of Va with BOC-Ser-NHS and crystallization of the product afforded BOC-Ser(BZL)-Gly-Gly-Pro-Leu-Val-$OCH_3 \cdot H_2O$ (VI), 1.4 g, 81%, mp 85–90°, R_f (S-I) 0.75, R_f (S-VI) 0.32, $[\alpha]^{26}\text{D}$ −70.7° (*c* 0.308, methanol). *Anal.* Calcd for $C_{36}H_{56}O_{10}N_6 \cdot 0.5H_2O$ (recrystallized sample, mp 85–88°): C, 58.58; H, 7.78; N, 11.19; H_2O, 1.2. Found: C, 58.32; H, 7.83; N, 11.31. A 1.1% weight loss was found upon drying to constant weight at 80° (0.2 mm).

D. **BOC-Gly-Asp(OBZL)-Ser(BZL)-Gly-Gly-Pro-Leu-Val-OCH_3 (VIII).** The BOC protecting group was removed from VI, affording HCl·H-Ser(BZL)-Gly-Gly-Pro-Leu-Val-OCH_3 (VIa), 100%, R_f (S-I) 0.45. VIa was coupled with BOC-Asp(OBZL)-NHS in the usual way, except for omission of alkali washing during the work-up procedure (in view of the known[11] base-catalyzed rearrangement of Asp-Ser sequences), yielding BOC-Asp(OBZL)-Ser-(BZL)-Gly-Gly-Pro-Leu-Val-OCH_3 (VII), 98%, R_f (S-I) 0.80, R_f (S-VI) 0.56. A solution of VII (1.58 g, 1.69 mmoles) in DME (4.0 ml) was treated with 6.1 *N* HCl–DME (1.24 ml, 7.6 mmoles) at 0° for 4 hr and worked up as previously indicated, leading to HCl·H-Asp-(OBZL)-Ser(BZL)-Gly-Gly-Pro-Leu-Val-OCH_3 (VIIa), 1.47 g, 99% yield, R_f (S-I) 0.50. VIIa was coupled with BOC-Gly-NHS as above, and the product, BOC-Gly-Asp(OBZL)-Ser(BZL)-Gly-Gly-Pro-Leu-Val-OCH_3 (VIII) crystallized from hexane–ethanol, 1.20 g, 77%, mp 122–130°, R_f (S-I) 0.74, R_f (S-VI) 0.16, $[\alpha]^{26}\text{D}$ −62.5° (*c* 0.320, methanol). *Anal.* Calcd for $C_{49}H_{70}O_{14}N_8$ (recrystallized sample, mp 130–133°: C, 59.14; H, 7.09; N, 11.26. Found: C, 58.90; H, 7.06; N, 10.99.

E. **HCl·H-Gly-Asp-Ser-Gly-Gly-Pro-Leu-Val-OCH_3 (IX).** Removal of the BOC group from VIII was carried out as described in part D, leading to HCl·H-Gly-Asp(OBZL)-Ser(BZL)-Gly-Gly-Pro-Leu-Val-OCH_3 (VIIIa) in 98% yield, R_f (S-I) 0.41. A solution of VIIIa (0.233 g, 0.25 mmole) in EtOH (45 ml) was hydrogenated over 10% palladium–charcoal (0.11 g) at 23° for 5.5 hr, consuming an equivalent volume of H_2. The suspension was filtered through Celite, the filtrate was evaporated, and the residue was dried over P_2O_5. The product, HCl·H-Gly-Asp-Ser-Gly-Gly-Pro-Leu-Val-OCH_3 (IX), 0.18 g, 96% yield, mp 151–156°, R_f (S-I) 0.11, was devoid of ultraviolet benzylic absorption at 257 mμ. Crystallization from EtOH–EtOAc led to granular crystals, mp 158–160°, $[\alpha]^{27}\text{D}$ −106° (*c* 0.057, H_2O). *Anal.* Calcd for $C_{30}H_{51}O_{12}N_{18}Cl_1 \cdot C_2H_5OH$: C, 48.20; H, 7.21; N, 14.05; Cl, 4.45. Found: C, 48.35; H, 6.98; N, 13.94; Cl, 4.48.

