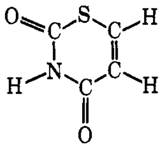
The Cis–Syn Photodimer of 1-Thiauracil. A Sulfur Analog of the Cis–Syn Photodimer of Uracil

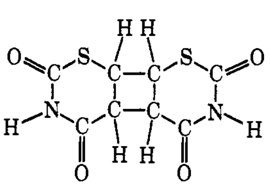
J. B. Bremner,[1a] R. N. Warrener,[1a] Elinor Adman,*[1b] and L. H. Jensen[1b]

Contribution from the Australian National University,
Canberra, A.C.T. 2600, Australia, and the Department of Biological Structure,
University of Washington, School of Medicine, Seattle, Washington 98105
Received November 17, 1970

Abstract: Irradiation of 1-thiauracil (3,4-dihydro-2,4-dioxo-2*H*-1,3-thiazine) (**1**) with ultraviolet light in the solid state is analogous to irradiation of certain naturally occurring pyrimidines and yields the cis–syn cyclobutane dimer **2**. Acid-solution photolysis of monomer and dimer leads to degradation of both. Spectral characteristics and the crystal structure of the dimer are similar to those of the uracil photodimer. The thiauracil dimer **2** crystallizes in space group $P2_1/a$ with cell parameters: $a = 26.718 \pm 0.003$, $b = 10.856 \pm 0.003$, $c = 6.951 \pm 0.0005$ Å, $\beta = 97.930 \pm 0.008°$. The structure was determined from 2737 diffractometer measured data and refined to an R of 0.032. The two dimers in the asymmetric unit each have a puckered cyclobutane ring and nonplanar heterocyclic rings. The crystal structure consists of molecules hydrogen bonded together with three moderate strength N–H···O bonds and one bifurcated weak N–H···O bond.

In view of the structural analogy of 3,4-dihydro-2,4-dioxo-2*H*-1,3-thiazine (1-thiauracil)[2a] (**1**) with uracil, and of the interest in recent years in the photochemistry of the heterocyclic constituents of nucleic acids,[2b,c] we have investigated the photochemistry of **1**, and the structure of its photoproduct **2**.


1


2

Experimental Section

Preparation of Cis–Syn Photodimer of 1-Thiauracil (2). A thin, solid film of 1-thiauracil (1.5 g) was deposited on the inside wall of a large round-bottomed flask (10 l.). A water-cooled Corex probe was inserted and irradiation (Hanovia U. V. S. 500-W Hg lamp) carried out for 27 hr in the absence of oxygen (Ar atmosphere). The resultant solid was dissolved in methanol (200 ml), mixed with silica gel (4.8 g), and evaporated to a dry powder. This residue was added to a column of silica gel (50 g, $^3/_4$ in. diameter column) and eluted with benzene–ethanol (19:1 v/v; 26 50-ml portions) followed by benzene–ethanol (9:1 v/v; four 50-ml portions). Evaporation yielded solid material which was crystallized from ethanol to yield the cyclobutane photodimer **2** (167 mg, 11%). Spectral and physical properties were: no distinct melting point, but darkens and slowly decomposes above 200°; nmr (DMSO-d_6–TMS) δ 4.00, 4.50 (complex m, 4 H, A_2X_2 pattern of cyclobutane protons), 3.30 (broad s, 2 H, N–*H*, exchanged with D_2O); ir $\nu_{\max}^{\text{Nujol}}$ 3300 (NH), 1720 and 1660 cm^{1} (C=O); uv $\lambda_{\max}^{\text{EtOH}}$ 217, 230 (sh) nm; $\lambda_{\max}^{0.1\ N\text{HCl}}$ 210, 235 nm.

Anal. Calcd for $C_8H_6N_2O_4S_2$: C, 37.22; H, 2.34. Found: C, 37.37; H, 2.69.

