indefinite self-association constants for deuterated and protiated *N*-methylacetamide) using the experimental values of Karasz and O'Reilly[7] for σ. In the former case $f = 0.5$ and in the latter case $f \simeq 1.0$. Upon examination of Figure 1 of Calvin, *et al.*,[3] which gives the variation of the specific rotation, $[\alpha]_D$, with temperature for hydrogen- and deuterium-containing solutions of PBG, it is seen that the deuterated sample is essentially all in the helical form at the transition temperature of the protiated sample (which corresponds to the temperature where $f = 0.5$). Similarly, the protiated sample is nearly all in the low-temperature random configuration at the temperature which corresponds to the transition temperature of the deuterated material. These results are consistent with mechanisms 3a–3c.

Thus, subject to other detailed interpretations of the thermally induced transition of PBG,[30] it would appear that the effect of deuteration on the transition temperature can be semiquantitatively understood in terms of an effect on peptide hydrogen bonds if use is made of the theory of Zimm and Bragg[27] and Applequist[28] and the effect of deuteration on the self-association of *N*-methylacetamide.

Acknowledgment. Preliminary aspects of this research were performed by Mr. Richard Jochman, National Science Foundation undergraduate research participant, during the summer of 1970.

(30) S. Hanlon and I. M. Klotz, *Biochemistry*, **4**, 37 (1965).

Effects of Sulfur Substituents on Base Stacking and Hydrogen Bonding. The Crystal Structure of 6-Thioguanosine Monohydrate[1]

Ulf Thewalt and Charles E. Bugg*

Contribution from the Institute of Dental Research and Laboratory of Molecular Biology, University of Alabama in Birmingham, Birmingham, Alabama 35233. *Received May 8, 1972*

Abstract: Crystals of 6-thioguanosine monohydrate are orthorhombic, space group $C222_1$, with $a = 12.272$ (1), $b = 6.904$ (1), and $c = 32.466$ (6) Å. X-Ray diffraction data were collected with an automated diffractometer. The crystal structure was solved by the heavy-atom method and refined by least squares to $R = 0.07$. The pattern of hydrogen bonding between bases is the same as that found in the crystal structures of guanine monohydrate and guanosine dihydrate. The bases form planar ribbons wherein adjacent thioguanine residues are related by screw axes and are joined by N(1)–H···N(7) and N(2)–H···S hydrogen bonds that have lengths of 2.96 and 3.27 Å, respectively. The parallel ribbons of bases are stacked with an interplanar separation of 3.4 Å. The base-stacking pattern involves a slight base overlap, with the sulfur atoms in close contact with purine rings of adjacent bases. The hydrogen-bonding and the base-stacking properties of thiopurines and thiopyrimidines are reviewed. The crystal structures of almost all other thio bases also involve hydrogen bonding to the sulfur substituents, and display stacking patterns in which the sulfur substituents are positioned in close contact with the ring systems of adjacent bases. It appears that replacement of carbonyl oxygen atoms by sulfur substituents affects hydrogen bond lengths, but has little additional effect on the solid state hydrogen-bonding patterns of purines and pyrimidines.

Sulfur derivatives of purines and pyrimidines occur as minor components of transfer ribonucleic acids (tRNA's).[2] Little is known about the function of these thio derivatives, but they probably play an important role in controlling the conformation of tRNA. Therefore, there has been considerable interest in the structural properties of thiopurines and thiopyrimidines.[3–20] Since secondary and tertiary structures of nucleic acids are largely controlled by base-stacking and hydrogen-bonding interactions, it is particularly important to understand the effects that sulfur substituents exert on these interactions.

In addition to the naturally occurring thio bases, a synthetic sulfur derivative, 6-thioguanine, can be incorporated into nucleic acids,[21–26] probably by sub-

(1) Supported by NIH Grants CA-12159, DE-02670, and RR-145. One of us (U. T.) thanks the Fulbright Commission for a travel grant.
(2) R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, N. Y., 1971.
(3) R. K. McMullan and M. Sundaralingam, *Biochem. Biophys. Res. Commun*, **43**, 1158 (1971).
(4) E. Shefter and T. I. Kalman, *ibid.*, **32**, 878 (1968).
(5) C. E. Bugg and U. Thewalt, *J. Amer. Chem. Soc.*, **92**, 7441 (1970).
(6) G. Hung-Yin Lin, M. Sundaralingam, and S. K. Arora, *ibid.*, **93**, 1235 (1971).
(7) J. Donohue, *J. Mol. Biol.*, **45**, 231 (1969).
(8) E. Shefter and H. G. Mautner, *J. Amer. Chem. Soc.*, **89**, 1249 (1967).
(9) E. Sletten, J. Sletten, and L. H. Jensen, *Acta Crystallogr., Sect. B*, **25**, 1330 (1969).
(10) G. M. Brown, *ibid., Sect. B*, **25**, 1338 (1969).
(11) E. Shefter, *J. Pharm. Sci.*, **57**, 1157 (1968).
(12) R. Srinivasan and R. Chandrasekharan, *Acta Crystallogr., Sect. B*, **24**, 1698 (1968).
(13) J. Donohue, *ibid., Sect. B*, **25**, 2418 (1969).
(14) S. Furberg and L. H. Jensen, *ibid., Sect. B*, **26**, 1260 (1970).
(15) G. Hung-Yin Lin and M. Sundaralingam, *ibid., Sect. B*, **27**, 961 (1971).
(16) W. Saenger and D. Suck, *ibid., Sect. B*, **27**, 2105 (1971).
(17) W. Saenger, *J. Amer. Chem. Soc.*, **94**, 621 (1972).
(18) W. Saenger and K. H. Scheit, *J. Mol. Biol.*, **50**, 153 (1970).
(19) W. Saenger and D. Suck, *ibid.*, **60**, 87 (1971).
(20) C. E. Bugg, J. M. Thomas, M. Sundaralingam, and S. T. Rao, *Biopolymers*, 10, 175 (1971).

stituting for guanine. After comparing the crystal structure of thioguanine with the crystal structures of other guanine derivatives, we concluded that guanine and thioguanine have similar hydrogen-bonding and base-stacking properties, except for differences in the lengths of their hydrogen bonds.[5] This finding prompted us to suggest that the biological properties of thioguanine, a metabolic inhibitor with antitumor activity,[21-27] are probably related to the effects that the sulfur substituent exerts on hydrogen-bond dimensions within nucleic acids.[5] The crystal structure of 6-thioguanosine (2-amino-6-mercaptopurine riboside, Figure 1) monohydrate was determined to gather additional information about the influence of sulfur substituents on base-stacking and hydrogen-bonding properties.