Compound IX (2.25 mg) was digested with a mixture of preincubated solutions of 0.1% leucine aminopeptidase and prolidase[24] (0.5 ml of each, 1 mg/ml of $MnCl_2 \cdot 4H_2O$, 0.025 *M* Tris buffer, pH 8.5, 37°, 69 hr). Thin layer chromatography (silica gel H; *n*-BuOH–HOAc–H_2O, 2:1:1, in milliliters; C_6H_5OH–H_2O, 3:1, in grams) of the digest accounted for all constituent amino acids and did not detect peptides with chlorine–tolidine. Samples of enzymic and acid (6 *N* HCl, 110°, 24 hr) digests were analyzed with the Beckman-Spinco analyzer. The amino acid ratios are given in Table I.

Acknowledgment. We thank Dr. George W. Anderson for helpful discussions, Dr. Susan Dowd for valuable assistance in the preparation of several intermediates, and Dr. Elizabeth Simons for help and advice in the enzyme hydrolysis studies. Amino acid analyses were carried out by Miss Mary Jane Becherer.

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[24] See footnote *b*, Table I.

The Crystal and Molecular Structure of 2,4-Dithiouracil[1]

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Contribution from the Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York, and the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut.

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Abstract: The molecular structure of 2,4-dithiouracil has been determined by X-ray diffraction. The crystals are monoclinic, space group $P2_1/c$, a = 4.247 A, b = 14.586 A, c = 9.907 A, β = 98.77°, and Z = 4. Evidence is presented that dithiouracil contains highly polarized thionamide groups, the polarization of these groups being position dependent. The C–S bond lengths were found to be 1.645 and 1.685 (±0.006) A. The packing of the molecules in the crystal lattice is dominated by S···(H)–N hydrogen bonds. The lengths of the hydrogen bonds appear to be a function of the degree of polarization about the sulfur atoms.

Numerous sulfur-substituted pyrimidines and purines have found applications as clinically useful drugs. In many of these it was noted that the position in which sulfur was introduced was crucial to biological activity. For instance, 2-thiouracil, but not 4-thiouracil, has useful antithyroid activity,[2] while 6-thioguanine and 6-mercaptopurine, but not the corresponding 2-thio compounds, exert antineoplastic action.[3] 4-Thiopteridines and -pyrimidines and 6-thiopurines, but not the 2-substituted isomers, bind to dihydrofolic acid reductase.[4] These differences in biological activity are paralleled by differences in the chemical reactivities of such compounds. Thus, the reaction of uracil with phosphorus pentasulfide yields only 4-thiouracil and 2,4-dithiouracil, but no 2-thiouracil,[5] while the reaction of 2,4-dithiouracil with ammonia yields only 2-thiocytosine, none of the 2-amino compound being formed.[6]

It has also been noted that the replacement of oxygen by sulfur[5] or by selenium[7] induced much greater bathochromic shifts in the ultraviolet spectra of 2,4-disubstituted pyrimidines (or the corresponding 2,6-disubstituted purines) when it took place in the 4 position of the pyrimidines (6 position of the purines) than when it

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[2] E. B. Astwood, A. Bissell, and A. M. Hughes, *Endocrinology*, **37**, 456 (1945).

[3] R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964).

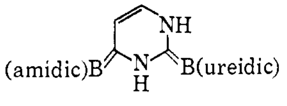
[4] J. R. Bertino and H. G. Mautner, unpublished data.

[5] G. B. Elion, *et al.*, *J. Am. Chem. Soc.*, **68**, 2137 (1946).

[6] G. H. Hitchings, *et al.*, *J. Biol. Chem.*, **177**, 357 (1949).

[7] H. G. Mautner, *J. Am. Chem. Soc.*, **78**, 5292 (1956).

took place in the 2 position of these compounds. This effect can be visualized readily by observing the colors of pyrimidines, pteridines, or purines in which sulfur or selenium has been introduced. Thus, 2-thio-4-oxo- or 2-seleno-4-oxopyrimidines are always colorless,[7-9] while 2-oxo-4-thiopyrimidines are always yellow and 2-oxo-4-selenopyrimidines are always orange or red. It was proposed[10] that sulfur and selenium could act as chromophoric groups only when they are present in NHC(=B)N= or NHC(=B)CH= ("amidic"), but not when they are present in NHC(=B)NH ("ureidic") groups.