Photolysis of 1-Thiauracil and 1-Thiauracil Photodimer in Acid Solution. In contrast to solid-film irradiation, short-wavelength irradiation of either monomer or dimer in acid solution leads to rapid degradation; irradiation (254 nm; Hanau NK6/20 Hg lamp) of the photodimer in dilute hydrochloric acid (0.1 *M*)[3] showed the immediate appearance of a band at 272 (sh) nm, corresponding to that of the monomer.[2a] Continued irradiation (1 hr) completely removed the monomer absorption and only end absorption below 200 nm remained. Controlled experiments with the monomer confirmed that it was rapidly destroyed under these irradiation conditions; ammonium chloride was the only degradation product isolated.

Separate experiments confirmed that the monomer and dimer were stable to acid solution in the absence of ultraviolet irradiation.

Crystallographic Data

Crystals of the photodimer of the 1-thiauracil prepared by the above methods were grown from ethanol, either by evaporation or by diffusion of ether into the ethanol solution. The clear platy crystals have the {100} faces most well developed, with (0$\bar{1}$0) and {012} faces also developed. For the crystal chosen for data collection, the thickness, *i.e.*, distance between {100} faces, is 0.095 mm; that between (01$\bar{2}$) and (0$\bar{1}$2), 0.50 mm, and between (0$\bar{1}\bar{2}$) and (012), 0.42 mm. The ethanol solution yielded two crystallographic modifications, presumably of the same dimer, which both had monoclinic space groups. A form with only one molecule per asymmetric unit was observed from Weissenberg photographs to be twinned; the second form, which predominated, proved to have two molecules in the asymmetric unit. The cell parameters (obtained from the least-squares refinement of the parameters derived from measurements with Mo Kα (λ = 0.7107 Å) radiation of the 2θ values for 22 reflections) of the second form are $a = 26.718 \pm 0.003$, $b = 10.856 \pm 0.003$, $c = 6.951 \pm 0.0005$ Å, $\beta = 97.930 \pm 0.008°$. With eight molecules in the unit cell, the calculated density is 1.718 g cm^{-3}. Reflections were observed for $h0l$ only when $h = 2n$ and for $0k0$ only when $k = 2n$, which indicates $P2_1/a$ as the probable space group.

Intensity data were collected to a 2θ limit of 50° with Mo Kα radiation on a four-circle manual diffrac-

---

[1] (a) Australian National University; (b) University of Washington.

[2] (a) R. N. Warrener and E. N. Cain, *Tetrahedron Lett.*, 3225 (1966); *Aust. J. Chem.*, 23, 51 (1970); (b) J. K. Setlow in "Current Topics in Radiation Research," Vol. II, M. Ebert and A. Howard, Ed., North Holland Publishing Co., Amsterdam, 1966, p 196; (c) E. Fahr, *Angew. Chem., Int. Ed. Engl.*, 8, 578 (1969).

[3] Dilute hydrochloric acid was chosen to avoid photohydration[4] of the monomer, so that the reaction could be followed by uv spectrophotometry.

[4] A. D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids," Pergamon Press, London, 1964, p 162.

tometer using an ω–2θ scan. The limits of the scan were calculated from the expression $A + B \tan \theta$, where $A = 1.46°$ and $B = 1.0°$. Backgrounds were measured by stationary counting at each limit of the scan for one-half the time spent in the peak scan. The take-off angle of the X-ray tube was $\sim$3.5°, the scan speed 2°/min. No attenuators were used for large reflections; coincidence loss corrections[5] were applied amounting to a 6% increase in the intensity for the largest reflections (60,000 dekacounts). Four standards were measured every 4 hr throughout the period of data collection. A plot of scale factors obtained from these indicated a linear decay of 6% over a period of 200 hr of X-ray exposure; however, it appeared later that the decay was, in fact, in the counter. Intensity returned to that observed in the initial data when the counter was replaced. The data were scaled by the average factor for each shift, and Lorentz and polarization factors were calculated and applied. Although the linear absorption coefficient, μ, is not large (5.24 cm^{-1}) the shape of the crystal necessitates a correction and it was made before the final cycles of refinement. Of 2737 data, 2209 were greater than twice their estimated standard deviation, where $\sigma_I = [(T + B) + k(T - B)^2]^{1/2}$ (T is total peak count, B the background, and k a factor [set = 0.01] encompassing machine instability and other factors which cause the variation in standards). The unobserved data were set equal to $2\sigma_F$ and coded as unobserved.