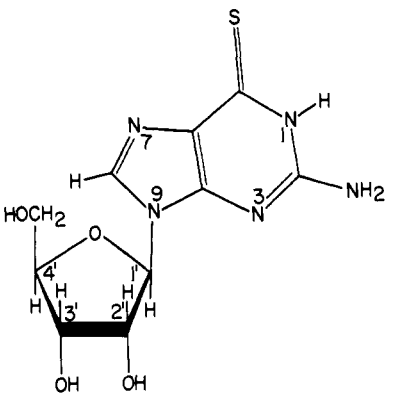

Figure 1. The structural formula of 6-thioguanosine.

Experimental Section

Thioguanosine monohydrate was crystallized as yellow needles by evaporating an aqueous solution containing thioguanosine and a few drops of dimethylamine. Weissenberg and oscillation photographs showed that the crystals are orthorhombic; the space group is $C222_1$ as indicated by the systematic absence of reflections hkl with $h + k$ odd and $00l$ with l odd. Most of the crystals produced Weissenberg photographs in which the spots were streaked considerably. To obtain a crystal for intensity measurements, a needle fragment with approximate dimensions of 0.2, 0.2, and 0.07 mm in the a, b, and c directions, respectively, was sliced from a larger crystal. Although this crystal was better than the others that we examined, it produced a $0kl$ Weissenberg photograph in which the spots were diffuse. The crystal was mounted on a Picker FACS-1 diffractometer with its a axis slightly inclined to the Φ axis of the diffractometer. Approximate cell parameters for use in collection of intensity data were caculated by a least-squares analysis of the angular settings for ten reflections in the range $29° < 2\theta < 54°$ (Cu Kα, λ = 1.5418 Å).

Intensity data were collected with the diffractometer, by use of a scintillation counter, nickel-filtered copper radiation, and a θ-2θ scanning technique. The scanning speed was 0.5°/min, and a 20-sec background measurement was performed at each terminus of the scans. Measurements were made for the 1285 independent reflections with $2\theta \leq 128°$. The 4 0 0, 0 2 0, and 0 0 12 reflections were selected as standards and were monitored periodically; the intensities of these reflections did not vary significantly during data collection.

Accurate values for the cell parameters were determined immediately after data collection, by a least-squares analysis of 2θ values for ten high angle reflections (Cu Kα_1, λ = 1.54051 Å); these cell parameters were not significantly different from those obtained prior to collecting intensity data. Crystal data are listed in Table I.

The intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0.03S)^2$, S being the scan counts. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the program ORABS.[28] Finally, the data were scaled by means of a Wilson[29] plot.

We arrived at a suitable trial structure by the heavy-atom method: the coordinates for the sulfur atom were found from a sharpened, three-dimensional Patterson map; the coordinates for the atoms of the purine ring, plus its immediate substituents, were obtained from a Fourier map phased with the sulfur atom; and the remaining nonhydrogen atoms were located in subsequent Fourier maps calculated by using phase angles derived from the known atoms. The trial structure was refined by use of a modified version of the full-matrix least-squares program ORFLS.[30] The quantity minimized was $\Sigma w(F_o^2 - F_c^2/k^2)^2$, where k is a scale factor and the weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms were from the International Tables for X-Ray Crystallography;[31] anomalous dispersion correction factors for these atoms were from Cromer and Liberman.[32] Hydrogen atom scattering factors were from Stewart, *et al.*[33] All hydrogen atoms were located in difference Fourier maps that were calculated during the latter stages of refinement. Final cycles of refinement included all positional parameters, along with anisotropic temperature factors for the heavy atoms, isotropic temperature factors for the hydrogen atoms, and Zachariasen's[34] isotropic extinction parameter g (as formulated by Coppens and Hamilton[35]). Because of the limited core storage capacity of the computer it was impracticable to refine all parameters simultaneously; consequently, the atoms of the thioguanine moiety were refined together, and the atoms of the ribose moiety and the water molecule were refined in the alternate cycles.

During the refinement, anomalous dispersion corrections were applied to the scattering factors of the nonhydrogen atoms. Both enantiomers were refined. The correct enantiomer (D(+) form) refined to R = 0.073 and a goodness-of-fit $((\Sigma w(F_o^2 - F_c^2/k^2)^2/(m - s))^{1/2}$, where m is the number of reflections used and s is the number of parameters refined) of 3.08. The unusually high values for the R index and the goodness-of-fit are probably mainly a consequence of the poor mosaic order of the crystal that was used

Table I. Crystal Data[a]

Stoichiometry	$C_{10}H_{13}N_5O_4S \cdot H_2O$
Z	8
Space group	$C222_1$
a, Å	12.272 (1)
b, Å	6.904 (1)
c, Å	32.466 (6)
ρ (calcd), g cm^{-3}	1.532
ρ (obsd), g cm^{-3}	1.53
μ, cm^{-1}	23.1

[a] The unit-cell parameters were measured at 25 ± 2°. The reported standard deviations are two times greater than those obtained from the least-squares analysis. The density was measured by flotation in a mixture of benzene and carbon tetrachloride.