B = S, Se

Application of Hückel molecular orbital calculations (LCAO–MO) to 2-thiouracil, 4-thiouracil, and 2,4-dithiouracil[10] yielded results accounting for the spectral properties of these compounds and predicted that the bond order of C–S and C–Se bonds should differ depending on whether they are located in the 2 or in the 4 position of 2,4-disubstituted pyrimidines.

Further interest in information about positional differences in the character of C–S bonds in the 2 and the 4 position of pyrimidines was raised by recent observations that a 2-thiopyrimidine[11] and 4-thiouridine[12] are present in soluble ribonucleic acid. Since these compounds presumably participate in the hydrogen bonds formed by s-RNA,[13] information about the relative bond order and the relative hydrogen-bonding ability of C–S bonds in the 2 and the 4 position of pyrimidines would have considerable biological relevance. Interest in such information is also raised by the postulate that the antineoplastic activity of 6-thioguanine might be related to the ability of this compound to be incorporated into deoxyribonucleic acid,[14] with the possible formation of unusual hydrogen bonds.[15] That 6-thioguanine incorporation occurs was proved[14] by the isolation of thioguanylic acid from nucleic acids isolated from Ehrlich ascites cells treated with the thiopurine.

That sulfur and selenium in heterocyclic compounds can act as powerful hydrogen bond acceptors—although to a lesser degree than oxygen—was established recently when a comparative study of the relative abilities of 2-pyridone, 2-pyridthione, and 2-pyridselenone to form hydrogen-bonded dimers was carried out.[16]

In view of the factors discussed above, an X-ray diffraction analysis of 2,4-dithiouracil appeared of considerable interest. Related studies have recently been carried out on 2,4-diselenouracil,[17] 2-thiouracil, and 2-selenouracil.[18]

Experimental Section

Crystals of 2,4-dithiouracil formed needles elongated about a from ethanol solutions. The following crystallographic data were obtained for these crystals: $a = 4.247 \pm 0.002$ A, $b = 14.586 \pm 0.005$ A, $c = 9.907 \pm 0.005$ A; $\beta = 98.77 \pm 0.05°$; volume = 606.55 A^3; D_x (calculated density) = 1.535 ± 0.001 g/cm^3, D_m (measured density) = 1.57 ± 0.02 g/cm^3; μ (linear absorption coefficient) = 6.7 cm^{-1}; absent reflections $h0l$ when l is odd, $0k0$ when k is odd; space group is $P2_1/c$.

The density was measured by flotation in a chloroform–bromobenzene mixture. The unit cell dimensions were determined by least-squares treatment of 40 general reflections measured with the General Electric XRD-6 spectrogoniometer and goniostat using a scintillation counter and Cu Kα radiation. The space group was determined to be $P2_1/c$ from the systematic absences and also by the successful refinement of the structure in this space group.

A crystal specimen (approximately 0.4 mm along a, 0.01 mm along b, and 0.02 mm along c) was mounted about a^* for the intensity collection on the diffractometer. The data were collected by the stationary crystal–stationary counter technique using balanced filters for Cu radiation. Of the 1020 accessible reflections in the 2θ range between 0 and 130°, 811 had intensities significantly greater than their background count. The intensities were corrected for $\alpha_1 - \alpha_2$ splitting in the manner described by Tulinsky, *et al.*,[19] and, with the appropriate Lorentz polarization factors, were converted to F^2. Because of the low absorption coefficient and the size of the crystal used, no absorption corrections were deemed necessary.

Structure Analysis. Weissenberg photographs showed that the crystals were "isomorphous" with those of 2,4-diselenouracil.[17] The final positional parameters obtained for the latter compound were then used to initiate least-squares refinement of this compound. For this purpose a modification, by Dr. G. Kartha, of the Gantzel, Sparks, and Trueblood block diagonal least-squares routine (ACA No. 317) was utilized. The initial cycles of refinement on the nonhydrogen atoms were carried out using a weighting scheme which simulates difference synthesis refinement, *i.e.*, wtg = $1/f_{(N)}$, where $f_{(N)}$ is the atomic scattering curve for nitrogen. A difference Fourier synthesis calculated after anisotropic temperature factors were introduced into the refinement (R value at this point was 6.5%) showed the four hydrogens with expected peak electron density (see Table II). The refinement was then continued with the hydrogens having isotropic temperature factors, until all the parameter shifts were less than one-fourth of their estimated standard deviations, which were calculated from the inverse of the diagonal elements of the normalized least-squares equations. In this final part of refinement, the weighting scheme was changed to wtg = 1/std dev of F^2, where the standard deviation of the structure factor was calculated using the method of Evans.[20] The complex part of the scattering curve for sulfur was included in the final cycles, as it has been demonstrated[21] that in certain cases it can have a significant influence on the phase angle of centric structures. The final R value for the 811 observed reflections was 5.7%. A list of the observed and calculated structure factors will be supplied with reprint requests. In all the above calculations the scattering curves for C, N, S, and H were taken from the International Tables for X-Ray Crystallography.[22] The final positional and thermal parameters for the various atoms are given in Tables I and II. Bond lengths and angles calculated from these parameters are shown in Figures 1 and 2, respectively. Figure 3 is a view of the structure down the a axis.