Solution of the Structure and Refinement

A sharpened, origin-removed Patterson map was calculated, using an overall B of 3.3 Å^2 and scale of 2.0 (for F's). A valuable clue in determining sulfur atom positions was provided by a large asymmetric peak in the $v = 0$ plane occurring about 3.5 Å from the origin. Since there are two molecules in the asymmetric unit and two sulfur atoms per molecule, this peak was taken to be the two intramolecular S–S vectors oriented in nearly the same way. This interpretation, coupled with examination of peaks on the Harker section ($v = 1/2$), led to the correct positions of the four sulfur atoms. Calculation of the structure factors with these four positions gave 0.57 for the discrepancy index, $R = \Sigma_{hkl}(||F_o| - |F_c||)/\Sigma_{hkl}|F_o|$. Five additional atomic sites were found in an F_o synthesis using phases based on the four sulfur sites; R decreased to 0.53. Including the locations of the remaining 23 nonhydrogen atoms found in the next F_o synthesis reduced R to 0.35.

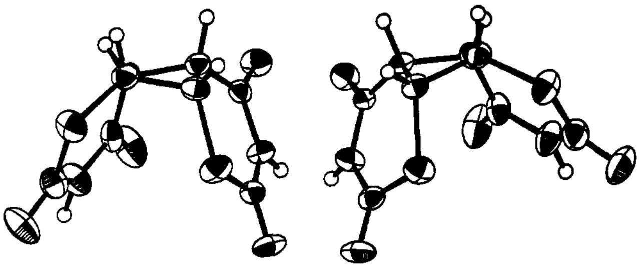


Figure 1. The asymmetric unit of the cis–syn photodimer of 1-thiauracil. Plot of atom positions with thermal ellipsoids at 50% probability level. Rings 2, 1, 4, and 3 go from left to right.

Least-squares refinement[6] of scale, positional, and thermal parameters (one cycle with isotropic, the second with anisotropic) decreased R to 0.105. Two stages of difference Fourier syntheses and further refinement yielded positions of all of the hydrogens although the difference Fourier peak corresponding to atom H(34) was quite asymmetric. Ultimately, this peak became regularly shaped in a difference Fourier synthesis using data both corrected for absorption and including approximately 100 remeasured reflections. Refinement of all positional and thermal parameters (anisotropic for nonhydrogen atoms and isotropic for hydrogen) was continued with the corrected data until the average Δ/σ was 0.1. The final R is 0.032, for the observed data.

Results

This study has revealed that 1-thiauracil is strikingly similar to uracil in its photoreactions, namely formation of cyclobutane-type dimers,[7] photoreversal of the dimer to monomer,[8] and degradation of monomer on solution photolysis.[9] The crystallographic results confirm that both photodimers from the solid state are the cis–syn isomeric form.

Figure 1 is a plot of the two molecules in the asymmetric unit, showing the loci of displacement due to thermal motion at the 50% probability level. The atomic coordinates and thermal parameters are listed in Table I. Figure 2 is a schematic drawing of the molecules showing bond distances and angles. Standard deviations in bond lengths are 0.003 Å for C–S, C–O, C–N, and C–C and 0.03 Å for C–H and N–H bonds.

Discussion

The structural features of the thiauracil photodimer are quite similar to those of the uracil photodimer.[10] The cyclobutane ring is puckered with a dihedral angle of 153° and the concomitant "twist" of the six-membered rings is 29°, compared with 155 and 24°, respectively, for the uracil dimer.

---

(5) E. Sletten, J. Sletten, and L. H. Jensen, *Acta. Crystallogr., Sect. B*, 25, 1330 (1969).