(21) M. E. Balis, "Antagonists and Nucleic Acids," American Elsevier, New York, N. Y., 1968, pp 167-169.
(22) G. A. LePage and N. Howard, *Cancer Res.*, **23**, 622 (1963).
(23) G. A. LePage and I. G. Junga, *ibid.*, **23**, 739 (1963).
(24) G. A. LePage, *ibid.*, **20**, 403 (1960).
(25) H. S. Kaplan, K. C. Smith, and P. Tomlin, *Nature (London)*, **190**, 794 (1961).
(26) D. H. Adams, *Biochem. J.*, **89**, 240 (1963).
(27) G. H. Hitchings and G. B. Elion, "Metabolic Inhibitors," Vol. I, J. H. Quastel and R. M. Hochster, Ed., Academic Press, New York, N. Y., 1963, pp 215-237.
(28) D. J. Wehe, W. R. Busing, and H. A. Levy, "ORABS, A Fortran Program for Calculating Single Crystal Absorption Corrections," Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962,, Report No. ORNL-TM-229.
(29) A. J. C. Wilson, *Nature (London)*, **150**, 151 (1942).
(30) W. R. Busing, K. O. Martin, and H. A. Levy, "ORFLS, A Fortran Crystallographic Least-Squares Program," Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962, Report No. ORNL-TM-305.
(31) "International Tables for X-Ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1962, pp 202-205.
(32) D. T. Cromer and D. Liberman, *J. Chem. Phys.*, **53**, 1891 (1970).
(33) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *ibid.*, **42**, 3175 (1965).
(34) W. H. Zachariasen, *Acta Crystallogr.*, **16**, 1139 (1963).
(35) P. Coppens and W. C. Hamilton, *ibid.*, *Sect. A*, **26**, 71 (1970).

Table II. Final Heavy-Atom Parameters and Their Standard Deviations[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S	15956 (11)	24596 (32)	04769 (4)	00316 (9)	02432 (42)	00074 (1)	−00040 (25)	−00001 (3)	00007 (10)
N(1)	0421 (4)	2528 (11)	−0215 (2)	0025 (3)	0212 (13)	0008 (1)	−0008 (8)	0003 (1)	−0002 (4)
C(2)	0206 (5)	2569 (12)	−0621 (2)	0035 (4)	0177 (15)	0007 (1)	0014 (9)	−0001 (1)	0001 (4)
N(2)	−0831 (4)	2562 (14)	−0743 (2)	0022 (3)	0352 (21)	0009 (1)	−0002 (10)	0000 (1)	−0010 (4)
N(3)	0992 (3)	2571 (10)	−0912 (1)	0020 (3)	0198 (14)	0008 (1)	0000 (7)	−0000 (1)	0003 (3)
C(4)	2013 (4)	2549 (12)	−0745 (2)	0024 (3)	0150 (15)	0008 (1)	0001 (9)	0003 (1)	0003 (4)
C(5)	2301 (4)	2532 (12)	−0332 (2)	0031 (4)	0154 (14)	0008 (1)	0002 (9)	0002 (1)	0002 (4)
C(6)	1454 (4)	2494 (11)	−0037 (2)	0031 (3)	0141 (13)	0008 (1)	−0010 (11)	−0002 (1)	−0000 (3)
N(7)	3404 (4)	2499 (9)	−0285 (2)	0022 (3)	0232 (13)	0009 (1)	0007 (9)	−0003 (1)	0009 (3)
C(8)	3787 (5)	2546 (17)	−0659 (2)	0020 (4)	0333 (23)	0007 (1)	0005 (10)	0000 (1)	0006 (5)
N(9)	2969 (4)	2586 (9)	−0957 (1)	0020 (3)	0229 (14)	0008 (1)	0005 (8)	−0001 (1)	0002 (3)
C(1′)	3148 (4)	2540 (11)	−1397 (2)	0023 (3)	0213 (16)	0007 (1)	0005 (8)	0002 (1)	−0010 (3)
O(1′)	3606 (4)	0717 (8)	−1500 (2)	0045 (3)	0237 (13)	0010 (1)	0006 (6)	0009 (1)	0004 (2)
C(2′)	3904 (5)	4058 (11)	−1565 (2)	0034 (4)	0151 (16)	0008 (1)	0011 (7)	0001 (2)	0000 (3)
O(2′)	3357 (5)	5808 (9)	−1607 (2)	0073 (4)	0145 (13)	0012 (1)	0014 (7)	0006 (1)	0003 (2)
C(3′)	4271 (5)	3096 (11)	−1971 (2)	0030 (4)	0202 (19)	0007 (1)	0013 (7)	0002 (1)	0003 (3)
O(3′)	3517 (4)	3368 (9)	−2293 (1)	0060 (4)	0206 (12)	0008 (1)	−0005 (6)	−0004 (1)	0009 (2)
C(4′)	4280 (5)	0972 (11)	−1860 (3)	0026 (4)	0209 (18)	0007 (1)	0006 (8)	0001 (1)	−0001 (3)
C(5′)	5418 (6)	0177 (13)	−1773 (3)	0039 (5)	0238 (22)	0010 (1)	0015 (8)	−0001 (2)	0002 (4)
O(5′)	5363 (4)	−1848 (7)	−1705 (2)	0046 (3)	0207 (14)	0009 (1)	0024 (5)	−0002 (1)	0005 (2)
O(W)	1406 (6)	1969 (11)	−2430 (2)	0058 (5)	0317 (20)	0013 (1)	−0005 (8)	0000 (2)	−0027 (3)

[a] The values for the sulfur atom have been multiplied by 10^5; all other values have been multiplied by 10^4. Temperature factors are in the form $T = \exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2\beta_{12}hk - 2\beta_{13}hl - 2\beta_{23}kl)$. The final value of the isotropic extinction parameter is $g = 0.014$ (5).

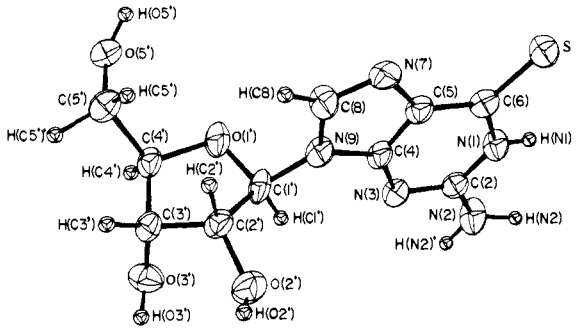

Figure 2. Conformation of 6-thioguanosine. The nonhydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50% probability. The hydrogen atoms are represented by spheres of 0.1-Å radius (this drawing was prepared by using the computer program ORTEP (C. K. Johnson (1965), "ORTEP, A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations," Oak Ridge National Laboratory, Oak Ridge, Tenn., Report No. ORNL-3794, revised)).

for the intensity measurements. The incorrect enantiomer (L(−) form) refined only to $R = 0.077$ and goodness-of-fit = 3.29.

