Jardetzky, C. D. (1962), *J. Amer. Chem. Soc. 84,* 62.

Kreishman, G. P., and Chan, S. (1971), *Biopolymers 10,* 159.

Lakshiminarayanan, A. V., and Sasisekharan, V. (1969), *Biopolymers 8,* 489.

Lapper, R. D., and Smith, I. C. P. (1973), *J. Amer. Chem. Soc. 95,* 2880.

Lavallee, D. K., and Coulter, C. L. (1973), *J. Amer. Chem. Soc. 95,* 576.

Mantsch, H. H., and Smith, I. C. P. (1972), *Biochem. Biophys. Res. Commun. 46,* 808.

Perahia, D., Saran, A., and Pullman, B. (1973), in Conformation of Biological Molecules and Polymers, Proceedings of the Vth Jerusalem Symposium, Bergmann, E. D., and Pullman, B., Ed., New York, N.Y., Academic Press, p 225.

Pullman, B., Perahia, D., and Saran, A. (1972), *Biochim. Biophys. Acta 269,* 1.

Remin, M., and Shugar, D. (1972), *Biochem. Biophys. Res. Commun. 48,* 636.

Rosenburg, J. M., Seeman, N. C., Kim, J. J. P., Suddath, F. L., Nicholas, H. B., and Rich, A. (1973), *Nature (London) 243,* 150.

Rubin, J., Brennan, T., and Sundaralingam, M. (1972), *Biochemistry 11,* 3112.

Saran, A., and Govil, G. (1971), *J. Theor. Biol. 33,* 407.

Sarma, R. H., Mynott, R. J., Wood, D. J., and Hruska, F. E. (1973), *J. Amer. Chem. Soc. 95,* 6457.

Sasisekharan, V. (1973), *in* Conformation of Biological Molecules and Polymers, Proceedings of the Vth Jerusalem Symposium, Bergmann, E. D., and Pullman, B., Ed., New York, N.Y., Academic Press, p 247.

Schleich, T., Blackburn, B. J., Lapper, R. D., and Smith, I. C. P. (1972), *Biochemistry 11,* 137.

Schweizer, M. P., and Robins, R. K. (1973), *in* Conformations of Biological Molecules and Polymers, Proceedings of the Vth Jerusalem Symposium, Bergmann, E. D., and Pullman, B., Ed., New York, N.Y., Academic Press, p 329.

Shefter, E., Barlow, M., Sparks, R. A., and Trueblood, K. N. (1969), *Acta Crystallogr., Sect. B 25,* 895.

Smith, I. C. P., Mantsch, H. H., Lapper, R. D., Deslauriers, R., and Schleich, T. (1973), *in* Conformations of Biological Molecules and Polymers, Proceedings of the Vth Jerusalem Symposium, Bergmann, E. D., and Pullman, B., Ed., New York, N.Y., Academic Press, p 381.

Suck, D., Manor, P. C., Germain, G., Schwalbe, C. H., Weimann, G., and Saenger, W. (1973), *Nature (London), New Biol. 246,* 161.

Sundaralingam, M. (1969), *Biopolymers 7,* 821.

Sundaralingam, M. (1973), *in* Conformations of Biological Molecules and Polymers, Proceedings of the Vth Jerusalem Symposium, Bergmann, E. D., and Pullman, B., Ed., New York, N.Y., Academic Press, p 381.

Tran-Dinh Son, and Chachaty, C. (1973), *Biochim. Biophys. Acta 335,* 1.

Tran-Dinh Son, Guschlbauer, W., and Guéron, M. (1972), *J. Amer. Chem. Soc. 94,* 7903.

Tran-Dinh Son, Thiery, J., Guschlbauer, W., and Dunand, J.-J. (1972), *Biochim. Biophys. Acta 281,* 289.

Wood, D. J., Hruska, F. E., Mynott, R. J., and Sarma, R. H. (1973), *Can. J. Chem. 51,* 2571.

Wood, D. J., Mynott, R. J., Hruska, F. E., and Sarma, R. H. (1973), *Fed. Eur. Biochem. Soc. 34,* 323.