The least-squares equation of the plane through the nonhydrogen atoms is

$$0.8017X - 0.1778Y + 0.5690Z = -0.3573 \text{ A}$$

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Table I. Final Positional and Thermal Parameters for the Nonhydrogen Atoms[a]

Atom	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	−3102 (13)	900 (4)	3794 (6)	704 (39)	60 (3)	81 (7)	−41 (20)	107 (27)	−16 (8)
C(2)	−2719 (14)	165 (4)	3020 (6)	542 (41)	40 (3)	89 (7)	60 (21)	10 (30)	−19 (9)
S(2)	−3859 (4)	−887 (1)	3295 (2)	924 (13)	42 (1)	127 (2)	−16 (6)	293 (8)	2 (2)
N(3)	−1091 (11)	377 (3)	1927 (4)	546 (33)	35 (2)	71 (5)	2 (16)	73 (22)	0 (7)
C(4)	−118 (13)	1227 (4)	1612 (6)	479 (39)	39 (3)	80 (7)	15 (20)	23 (27)	10 (8)
S(4)	1781 (4)	1361 (1)	252 (2)	714 (11)	45 (1)	94 (2)	−9 (6)	121 (7)	13 (2)
C(5)	−652 (15)	1954 (4)	2492 (6)	694 (48)	34 (3)	115 (8)	−56 (22)	89 (33)	−5 (9)
C(6)	−2195 (15)	1774 (4)	3575 (6)	797 (50)	41 (3)	117 (8)	−74 (23)	109 (34)	−47 (9)

[a] Values multiplied by 10^4. The temperature factor is of the form $\exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - \beta_{12}hk - \beta_{13}hl - \beta_{23}kl)$. The standard deviation for the various parameters are in the parenthesis and are multiplied by 10^4.

where X, Y, and Z are in angstroms measured along a, b, and c^*. Within experimental error the molecule is planar. The deviations of the atoms from this plane are shown in Table III.

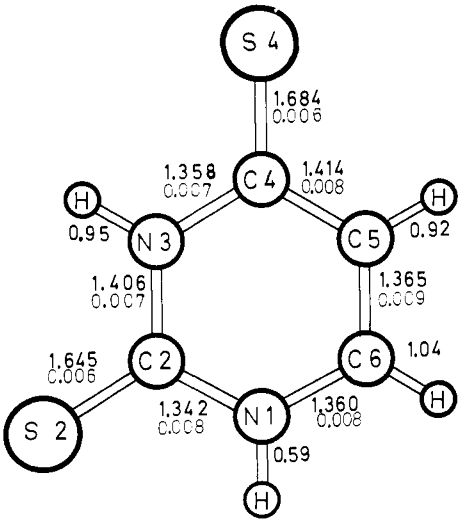

Figure 1. Intramolecular distances.

The magnitude and direction of the thermal vibration of the nonhydrogen atoms are listed in Table IV. A complete rigid body analysis of the molecule was not undertaken. The thermal parameters of the hydrogens are much lower than those of the respective atoms to which they are attached. Jensen and Sundaralingam[23] have made similar observations and reasoned that in the bonded hydrogen atom there appears to be a greater localization of electron density than that calculated using the scattering curve for the free atom. This results in a lower thermal parameter to compensate for the difference in electron density.