During the final cycle of refinement with the correct enantiomer, the average parameter shift was less than one-fifth of the estimated standard deviation, and no parameter shifted more than one-third of its estimated standard deviation. A final three-dimensional electron density difference map showed several peaks and troughs of magnitudes ranging from 0.4 to 0.7 electron/Å^3 in the vicinity of the sulfur atom; there were no other fluctuations with magnitudes that exceeded 0.4 electron/Å^3.

Results

Table II lists the final heavy-atom parameters and their estimated standard deviations. Table III gives the hydrogen atom parameters and their estimated standard deviations. The estimated standard deviations in positional coordinates are approximately 0.001 Å for the sulfur atom, 0.006 Å for the carbon, oxygen, and nitrogen atoms, and 0.08 Å for the hydrogen atoms.[36]

(36) Observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-8889. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

Table III. Final Hydrogen-Atom Parameters and Their Standard Deviations[a]

Atom	x	y	z	B, Å^2
H(N(1))	−008 (5)	241 (12)	−005 (2)	3.7 (1.5)
H(N(2))	−147 (9)	276 (17)	−055 (3)	11.7 (3.4)
H(N(2))′	−099 (7)	262 (15)	−103 (3)	8.8 (2.6)
H(C(8))	447 (5)	264 (10)	−075 (2)	2.7 (1.3)
H(C(1′))	234 (5)	274 (10)	−150 (2)	2.7 (1.2)
H(C(2′))	471 (5)	407 (9)	−135 (2)	3.0 (1.3)
H(O(2′))	356 (9)	675 (14)	−163 (3)	5.5 (3.3)
H(C(3′))	509 (8)	352 (13)	−202 (2)	6.6 (2.3)
H(O(3′))	355 (4)	459 (8)	−238 (1)	0.5 (1.0)
H(C(4′))	398 (4)	022 (8)	−211 (2)	1.1 (1.1)
H(C(5′))	559 (5)	084 (9)	−154 (2)	1.3 (1.2)
H(C(5′))′	588 (8)	079 (16)	−204 (3)	8.3 (2.7)
H(O(5′))	547 (7)	−242 (14)	−146 (3)	6.3 (2.3)
H(OW)	185 (6)	211 (13)	−238 (2)	2.0 (2.2)
H(OW)′	105 (6)	265 (11)	−223 (2)	3.6 (1.6)

[a] The positional parameters have been multiplied by 10^3.

The conformation and heavy-atom thermal ellipsoids of 6-thioguanosine are shown in Figure 2. As in most other structures of nucleosides and nucleotides,[37,38] the conformation around the glycosidic linkage is anti; the torsion angle χ_{CN}[38] (O(1′)–C(1′)–N(9)–C(8)) is 65.7°. The ribose ring assumes the C(2′) endo conformation:[39] atoms C(1′), O(1′), C(4′), and C(3′) are coplanar with no deviations from the plane in excess of 0.02 Å, and atom C(2′) is displaced 0.56 Å from the plane of the four other ring atoms. The conformation about the C(4′)–C(5′) bond is gauche–trans;[39,40] the torsion angle Φ_{OO}(O(5′)–C(5′)–C(4′)–O(1′)) is 64.3°, and the torsion angle Φ_{OC}(O(5′)–C(5′)–C(4′)–C(3′)) is −175.4°. The nine atoms of the purine ring are coplanar within experimental error; S, N(2), and C(1′) are displaced from the least-squares purine plane by 0.01, 0.01, and 0.05 Å, respectively.

(37) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).
(38) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).
(39) M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).
(40) E. Shefter and K. N. Trueblood, *Acta Crystallogr.* **18**, 1067 (1965).

Table IV. Bond Lengths Involving Only Nonhydrogen Atoms[a]

Atoms	Distance, Å	Atoms	Distance, Å
N(1)–C(2)	1.343	C(8)–N(9)	1.394
N(1)–C(6)	1.393	N(9)–C(1′)	1.446
C(2)–N(2)	1.333	C(1′)–O(1′)	1.418
C(2)–N(3)	1.352	C(1′)–C(2′)	1.503
N(3)–C(4)	1.365	O(1′)–C(4′)	1.445
C(4)–C(5)	1.387	C(2′)–O(2′)	1.388
C(4)–N(9)	1.360	C(2′)–C(3′)	1.541
C(5)–C(6)	1.415	C(3′)–O(3′)	1.409
C(5)–N(7)	1.362	C(3′)–C(4′)	1.501
C(6)–S	1.679	C(4′)–C(5′)	1.528
N(7)–C(8)	1.301	C(5′)–O(5′)	1.417

[a] The estimated standard deviations are about 0.008 Å.

Table V. Bond Angles Involving Only Nonhydrogen Atoms[a]

Atoms	Angle, deg	Atoms	Angle, deg
C(6)–N(1)–C(2)	125.8	C(4)–N(9)–C(1′)	129.0
N(1)–C(2)–N(3)	123.2	C(8)–N(9)–C(1′)	125.2
N(1)–C(2)–N(2)	118.6	N(9)–C(1′)–O(1′)	108.2
N(2)–C(2)–N(3)	118.2	N(9)–C(1′)–C(2′)	116.0
C(2)–N(3)–C(4)	112.1	O(1′)–C(1′)–C(2′)	106.7
N(3)–C(4)–N(9)	126.3	C(1′)–O(1′)–C(4′)	108.1
N(3)–C(4)–C(5)	128.2	C(1′)–C(2′)–O(2′)	110.1
C(5)–C(4)–N(9)	105.5	C(1′)–C(2′)–C(3′)	101.0
C(4)–C(5)–C(6)	117.8	O(2′)–C(2′)–C(3′)	115.6
C(4)–C(5)–N(7)	111.3	C(2′)–C(3′)–O(3′)	112.6
C(6)–C(5)–N(7)	130.9	C(2′)–C(3′)–C(4′)	102.6
N(1)–C(6)–C(5)	112.9	O(3′)–C(3′)–C(4′)	108.1
N(1)–C(6)–S	120.5	O(1′)–C(4′)–C(3′)	107.8
C(5)–C(6)–S	126.7	O(1′)–C(4′)–C(5′)	109.2
C(5)–N(7)–C(8)	104.7	C(3′)–C(4′)–C(5′)	113.6
N(7)–C(8)–N(9)	112.8	C(4′)–C(5′)–O(5′)	109.9
C(4)–N(9)–C(8)	105.7		

[a] The estimated standard deviations are about 0.6°.