Hydrogen Bonding Abilities of 2,4-Dithiouridine Derivatives[†]

J. Pitha* and K. H. Scheit

ABSTRACT: The base pairing ability of a di-2,4-thiouridine derivative was studied in carbon tetrachloride solutions by the methods of infrared spectroscopy. The strength of the association by hydrogen bonding was found to decrease in the following order: adenine-uracil, adenine-di-2,4-thiouracil, uracil-uracil, and di-2,4-thiouracil-di-2,4,-thiouracil. These findings contrast with the previously demonstrated fact that poly(s^2s^4U) is strongly self-associated and does not form a complex with poly(A). To correlate these results, it is proposed that long range stabilizing forces are acting between the di-2,4-thiouracil residues in polynucleotide chains. This assumption also explains the existence of an ordered structure in the alternating copolymer poly(s^2s^4U-A).

The stability of polynucleotide structures and their complexes depends on a number of different molecular interactions. The importance of hydrogen bonding was recognized earliest; the vertical interaction between bases, the restricted rotation around the chemical bonds in the polymer backbone, and ion-ion interactions were fully recognized and studied later (Felsenfeld and Miles, 1967; Inners and Felsenfeld, 1970; Pullman and Pullman, 1969; Eichhorn, 1973, and references therein). Recently one of us reported an interesting change in the properties of poly(U) occurring when the oxygens of uracil are substituted by sulfur atoms. Poly(s^2s^4U),[1] unlike poly(U) or poly(s^4U), forms a very sta-

---

[†] From the Laboratory of Molecular Aging, National Institutes of Health, National Institute of Child Health and Human Development, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224, and Max-Planck-Institut für Biophysikalische Chemie, Karl-Friedrich-Bonhoeffer-Institut, Abteilung Molekulare Biologie, Göttingen. *Received March 25, 1974.*

[1] Abbreviations used are: $Ac_3s^2s^4U$, tri-2′,3′,5′-*O*-acetyl-2,4-dithiouridine; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid). Position of thio substitution is noted by a numerical index; ν(XH), stretching vibration of X–H bond.

ble structured complex in solutions and does not interact with poly(A) (Bähr *et al.*, 1973). The possibility of using measurement of the association of the substituted base derivatives in nonpolar solvent for assessing the role of hydrogen bonding in this surprising change of polynucleotide properties prompted us to the present infrared study.

Materials and Methods

9-Stearyladenine and 1-stearyluracil were prepared following the procedure of Browne *et al.* (1968); preparation of the $Ac_3s^2s^4U$ was described previously (Veda *et al.*, 1968); the other compounds used were purchased from Sigma Co., St. Louis, Mo., and Cyclo Chemical Co., Los Angeles, Calif.

Infrared spectra were measured on a Beckman IR12 spectrometer. The instrument was constantly flushed by air dried with a pneumatic dryer; furthermore, the air in the cell compartment was pumped through a silica gel drying column. The wave number scale of the instrument was calibrated using ammonia and polystyrene; the transmittance scale was rechecked periodically using a Beckman rotating sector of 25.1% transmission. To suppress interferences caused by humidity, the solutions after preparation were stored overnight in volumetric flasks over P_2O_5 in a desiccator and then brought precisely to the desired volume using similarly dried solvent. For the measurements infrasil cells 2, 10, and 40 mm long were used. Water at 17.5° was circulated through the cell holder; this was sufficient to maintain a solution temperature of 20 ± 0.5° as measured directly in 1-cm cells. The spectra of all solutions were recorded twice. For studies of base association equilibria, deuteriochloroform (Hamlin *et al.*, 1965), chloroform (Pitha *et al.*, 1966; Kyogoku *et al.*, 1967), or carbon tetrachloride (Küchler and Derkosch, 1966) were used as solvents. The stability and high transparency of CCl_4 led us to use the last solvent. Küchler and Derkosch studied the system of 5′-*O*-trityl-2′,3′-*O*-isopropylideneuracil and 5′-*O*-trityl-2′,3′-*O*-isopropylideneadenosine under the same conditions. To establish a general correlation with their data we made measurements of the system 1-stearyluracil and 5′-*O*-acetyl-2′,3′-*O*-isopropylideneadenosine. There is approximate agreement in the wave numbers of the absorption maxima of the complex: in our experiments we found 3492, 3324, and 3258 cm^{-1}; Küchler and Derkosch found 3485, 3325, and 3255 cm^{-1}. Our association constant was found to be somewhat higher (800 l./mol^{-1}) than that studied by Küchler and Derkosch (590 l./mol^{-1}).