Table II. Atomic Positional and Thermal Parameters for Hydrogens from Least-Squares Refinement[a]

Atom	x/a	y/b	z/c	B, A^2	Electrons, A^3
N(1)H	−0.3321	0.0805	0.4350	0.9	0.30
N(3)H	−0.0842	−0.0107	0.1317	−0.3	0.40
C(5)H	−0.0018	0.2531	0.2302	0.8	0.39
C(6)H	−0.2449	0.2311	0.4233	1.5	0.34

[a] Peak heights from a difference Fourier synthesis are shown. The average estimated standard deviations for positional and thermal parameters are 0.08 and 1.2 A, respectively (this is twice that calculated from the diagonal elements of the least-squares matrix).

Discussion

The location of the hydrogens on the ring nitrogens, as in the related study on 2-thiouracil,[18] confirms the report[24] that pyrimidthiones, to an even greater extent than pyrimidones, should be considered as being amides and not as being enols. The bond angles and lengths for the hydrogen atoms are similar within experimental error to those found in a variety of structure determinations, with the exception of the N(1)–H bond. The

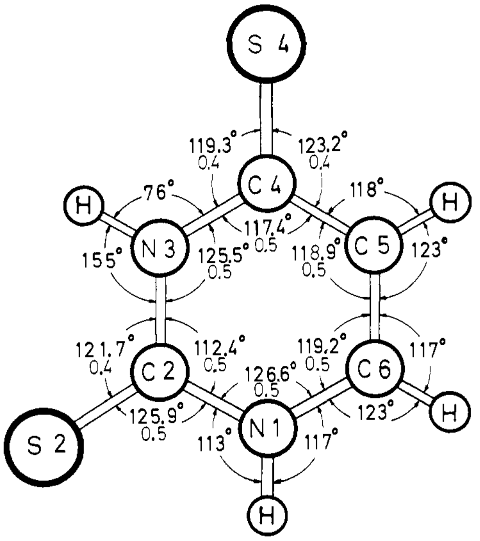

Figure 2. Intramolecular angles.

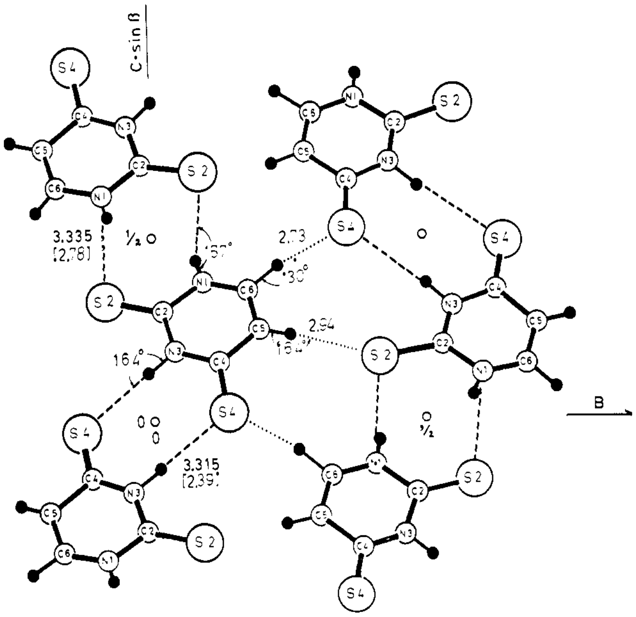

Figure 3. Projection down the a axis.

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Table III. Deviations of the Atoms from Least-Squares Plane Calculated through Nonhydrogen Atoms

Atom	Displacement, A
N(1)	0.010
C(2)	−0.008
S(2)	−0.003
N(3)	0.013
C(4)	−0.014
S(4)	0.003
C(5)	0.002
C(6)	−0.004
N(1)H	0.204
N(3)H	−0.043
C(5)H	−0.003
C(6)H	0.059

Table IV. Principal Axes, *i*, of Anisotropic Temperature Factors and Their Direction Cosines Relative to the Orthonormal Axes *a*, *b*, and *c**[a]