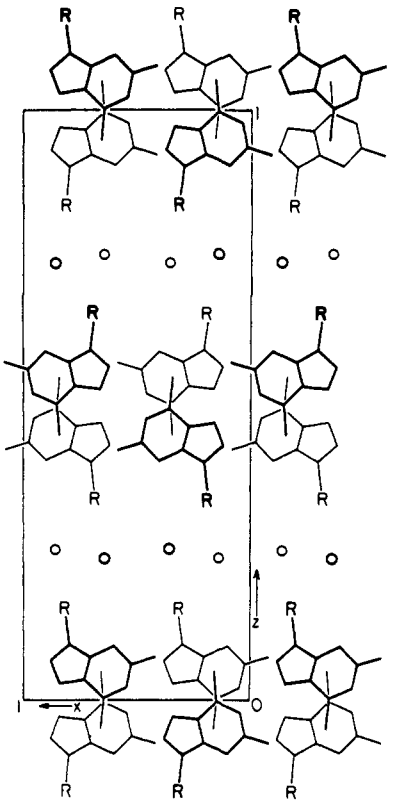

Figure 3. The crystal packing as viewed down the *b* axis. The molecules represented by the heavy lines are at $y = 0.75$ and those represented by the light lines are at $y = 0.25$. Ribose moieties are represented by the letter R and water molecules are represented by circles.

Table IV lists bond lengths and Table V lists bond angles. The bond lengths and angles are in agreement with those found for guanosine dihydrate.[41,42]

range from 0.82 to 1.02 Å with an average value of 0.93 Å, and the O–H bond lengths range from 0.57 to 0.91 Å with an average value of 0.79 .

The crystal packing is depicted in Figure 3. Thioguanine moieties are hydrogen bonded around screw

Table VI. Hydrogen Bond Distances and Angles

Donor atom	Hydrogen atom	Acceptor atom[a]	Distances, Å: Donor–acceptor	Distances, Å: Acceptor–hydrogen	Donor–hydrogen–acceptor angle, deg
N(1)	H(N(1))	N(7), b	2.961	2.16	167
N(2)	H(N(2))	S, b	3.274	2.39	145
N(2)	H(N(2))′	O(2′), c	3.216	2.39	144
O(2′)	H(O(2)′)	O(5′), d	2.963	2.42	135
O(3′)	H(O(3)′)	O(W), e	2.646	1.76	177
O(5′)	H(O(5)′)	N(3), f	2.715	1.90	152
O(W)	H(OW)	O(3′), a	2.800	2.24	166
O(W)	H(OW)′	O(5′), g	2.803	1.94	158

[a] Symmetry codes: a, x, y, z; b, $x - 1/2, -y + 1/2, -z$; c, $x - 1/2, y - 1/2, z$; d, $x, y + 1, z$; e, $-x + 1/2, y + 1/2, -z - 1/2$; f, $x + 1/2, y - 1/2, z$; g, $x - 1/2, y + 1/2, z$.

Apparently, replacing the carbonyl oxygen atom of guanosine with a sulfur substituent has little effect on the bond lengths and angles within the purine ring. The C–S bond length of 1.679 Å is in agreement with that found for other thiopurines and thiopyrimidines.[6] The C–H bond lengths range from 0.89 to 1.20 Å with an average value of 1.04 Å, the N–H bond lengths

(41) U. Thewalt C. E. Bugg, and R. E. Marsh, *Acta Crystallogr. Sect. B*, **26**, 1089 1970).

(42) C. E. Bugg, U. Thewalt, and R. E. Marsh, *Biochem. Biophys. Res. Commun.*, **33**, 436 (1968).

axes to form planar ribbons of bases running in the *a* direction and lying nearly parallel to the *ac* plane. The ribbons of bases are stacked in the *b* direction. The ribose moieties and the water molecules are arranged in channels between stacks of bases. Thus, the crystal structure consists of successive layers of bases, sugars, and water molecules, with the layers running parallel to the *ab* plane.

The hydrogen-bonding scheme is described in Table VI. All of the hydrogen atoms that are covalently bonded to nitrogen or oxygen atoms participate in

Table VII. Hydrogen Bond Distances and Angles Involving Sulfur Acceptors in Crystal Structures of Thiopurines and Thiopyrimidines

Compound	Donor atom	Distances, Å		Donor–H–sulfur angle, deg
		Donor–S	H–S	
6-Thioguanosine	N(2)	3.274 (8)	2.39 (8)	145 (5)
6-Thioguanine[a]	N(2)	3.327 (5)	2.46 (4)	171 (3)
	N(7)	3.303 (5)	2.27 (4)	157 (3)
2-Thiocytidine[b]	N(4)	3.467 (5)	2.75 (5)	139 (4)
	O(water)	3.267 (5)	2.74 (5)	120 (4)
2,4-Dithiouracil,[c] atom S(2)	N(1)	3.335 (6)	2.78 (8)	157 (5)
2,4-Dithiouracil,[c] atom S(4)	N(3)	3.315 (6)	2.39 (8)	164 (5)
6-Mercaptopurine[d,e]	O(water)	3.373 (2)	2.58 (2)	162 (1)
6-Mercaptopurine riboside[f]	O(3′)	3.133 (9)	2.27 (9)	142 (5)
2-Mercapto-6-methylpurine[g,h]	O(water)	3.26 (3)		
	N(1)	3.37 (3)		
2-Thiocytosine,[i] molecule A	N(4)	3.408 (2)	2.44 (2)	176 (1)
	N(4)	3.551 (2)	2.66 (2)	171 (1)
2-Thiocytosine,[i] molecule B	N(4)	3.345 (2)	2.41 (2)	176 (1)
	N(4)	3.466 (2)	2.58 (2)	168 (1)
2,4-Dithiouridine,[j] atom S(2)	O(5′)	3.218 (5)		
2,4-dithiouridine,[j] atom S(4)	O(5′)	3.476 (5)		
	N(3)	3.330 (5)	2.41 (6)	158 (5)
3′-*O*-Acetyl-4-thiothymidine[k]	O(5′)	3.227 (6)	2.3 (1)	156 (7)
1-β-Arabinofuranosyl-4-thiouracil[l]	O(2′)	3.311 (5)	2.32 (7)	173 (6)
	O(3′)	3.343 (5)	2.45 (7)	142 (6)

[a] Reference 5. [b] Reference 6. [c] Reference 8. [d] Reference 9. [e] Reference 10. [f] Reference 11. [g] Reference 12. [h] Reference 13. [i] Reference 14. [j] Reference 15. [k] Reference 16. [l] Reference 17.