Stearyl derivatives of bases dissolve very easily in hot organic solvents and precipitate from the concentrated solution only after prolonged standing at room temperature. The precipitation of these compounds may go through some micellar stages, a process which would considerably complicate their associative equilibria. Therefore, the stearyl derivatives were used only to reconfirm the results obtained with other derivatives.

Results

$Ac_3s^2s^4U$ in dilute carbon tetrachloride solution has two absorption bands in the ν(X–H) region in addition to the C–H absorption. One at 3536 cm^{-1} is an overtone of the ν(C=O) of the acetyl groups; the other at 3364 cm^{-1} is ν(NH) of the monomeric specie. With increasing concentration the apparent extinction coefficient of this band decreases and a new band appears at 3224 cm^{-1} as shown in Figure 1. $Ac_3s^2s^4U$ is apparently much less associated than a similar uridine derivative (Küchler and Derkosch, 1966). In the self-association of the latter the "closed" dimer (having both nitrogen bonded hydrogens engaged in hydrogen bonding) was deduced to be the main species: no absorption bands indicating the presence of an "open" dimer was detected and data fit the proper equilibrium (Küchler and Derkosch, 1966). Within the limitation imposed by the smaller self-association and by the low solubility no discernible qualitative difference was found between the self-association of the uridine and of the di-2,4-thiouridine derivatives; in Figure 1 curves corresponding to a dimerization constant of 10 l. mol^{-1} were drawn. In crystals of s^2s^4Ura and s^2s^4U, symmetrical N_3–H · S bonds are formed similar to the one found in uracil derivatives crystals (Shefter and Mautner, 1967; Lin and Sundaralingam, 1971; Saenger and Suck, 1971).

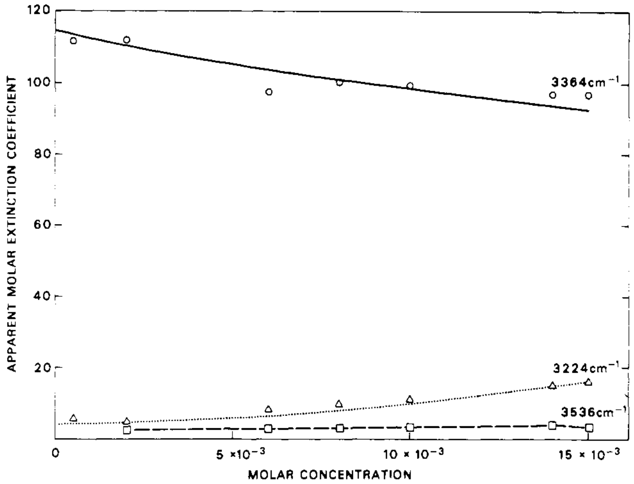

FIGURE 1: Dependence of the apparent molar extinction coefficients on concentration of $Ac_3s^2s^4U$ in carbon tetrachloride at 20°. Band at 3364 cm^{-1} is ν(NH) of the monomer, at 3224 cm^{-1} is ν(NH) of the dimeric species. The corresponding lines are the ones calculated for the dimerization with k_{dim} = 10 l. mol^{-1} [obtained by initial slope method—Liddel and Becker, 1957]. The band at 3536 cm^{-1} is an overtone of ν(C=O) of the acetyl groups and the corresponding extinction coefficient does not vary with concentration.

$Ac_3s^2s^4U$ forms a hydrogen-bonded complex with adenosine derivatives; 9-stearyladenine was used (see Figure 2). Absorption bands of the complex are at 3494, 3324, 3258, and 3170 cm^{-1} (upper panel); a comparison of the spectra of the complex with that of 9-stearyladenine alone (lower panel) shows that the association between the derivatives of dithiouridine and adenine is stronger than the self-association of the latter. A similar situation was found for the combination of 5′-*O*-acetyl-2′,3′-*O*-isopropylideneadenosine and $Ac_3s^2s^4U$. These results and results of a Job plot (Job, 1928, Figure 3) demonstrate that both the amino group of adenosine derivatives (absorption bands 3534 and 3421 cm^{-1} or 3536 and 3422 cm^{-1}, respectively) and the N–H bond of dithiouridine derivative (3364 cm^{-1}) are involved in hydrogen bonding. Comparison of the published spectra shows that the association between di-2,4-thiouridine and adenine derivatives is weaker than that of uridine and adenine derivatives (a value of 590 l. mol^{-1} for this association was obtained by Küchler and Derkosch, 1966) and stronger than the self-association of adenine derivatives (80 l. mol^{-1}, Küchler and Derkosch, 1966). In order to obtain an approximate value for the association constant, the stoichiometry of the complex must be known. The results of Figure 3 suggest a 1:1 ratio but the magnitude of the observed