Atom	*i*	Rmsd	l_1	l_2	l_3
N(1)	1	0.267	0.591	−0.770	0.238
	2	0.240	0.789	0.614	0.028
	3	0.193	0.168	0.171	−0.971
C(2)	1	0.248	−0.713	−0.558	0.425
	2	0.200	−0.578	0.125	−0.806
	3	0.186	−0.397	0.821	0.411
S(2)	1	0.303	−0.815	0.037	−0.578
	2	0.220	−0.441	0.608	0.660
	3	0.209	−0.376	−0.793	0.480
N(3)	1	0.221	−0.986	−0.032	−0.165
	2	0.195	−0.025	−0.999	−0.044
	3	0.184	−0.166	−0.040	0.986
C(4)	1	0.214	−0.881	−0.047	0.047
	2	0.211	−0.261	−0.879	−0.400
	3	0.186	−0.395	0.475	−0.786
S(4)	1	0.253	−0.957	0.041	−0.287
	2	0.226	−0.127	0.830	0.543
	3	0.201	−0.260	−0.556	0.789
C(5)	1	0.254	−0.942	0.300	−0.152
	2	0.236	−0.168	−0.029	0.985
	3	0.186	−0.292	−0.953	−0.078
C(6)	1	0.278	−0.773	0.438	−0.458
	2	0.250	−0.615	−0.344	0.709
	3	0.177	−0.153	−0.830	−0.536

[a] The root-mean-square vibrational displacements (rmsd) are given in A along *i*.

shorter distance found for this N–H bond and its large deviation from the least-squares plane of the molecule (see Table IV) most probably reflect the difficulty in accurately positioning such a light atom in a structure dominated by such a "heavy" atom as sulfur.

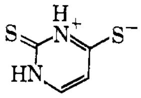
Table V compares the bond lengths computed for 2,4-dithiouracil by means of Hückel LCAO-MO calculations[10] with the values derived from X-ray diffraction measurements described above. As in the recent study of Tsernoglou[18] dealing with 2-thiouracil, discrepancies between calculated and experimental data were observed. For instance, while the length of the C(2)–S(2) bond is in agreement with the predicted value, the C(4)–S(4) bond is somewhat longer than the calculated value, while the N(3)–C(4) length was shorter. These results can best be accounted for by assuming that the sulfur in the 4 position is in the zwitterionic amidic configuration, —$^+$NH=C(—S$^-$)—, to a greater extent than the sulfur in the 2 position. The greater degree of polarization about the 4 position is also strikingly demonstrated when the bond lengths of the molecule are compared with those found in uracil,[25] thymine,[26] and 2-thiouracil.[18] The N(1)–C(2) and N(3)–C(4) bond distances in the latter three structures are respectively 1.344 and 1.374 A for uracil, 1.355 and 1.391 A for thymine, and 1.357 and 1.376 A for 2-thiouracil, which strongly suggest a significant shortening of the N(3)–C(4) distance in 2,4-dithiouracil owing to the introduction of sulfur in the 4 position.

Table V. Comparison of Observed Bond Distances with Those Calculated by Molecular Orbitals

Bond	Calcd value, A[a]	Obsd length, A
N(1)–C(2)	1.39	1.342
C(2)–N(3)	1.38	1.406
N(3)–C(4)	1.39	1.358
C(4)–C(5)	1.45	1.414
C(5)–C(6)	1.37	1.365
C(6)–N(1)	1.36	1.360
C(2)–S(2)	1.64	1.645
C(4)–S(4)	1.65	1.685

[a] The bond orders calculated in ref 10 were converted to bond lengths using values for single and double bonds given in L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960.

In both 2,4-dithiouracil and 2,4-diselenouracil the carbon–sulfur or carbon–selenium bond in the 4 position is significantly longer than in the 2 position, also reflecting a greater degree of sulfur (or selenium) polarization in the 4 as compared to the 2 position, and suggesting an important contribution of the resonance form



Similarly, it can be seen in the packing diagram (Figure 3) that hydrogen bonds involving S(4) are significantly shorter than those involving S(2). Again, the results obtained with 2,4-dithiouracil are consistent with the findings for the diseleno analog.

Aside from hydrogen bonds, there appears to be one intermolecular distance that is significantly shorter than a normal van der Waals contact. This is the 2.73-A contact between S(4) and the hydrogen attached to C(6) (see Figure 3). The sum of the van der Waals radii for H and S is 3.05 A. It may be possible that a hydrogen-bonding mechanism similar to that proposed by Sutor[27] for C–H· · O systems is operative in this structure. It is worthy to note that in the crystal structures of barium uridine phosphate,[28] adenosine uridine phosphate,[29] and calcium thymidylate,[30] a short intermolecular contact was found between the pyrimidine residue's C(6) hydrogen and a sugar oxygen.