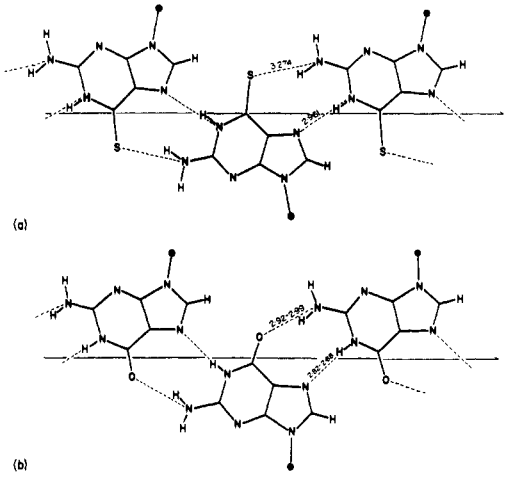

Figure 4. Hydrogen bonding between bases in the crystal structures of (a) thioguanosine monohydrate and (b) guanine monohydrate[42,44] and guanosine dihydrate.[41,42] The bases are arranged in planar ribbons within which adjacent bases are related by twofold screw axes. The ranges for the hydrogen bonds in the guanine and guanosine structures are shown. This same hydrogen-bonding scheme is also found in the crystal structure of 8-azaguanine monohydrate.[45,46]

hydrogen bonding. As shown in Figure 4a, adjacent thioguanine moieties within the ribbons of bases are joined by N(1)–H···N(7) and N(2)–H···S hydrogen bonds. Ribose oxygen atom O(5′) is hydrogen bonded to atom N(3) of a neighboring nucleoside. The other hydroxyl groups are involved in hydrogen bonding to adjacent ribose moieties and to the water molecule.

Discussion

An outstanding feature of this crystal structure is the hydrogen bonding between thioguanine residues. In agreement with earlier conclusions that sulfur atoms are good hydrogen bond acceptors,[5,7–9,43] the bases in this crystal structure are joined by N–H···S hydrogen bonds. As shown in Figure 4, the pattern of hydrogen bonding between bases is the same as that found in the crystal structures of guanosine dihydrate,[41,42] guanine monohydrate,[42,44] and 8-azaguanine monohydrate,[45,46] wherein the guanine residues are also hydrogen bonded around screw axes and are linked by N(2)–H···O and N(1)–H···N(7) hydrogen bonds. Therefore, it appears that replacement of the carbonyl oxygen atom of guanine by a sulfur substituent has little effect on the hydrogen-bonding capabilities of the base. However, the hydrogen bonds between thioguanine bases are considerably longer than the corresponding hydrogen bonds between guanine bases. Figure 4a shows that the N(2)–H···S and N(1)–H···N(7) hydrogen-bond lengths are 3.30 and 2.95 Å, respectively. In contrast,the guanine bases are joined by N(2)–H···O(6) and N(1)–H···N(7) hydrogen bonds that have lengths of about 2.90 and 2.83 Å, respectively. These results are consistent with those of other crystal-structure studies which demonstrate that hydrogen bonds involving sulfur acceptors are considerably longer than those involving oxygen acceptors.[5,7,43]

Table VII lists the lengths and angles for those hydrogen bonds that involve sulfur acceptors in other crystal structures of thiopurines and thiopyrimidines. In agreement with the crystal structure of 6-thioguanosine, N–H···S hydrogen bonds range from 3.2 to 3.6 Å in length. The data presented in Table VII leave little doubt about the importance of hydrogen bonding to sulfur acceptors in the crystal structures of thiopurines and thiopyrimidines. Assuming that the van der Waal's radius of hydrogen is 1.2 Å[47]

(43) W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968.

(44) U. Thewalt, C. E. Bugg, and R. E. Marsh, *Acta Crystallogr., Sect. B*, 27, 2358 (1971).

(45) W. N. MacIntyre, P. Singh, and M. S. Werkema, *Biophys. J.*, 5, 697 (1965).

(46) J. Sletten, E. Sletten, and L. H. Jensen, *Acta Crystallogr., Sect. B*, 24, 1692 (1968).

(47) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, p 260.

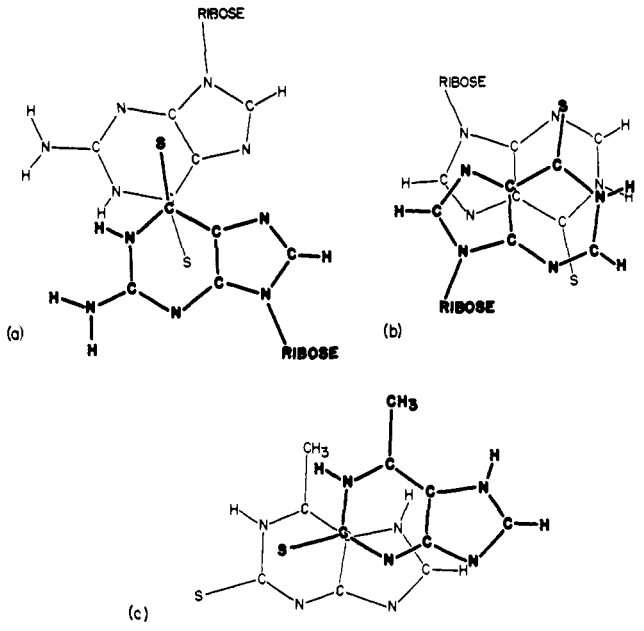

Figure 5. Base stacking patterns in crystals of thiopurines, as viewed perpendicular to the plane of the upper base, which is represented by the darker lines: (a) 6-thioguanosine monohydrate, interplanar spacing = 3.4–3.5 Å; dihedral angle = 0°; (b) 6-thiopurine riboside,[11] interplanar spacing = 3.5–3.7 Å; dihedral angle = 3°; (c) 2-mercapto-6-methylpurine monohydrate,[12,13] interplanar spacing = 3.38 Å.