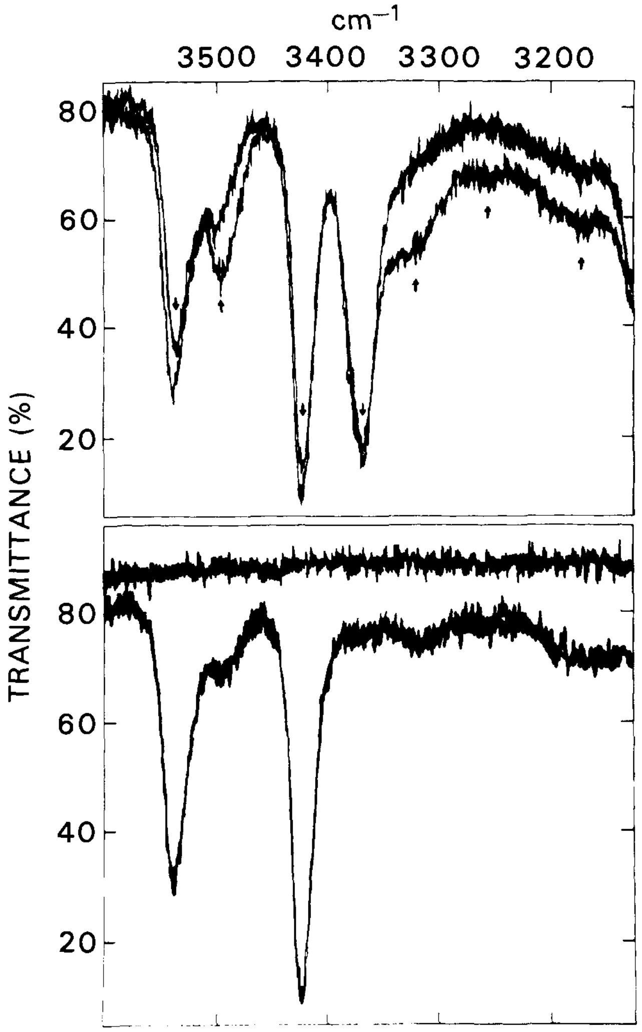

FIGURE 2: Upper panel: Recording of the infrared spectrum of a mixture of 9-stearyladenine and $Ac_3s^2s^4U$ (1:1 ratio) each at 5.34 mM in a 1-cm cell (noted by arrows) and at 5.34/4 mM (1.34 mM) in a 4-cm cell. Lower panel: Infrared spectrum of 9-stearyladenine at 5.34 mM in a 1-cm cell. The solvent-solvent base line, common for both 4- and 1-cm cells, is given in the lower panel.

changes does not allow a definite conclusion. If a 1:1 stoichiometry is assumed for the complex, a value of 240 l. mol^{-1} is obtained for the association constant of di-2,4-thiouridine with adenine derivatives. In this calculation absorbance at 3536 cm^{-1} of 5′-*O*-acetyl-2′,3′-*O*-isopropylideneadenosine was used and corrections were introduced for both the band overlap (Seshadri and Jones, 1963) and

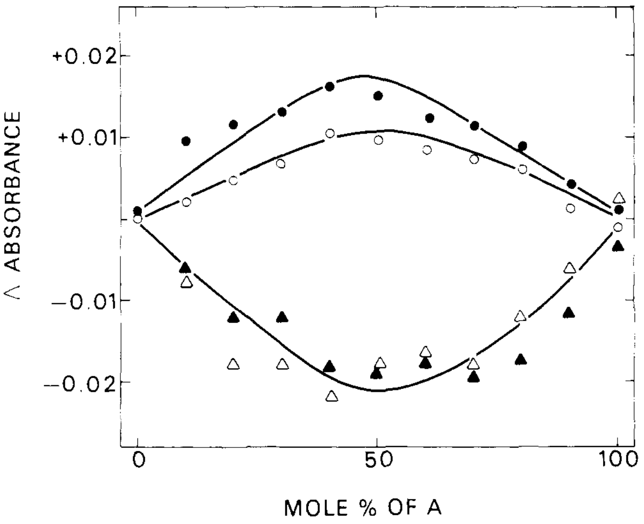

FIGURE 3: The continuous variation study of the $Ac_3s^2s^4U$ and 5′-*O*-acetyl-2′,3′-*O*-isopropylideneadenosine system (1-cm cell, 20°, carbon tetrachloride, 2 mM total concentration). Change in absorbance at 3495 cm^{-1} (●), at 3324 cm^{-1} (○), at 3536 cm^{-1} (▲), and at 3365 cm^{-1} (△).

the dimerization of the adenine derivative (Kyogoku *et al.*, 1967; Küchler and Derkosch, 1966).