The crystal structures of 2,4-dithiouracil and that of the seleno analog are virtually identical in their packing and resonance. Similarly, Tsernoglou reported, in comparing 2-thiouracil and 2-selenouracil, "The two structures are very similar, indeed, as far as their uracil

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part is concerned, they are more similar to each other than any two thymine or uracil containing structures of which I am aware."[18] This finding strongly supports our suggestion[31,32] that isologous sulfur and selenium compounds should be isosteric to such an extent that differences in biological activity should be ascribable not to differences in their ability to fit receptor sites, but rather to differences in electron distribution, thus adding further interest to the synthesis and biological study of sulfur and selenium isologs related to biologically active compounds.

The results reported here are in agreement with the prediction[10] that bond length and bond order of carbon–sulfur bonds in pyrimidines should be position dependent. It was also shown that the bond lengths of sulfur–hydrogen bonds were position dependent. It would be tempting to extrapolate from these data to the differences in chemical reactivity and biological activity of positional isomers of thiopyrimidines already noted; however, such speculations would seen to be premature in view of the lack of knowledge about the mechanisms involved.

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Electron Paramagnetic Resonance Studies of the Role of Solvent in Chlorophyll-Photosensitized One-Electron-Transfer Reactions Involving Quinones and Hydroquinones[1]

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Abstract: Chlorophyll-photosensitized one-electron-transfer reactions involving quinones and hydroquinones have been carried out in a variety of solvents. Two principal conclusions have emerged: (a) solvent electrons are not involved in semiquinone radical formation; (b) carbonyl-containing solvents form complexes with the semiquinone radicals causing marked changes in spin density distributions and thus in the hyperfine structure of the epr spectrum. Experiments using β-carotene as a quencher demonstrate that the chlorophyll triplet state is involved in both oxidation and in reduction processes.

We have been interested in understanding the mechanism of porphyrin-photosensitized, one-electron-transfer reactions as a means of obtaining insight into the role of chlorophyll in photosynthesis.[2-5] Our previous work has demonstrated the reversible formation of semiquinone free radicals upon illumination of degassed ethanol solutions of porphyrins and quinones or hydroquinones. It was proposed that radical formation is the result of a single electron transfer between electronically excited porphyrin and an oxidant or reductant. The present paper is a result of attempts to assess the role of the solvent in these reactions. This work has provided us with further insight into the mechanism of semiquinone formation in these systems.

Experimental Section

Electron paramagnetic resonance (epr) spectra were obtained with a Varian 100-kc modulation spectrometer, equipped with a V-FR2200 Fieldial unit and a TE_{102} cavity. A Leeds and Northrup Speedomax G recorder was used to monitor the output of the spectrometer.

For irradiation involving porphyrin excitation, a 500-w tungsten filament projection lamp was focused through a water bath containing an infrared-absorbing heat filter and through a Corning-No. 3-66 filter (which passes only wavelengths longer than 5500 A) into the cavity of the epr spectrometer.

For irradiation involving quinone excitation, a high-pressure mercury arc (Osram HBO-100w/2) was focused into the epr spectrometer cavity by means of two quartz condensing lens.

Chlorophyll *a* was prepared by the method of Anderson[6] from spinach leaves. Pheophytin (*a* and *b*) and hematoporphyrin were obtained from Fluka AG and protoporphyrin IX was obtained from Calbiochem. All were used without further purification. *p*-Benzoquinone and 1,4-naphthoquinone were obtained from Eastman and purified by sublimation. Hydroquinone and 1,4-naphthalenediol (Eastman) were recrystallized from water. Propionaldehyde, cyclohexanone, aceptophenone, and dimethylacetamide were redistilled before use. Acetic acid (Du Pont, reagent grade), ethanol (U.S.I., absolute), monochloroacetic acid (Mallinckrodt AR), dichloroacetic acid (Matheson Coleman and Bell, Reagent Grade), trichloroacetic acid (Eastman), benzoic acid (Mallinckrodt AR), formic acid (Mallinckrodt, 97–100%), and potassium acetate (Mallinckrodt AR) were used without further purification.

Results and Discussion

In Figure 1a is shown a typical epr spectrum obtained by illuminating a degassed solution of chlorophyll *a* and *p*-benzoquinone in ethanol with red light.[2] The spectrum is that characteristic of the *p*-benzosemiquinone free radical as observed, for example, by chemical reduction of *p*-benzoquinone in aqueous base. The

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