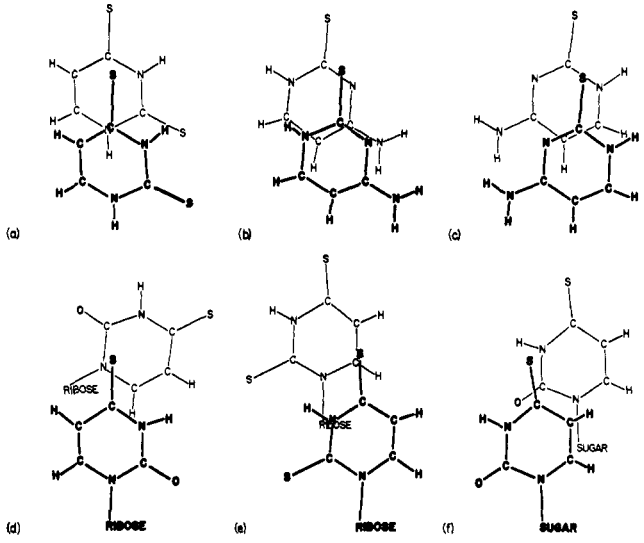

Figure 6. Base stacking patterns in crystals of thiopyrimidines as viewed perpendicular to the plane of the upper base, which is represented by the darker lines: (a) 2,4-dithiouracil,[8] interplanar spacing = 3.41 Å; (b) 2-thiocytosine (molecule A),[14] interplanar spacing = 3.51 Å; (c) 2-thiocytosine (molecule B),[14] interplanar spacing = 3.40 Å; (d) 4-thiouridine hydrate,[15] interplanar spacing = 3.5–3.9 Å, dihedral angle = 12°; (e) 2,4-dithiouridine monohydrate,[15] interplanar spacing = 3.51 Å; (f) 1-β-D-arabinofuranosyl-4-thiouracil monohydrate,[17] interplanar spacing = 3.52 Å (this pattern is also found in the crystal structure of 3′-*O*-acetyl-4-thiothymidine[16] in which the interplanar spacing is 3.68 Å).

and that of sulfur is 1.75–1.85 Å[9,47] one would expect normal H···S van der Waal's spacings of 2.9–3.1 Å. As shown in Table VII, the H···S distances in these crystal structures range from 2.3 to 2.8 Å, and are therefore significantly shorter than would be expected in the absence of hydrogen bonding. Also, the donor-hydrogen···acceptor angles are within the ranges typical for hydrogen bonds.[37,43] The only published thiopurine or thiopyrimidine crystal structures[48] in which the sulfur atoms do not accept at least one hydrogen bond are those of 4-thiouridine hydrate[18] and the 1-methyl-4-thiouracil–9-methyladenine complex.[19]

As in crystal structures of other purine and pyrimidine derivatives,[20,42] base stacking is a prominent feature in the crystal structure of 6-thioguanosine monohydrate. Figure 5a depicts the stacking pattern of thioguanosine. Thioguanine bases are stacked with their sulfur substituents positioned in close contact with the pyrimidine moieties of adjacent purine rings. In the crystal structure of 6-thioguanine,[5] the stacking pattern also involves contact between the sulfur substituent and the pyrimidine moiety of a neighboring base. Figures 5b and 5c show that this general type of stacking pattern is found in the crystal structures of other thiopurines. Of the published thiopurine crystal structures, 6-mercaptopurine monohydrate[9,10] is the only one in which the stacking pattern does not involve close contacts between the sulfur substituent and the ring system of an adjacent base. Figure 6 shows the stacking patterns in the crystal structures of thiopyrimidines. As with thiopurines, the stacking patterns are such that sulfur substituents are in close contact with pyrimidine rings of neighboring bases.

(48) In this review, only those bases containing sulfur substituents in the thione form were considered. Those containing disulfide groups or thiomethyl substituents were not considered.

Of the published crystal structures of thiopyrimidines, 2-thiocytidine dihydrate[6] and the 1-methyl-4-thiouracil–9-methyladenine[19] complex are the only ones in which the stacking patterns deviate from the type shown in Figure 6.

It was noted earlier that the stacking patterns of thio bases are similar to those found for purines and pyrimidines possessing carbonyl oxygen atoms in place of sulfur substituents.[5,20] This was particularly apparent for thioguanine, since the stacking pattern in its crystal structure is almost identical with that in the crystal structures of guanine bases.[5] Purines and pyrimidines that possess carbonyl groups usually stack with patterns in which the carbonyl oxygen atoms form close contacts with the ring systems of adjacent bases.[20] For example, the stacking patterns commonly found for thymine, uracil, and cytosine bases are essentially the same as the patterns shown in Figure 6, except that carbonyl oxygen atoms, rather than sulfur atoms, overlap adjacent pyrimidine rings.

The results described here indicate that substitution of sulfur atoms for carbonyl oxygen atoms has little effect on hydrogen-bonding patterns of purines and pyrimidines. However, because of differences in the lengths of C–S and C–O covalent bonds (1.7 *vs.* 1.2 Å) and in the lengths of N–H···S and N–H···O hydrogen bonds (3.3 *vs.* 2.9 Å), replacement of carbonyl oxygen atoms by sulfur substituents has a major effect on the dimensions of pairs of hydrogen-bonded bases. These effects are clearly illustrated in Figure 4, which shows that, despite the similarity of the hydrogen bonding between bases in thioguanosine monohydrate and that between the bases in the crystal structures of several guanine derivatives, the pairs of hydrogen-bonded bases have different dimensions. As we suggested earlier, guanine–cytosine and thioguanine–

cytosine base pairs would also have different dimensions, and these differences might result in considerable distortions in the secondary structure of nucleic acids containing thioguanine.[5] Structural distortions caused by the substitution of thioguanine for guanine might be of sufficient magnitude to disrupt the normal biological functioning of nucleic acids, and to account for the antimetabolite activity of thioguanine. The thio bases of tRNA might also disrupt the regularity of hydrogen-bonded regions by affecting hydrogen bond lengths, thus contributing to the control of tRNA conformation.