Discussion

In a number of cases the strength of hydrogen bonding between bases correlates at least qualitatively with the stability of the corresponding helical complexes. Thus the guanosine · cytosine or 2,6-diaminopurine · uracil base pairs are more stable than the uracil · adenine pair. With the corresponding polynucleotides we find the same sequence poly(G) · poly(C) and poly(n^2A) · polyU complexes are more stable than poly(A) · poly(U) (Felsenfeld and Miles, 1967 and references therein).

Study of the sulfur analogs shows a somewhat different situation; pertinent results are summarized in Table I. In nonpolar solution the self-association of dithio analog by base pairing is by an order of magnitude weaker than that of uracil. This is in accord with the previous finding that hydrogen bonds in which sulfur atoms function as proton acceptors are usually weaker than similar bonds where N or O atoms act as acceptors (Donohue, 1969; Shefter and Mautner, 1967). As the dithio analog is more acidic than uracil (by 1.8 pK_a units) the observed decrease in association must be due to the lower affinity of the sulfur atoms for accepting H bonds. The base pairing between the dithio analog

Table I: The Relative Strength of Interactions.

Nucleoside derivatives in nonpolar solution	$s^2s^4U \cdot s^2s^4 < U \cdot U < s^2s^4U \cdot A < U \cdot A$ (10)[a] (110)[a] (240)[a] (590)[a]
Homopolymers in aqueous solution	poly(s^2s^4U) · poly(A) < poly(U) · poly(U) < (not formed) (10)[b] poly(U) · poly(A) < poly(s^2s^4U) · poly(s^2s^4U) (56)[b] (90)[b]
Alternating copolymers in aqueous solution	poly(A · U) ≅ poly(A · s^2s^4U) (65)[c] (61)[c]

[a] Approximate association constants [l. mol^{-1}] of the substituted nucleosides at 20° in carbon tetrachloride solution; data: this work and Küchler and Derkosch (1966). [b] Melting temperature of the complex in 0.1 M NaCl, pH 7. [c] Melting temperature of the structure in 0.01 M NaCl, pH 7.

and adenine derivative is decreased to about half of that for derivatives of uracil and adenine. Apparently with the described increase of acidity the strength of the hydrogen bond between the N_3–H group of uracil and adenine nitrogen also increases, which compensates for the weaker bond between the amino group of adenine and sulfur atom of the analog.

The observed stability sequence is reversed on the polynucleotide level. The insertion of the weakest observed base pair $s^2s^4U \cdot s^2s^4U$ into the polynucleotide backbone produces a very stable structure, which according to some evidence may have double helical form (Bähr *et al.*, 1973). The stability of this structure is apparently so high that no complex is formed between the poly(s^2s^4U) and poly(A). The present results show that the instability of the complex is not caused by the low stability of the $s^2s^4U \cdot A$ base pair. The stability of the U · U pair in nonpolar solution (Table I) is lower and nevertheless poly(rU) forms a double helical structure in aqueous solution. Further support of such reasoning comes also from the study of alternating copolymer complexes—the structured form of poly(s^2s^4U-A) is nearly as stable as that of poly(U-A) and thus the base pair of $s^2s^4U \cdot A$ apparently can exist on a polynucleotide level in that case (K. H. Scheit, unpublished results).

The observed phenomena can be explained on the basis of strong interaction between the s^2s^4U bases which stabilize the helical structures. Such forces must manifest themselves not only between the stacked bases, but also at longer range and notably even between the bases located in opposing strands. Thus the hypothetical complex poly(s^2s^4U) · poly(A) dissociates into free poly(A) and poly(s^2s^4U) · poly(s^2s^4U), as the latter complex is stabilized by forces acting between both strands; apparently hydrogen bonding forces are not strong enough to prevent such a reaction. In the association of alternating copolymer poly(s^2s^4U-A) these forces act in the same direction and the structure is sustained.

