. [ϕ] is molecular rotation per residue, defined as [ϕ] = [M]/4 for the disulfides of ApTU and GpTU and [ϕ] = [M]/2 for bis(4-deoxy-4-uridinyl) disulfide. Insert: The ORD of 4-thiouridine (—) and its disulfide (......) at pH 6.

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noticed either on going from the dinucleoside monophosphates to the corresponding disulfides or on ionization to the anionic form of the 4-thiouridine residue. ApTU and GpTU were converted to the corresponding disulfides by treating the aqueous solutions at pH 5–6 with 1.0–1.1 equiv of a standardized dilute iodine solution (10^{-3} M I_2–KI) in an ice bath. This oxidation procedure is substantially the same as those reported by Lipsett and Doctor.[14] Disulfide formation can be followed spectrophotometrically (see Table II) and unambiguously demonstrated by enzymatic hydrolysis with RNase T_2.

Like other dinucleoside monophosphates, ApTU and GpTU exhibit the ORD spectra[16] characteristic of dissymmetric conformations formed by strong base–base interactions. Data at pH 5.8 shown in Figure 1 should be useful for the sequence determination of a 4-thiouridine-containing oligonucleotide obtainable on the RNase IA digestion of *E. coli* tRNA, when combined with the known ORD data of other dinucleoside monophosphates,[17] since nearest neighbor calculations of a given oligonucleotide have been proven to provide a sequence-dependent feature of the ORD pattern in fair agreement with experimental curves.[6-9]

The frequency shifts of the ORD spectra of the disulfides relative to the dinucleoside monophosphates are consistent with the uv absorption behaviors observed during the same structural transformations (Figure 2). However, if one remembers a large increase in the rotatory power for the transformation of 4-thiouridine to its disulfide,[13] the extent to which the magnitudes of the rotation per residue were altered on going from ApTU and GpTU to their disulfides indicates that electrostatic and geometric situations in the disulfides might be expected to force a disruption of the stacking interactions.

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(16) The ORD spectra were measured at 25° in a 1-cm quartz cell placed in a water-jacketed housing for temperature control with a Jasco Model ORD/UV-5 autorecording spectropolarimeter. Rotations are expressed either in molar rotation, [M], or in molar rotation per residue, [ϕ], defined as

$$[\phi] = \frac{[M]}{n} = \frac{100\alpha\varepsilon_\lambda}{n A_\lambda}$$

where n is the number of base residues in a molecule (see captions for Figures 1 and 2), α is the observed rotation in degrees, and A_λ and ε_λ are the absorbancy of a sample solution and molar extinction coefficient at a given wavelength.

(17) The ORD data for guanylate dimer at 25°, ionic strength 0.1, have been supplemented and will be reported in part VIII in this series.

(18) To whom correspondence should be addressed.

Shinkichi Irie, Fujio Egami[18]
Department of Biophysics and Biochemistry
Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan

Yasuo Inoue
Department of Chemistry, Tohoku University
Sendai, Japan

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Book Reviews

Electrochemistry. By C. W. Davies, D.Sc., F.R.I.C., Senior Research Fellow, University of Surrey, Professor Emeritus, University of Wales. Philosophical Library, Inc., 15 East 40th St., New York, N. Y. 1968. xii + 234 pp. 16.5 × 23 cm. $20.00.

Although there is no dearth of texts in English on electrochemistry, the subject is rarely taught as a separate discipline in American universities. The present book is intended to serve as an introductory text. The author is no newcomer to the field and has contributed considerably to the interpretation of conductance in aqueous medium of electrolytes composed of higher valent ions and to ion association (see C. W. Davies, "Conductivity of Solutions," 1930, and "Ion Association," 1962). The organization of the new text is conventional, starting with a brief introduction of the theory of electrolytic dissociation, followed by chapters on thermodynamics (only 4.5 pp), conductivity, ion transport, activity coefficients, incomplete dissociation, acid-base equilibria, solubility, electrical double layer, colloids, emf, electrode potentials, concentration cells, pH, electrolysis, overvoltage, kinetics of electrode processes, nonaqueous solutions, and fuel cells. The style and treatment are clear and exact. There are no literature references in the text, but at the end of several chapters is a brief addendum with "recommended reading." These lists are not very selective. It is difficult to define in an elementary text the limitations in the treatment of the various topics. Many teachers would appreciate a more detailed discussion of Stoke's law (p 49) and of modern views on the structure of water. The chapters on nonaqueous solutions and ionic melts are rather sketchy and not quite up-to-date. The statement that the glass electrode does not behave satisfactorily in nonaqueous media is—to say the least—misleading. These critical comments are of a relatively minor nature as compared to the virtues of the book. If the price were not prohibitive, it would serve well as an introductory text for undergraduates.

In a foreword L. L. Shreir states that the book should also provide a suitable introduction to a series of "International Monographs" of which the present book is the first volume.

I. M. Kolthoff
School of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455

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