Interactions of Divalent Metal Ions with Inorganic and Nucleoside Phosphates. I. Thermodynamics

Cheryl Miller Frey and John E. Stuehr*

Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received October 19, 1971*

Abstract: Thermodynamic data are reported for 11 nucleotide and phosphate systems: orthophosphate, pyrophosphate, and tripolyphosphate; ribose phosphate; adenosine 5′-mono-, di-, and triphosphates; adenosine 2′-monophosphate; cytidine 5′-mono-, di-, and triphosphates; pK_a values of the free acids and stability constants with Ni(II) and Mg(II) are reported at 15° and $I = 0.1$. Magnesium behaves similarly with all systems containing the same number of phosphates, indicating at the most a very weak interaction between Mg(II) and the nucleotide ring. For nickel, a number of differences are noted, including ring specificity and the appearance of 1:2 as well as 1:1 complexes in some instances. These results are interpreted on the basis of various structural possibilities.

Due in part to the role of nucleotides and their metal ion complexes as substrates in enzyme-catalyzed reactions, considerable interest in recent years has focused upon the stability of metal ion complexes formed with the adenine nucleotides.[1-11] Unfortunately, various investigations have been carried out under differing conditions of temperature, ionic strength, and supporting electrolyte. As a consequence, it is often difficult to make detailed comparisons of the results from different investigations. In addition, nucleotides other than the adenine series are only sparcely represented in the literature.[12,13]

We report here pK_a values and stability constants for nickel and magnesium for a series of inorganic phosphates, adenine nucleotides, and cytosine nucleotides in 0.1 *M* KNO_3 at 15°. The metal ions Mg(II) and Ni(II) were chosen for study because they might be expected to behave differently with respect to ring binding.[14-16] The purpose of this research was to study the influence of the following parameters on the thermodynamic behavior: (1) length of phosphate backbone; (2) base composition, adenine series *vs.* cytosine series; (3) nucleoside phosphates *vs.* inorganic phosphates; (4) Ni(II) *vs.* Mg(II). The supporting electrolyte was chosen primarily to have results to compare with the large body of data already available for the adenine nucleotides. In addition, titrations were carried out at several different metal:ligand ratios.

Critical to an analysis of potentiometric data is a consideration of the possible existence of various species, including not only those of the ligand, but also the metal–ligand complex. The formation of ML and MHL (protonation on the phosphate)[14] is well known in the adenine nucleotides and the inorganic phosphates.[17,18] In addition, there is considerable evidence for self-association, or base stacking (*i.e.*, L_2 formation), in many nucleotides and nucleosides.[19-25] Recently there has also been speculation about the formation of M_2L_2 and ML_2 complexes involving base-stacked ligands in the adenine nucleotides.[26,27] We have focused our attention on the possibility of these species in the analysis of our potentiometric data.

(1) M. M. T. Khan and A. E. Martell, *J. Amer. Chem. Soc*, **88**, 668 (1966).
(2) M. M. T. Khan and A. E. Martell, *ibid.*, **89**, 5586 (1967).
(3) M. M. T. Khan and A. E. Martell, *J. Phys. Chem.*, **66**, 10 (1962).
(4) M. M. T. Khan and A. E. Martell, *J. Amer. Chem. Soc.*, **84**, 3037 (1962).
(5) A. E. Martell and G. Schwarzenbach, *Helv. Chim. Acta*, **39**, 653 (1956).
(6) P. George, R. C. Phillips, and R. J. Rutman, *Biochemistry*, **2**, 508 (1963).
(7) R. C. Phillips, P. George, and R. J. Rutman, *J. Amer. Chem. Soc.*, **88**, 2631 (1966).
(8) R. Phillips, *Chem. Rev.*, **66**, 501 (1966).
(9) R. M. Smith and R. A. Alberty, *J. Phys. Chem.*, **60**, 180 (1956).
(10) W. J. O'Sullivan and D. D. Perrin, *Biochemistry*, **3**, 18 (1964).
(11) M. S. Mohan and G. A. Rechnitz, *J. Amer. Chem. Soc.*, **92**, 5839 (1970).
(12) E. Walaas, *Acta Chem. Scand.*, **12**, 528 (1958).
(13) R. C. Phillips, P. Eisenberg, P. George, and R. J. Rutman, *J. Biol. Chem.*, **240**, 4393 (1965).
(14) M. Cohn and T. R. Hughes, *ibid.*, **235**, 3250 (1960).
(15) M. Cohn and T. R. Hughes, *ibid.*, **237**, 176 (1962).
(16) P. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).
(17) A. Martell and G. Schwarzenbach, *ibid.*, **39**, 653 (1956).
(18) G. Hammes and M. L. Morell, *J. Amer. Chem. Soc.*, **86**, 1497 (1964).
(19) P. O. P. Ts'o, J. S. Melvin, and A. C. Olson, *ibid.*, **85**, 1289 (1963).
(20) P. O. P. Ts'o and S. Chan, *ibid.*, **86**, 4176 (1964).
(21) S. Chan, M. Schweizer, P. O. P. Ts'o, and G. Helmkamp, *ibid.*, **86**, 4182 (1964).
(22) M. Schweizer, S. Chan, and P. O. P. Ts'o, *ibid.*, **87**, 5241 (1965).
(23) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *ibid.*, **89**, 3612 (1967).
(24) O. Jardetzky, *Biopolymers Symp.*, **1**, 501 (1964).
(25) M. Schweizer, A. Broom, P. O. P. Ts'o, and D. Hollis, *J. Amer. Chem. Soc.*, **90**, 1042 (1968).
(26) N. A. Berger and G. L. Eichhorn, *Biochemistry*, 10, 1847 (1971).
(27) H. Sternlicht, D. Jones, and K. Kustin, *J. Amer. Chem. Soc.*, **90**, 7110 (1968).