A somewhat similar situation may be found in halogenated polynucleotides. Unpublished data of Shoup, Miles, and Becker (Howard *et al.*, 1969) show that base pairing between inosine and cytidine derivatives (as measured in dimethylformamide) is slightly stronger than that between inosine and 5-bromocytidine derivatives. On the other hand, the complex poly(rC) · poly(rI) is less stable than the complex between poly-5-bromocytidylate and polyinosinate (Michelson and Monny, 1967; Howard *et al.*, 1969; Folayan and Hutchinson, 1974).

Several intermolecular interactions may contribute to the described stabilization of helical structures. With halogenated polynucleotides, the concomitant increase in polarizability may be the main factor for the helix stabilizing forces (Howard *et al.*, 1969; Folayan and Hutchinson, 1974). With the sulfur analogs the situation may be similar. An increase in polarizability strengthens both monopole-induced dipole and dispersion interactions; the former is inversely proportional to the third power of intermolecular distances, the latter to the sixth power (Pullman and Pullman, 1969).

The importance of such long range interactions has been recognized, and its existence is documented by extensive experimental studies (Felsenfeld and Miles, 1967; Pullman and Pullman, 1969; Ts'o, 1968; Morikawa *et al.*, 1973). The results described here indicate that in the field of nucleic acids this interaction with at least some analogs can become a dominant force in determining which of the possible complexes will be formed.

Acknowledgment

We thank Dr. G. L. Eichhorn for his interest and discussion and Mr. G. D. Press for the assistance with the measurements of spectra.

References

Bähr, W., Faerber, P., and Scheit, K. H. (1973), *Eur. J. Biochem. 33,* 535.

Browne, D. T., Eisinger, J., and Leonard, N. J. (1968), *J. Amer. Chem. Soc. 90,* 7302.

Donohue, J. (1969), *J. Mol. Biol. 45,* 231.

Eichhorn G. L. (1973), *in* Inorganic Biochemistry, Eichhorn, G. L., Ed. Amsterdam, Elsevier, p 1210.

Felsenfeld, G., and Miles, H. T. (1967), *Annu. Rev. Biochem. 36,* 407.

Folayan, J. D., and Hutchinson, D. W. (1974), *Biochim. Biophys. Acta 340,* 194.

Hamlin, R. M. Jr., Lord, R. C., and Rich, A. (1965), *Science 148,* 1734.

Howard, F. B., Frazier, J., and Miles, H. T. (1969), *J. Biol. Chem. 244,* 1291.

Inners, L. D., and Felsenfeld, G. (1970), *J. Mol. Biol. 50,* 373.

Job, P. (1928), *Ann. Chim.* (*Paris*) *9,* 113.

Küchler, E., and Derkosch, J. (1966), *Z. Naturforsch. B 21,* 209.

Kyogoku, Y., Lord, R. C., and Rich, A. (1967), *J. Amer. Chem. Soc. 89,* 496.

Liddel, U., and Becker, E. D. (1957), *Spectrochim. Acta 10,* 70.

Lin, G. H., and Sundaralingam, M. (1971), *Acta Crystallogr., Sect. B 27,* 961.

Michelson, A. M., and Monny, C. (1967), *Biochim. Biophys. Acta 149,* 88.

Morikawa, K., Tsuboi, M., Takahashi, S., Kyogoku, Y., Mitsui, Y., Intaka, Y., and Thomas, G. J. (1973), *Biopolymers 12,* 799.

Pitha, J., Jones, R. N., and Pithova, P. (1966), *Can. J. Chem. 44,* 1045.

Pullman, B., and Pullman, A. (1969), *Prog. Nucleic Acid Res. Mol. Biol. 9,* 372.

Saenger, W., and Suck, D. (1971), *Acta Crystallogr., Sect. B 27,* 1178.

Seshadri, R. S., and Jones, R. N. (1963), *Spectrochim. Acta 19,* 1013.

Shefter, E., and Mautner, H. G. (1967), *J. Amer. Chem. Soc. 89,* 1249.

Ts'o, P. O. P. (1968), *in* Molecular Association in Biology, Pullman, B., Ed., Academic Press, p 39.

Veda, T., Tida, K., Ikeda, K., and Mizumo, Y. (1968), *Chem. Pharm. Bull. 16,* 1788.