(6) In the full-matrix least-squares refinement the quantity minimized was $\Sigma_{hkl}w(|F_o| - |F_c|)^2$ in which weights were defined as follows: $1/\sqrt{w} = \sigma_F = \sigma_I/2\,(ILp)^{1/2}$ where I = net intensity, Lp = Lorentz and polarization correction. Reflections coded[2] as unobserved were included only when calculated to be greater than the cutoff value. In later stages of refinement the number of parameters exceeded the storage capacity of the computer, so the parameters were divided into three groups: group 1, all S, C(5), C(6), H(5), and H(6); group 2, all S, C(2), O(2), N(3), and H(3); group 3, all S, C(4), O(4), N(3), H(3), and C(5). Scattering factors used were as follows: sulfur, B. Dawson, *Acta Crystallogr.*, 13, 403 (1960); oxygen, J. Bergius, I. Haanappel, M. Potters, B. O. Loopstra, C. H. MacGillavry, and A. L. Veenendaal, *ibid.*, 8, 478 (1955); nitrogen and carbon, R. McWeeney, *ibid.*, 4, 513 (1951); hydrogen, R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 42, 3175 (1965). A list of observed and calculated structure factors has been deposited as Document No. NAPS–01358 with the ASIS National Auxiliary Publications Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. A copy may be secured by citing the document number and by remitting $2.00 for microfiche or $5.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS.

(7) G. M. Blackburn and R. J. H. Davies, *Tetrahedron Lett.*, 4471 (1966). Other forms of the uracil cyclobutane dimer have been found by other sensitized and nonsensitized solution photolyses (C. L. Greenstock and H. E. Johns, *Biochem. Biophys. Res. Commun.*, 30, 21 (1968)), but the predominant form found in the solid state is the cis–syn type. Other forms of the 1-thiauracil dimer have not been sought.

(8) K. C. Smith, "Photophysiology," Vol. II, A. C. Giese, Ed., Academic Press, New York, N. Y., 1964, p 352.

(9) W. E. Conrad, *Radiat. Res.*, 1, 523 (1954); E. Ben-Hur and I. Rosenthal, *Photochem. Photobiol.*, 11, 163 (1970), and references therein.

(10) E. Adman and L. H. Jensen, *Acta Crystallogr., Sect. B*, 26, 1326 (1970); E. Adman, M. P. Gordon, and L. H. Jensen, *Chem. Commun.*, 1019 (1968).

Table I. Positional and Thermal Parameters for Cis–Syn 1-Thiauracil Dimer

	Fractional coordinates			Thermal parameters[a] (B in Å^2)					
Atom	x	y	z	B_{11} (or B)	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
S(11)	0.09499	0.22418	0.4802	4.47	2.18	3.72	0.60	1.43	1.05
O(21)	0.06402	0.3054	0.7870	4.78	5.11	2.65	0.16	1.23	1.71
C(21)	0.07115	0.3365	0.6258	2.42	2.82	2.43	−0.19	0.22	0.59
N(31)	0.06091	0.4530	0.5583	3.15	2.40	1.96	0.11	0.67	0.18
O(41)	0.05479	0.6112	0.3517	3.79	2.13	3.10	0.86	0.48	0.40
C(41)	0.06976	0.5079	0.3859	1.95	2.21	1.95	−0.21	−0.12	−0.00
C(51)	0.09854	0.4377	0.2515	2.52	2.05	2.01	0.29	0.42	0.39
C(61)	0.10107	0.2960	0.2507	3.16	2.21	2.03	0.05	0.04	−0.12
S(12)	0.20567	0.20271	0.3090	3.69	3.16	3.51	1.34	0.30	−0.46
O(22)	0.26123	0.2104	0.6406	4.26	3.68	5.43	1.38	−1.54	−0.23
C(22)	0.22980	0.2676	0.5329	2.91	3.02	3.81	0.38	0.19	−0.08
N(32)	0.21387	0.3813	0.5869	3.41	2.88	3.94	0.60	−0.43	−0.60
O(42)	0.16860	0.5521	0.5801	3.97	3.58	5.82	1.17	−0.63	−1.82
C(42)	0.17948	0.4631	0.4926	1.96	2.14	3.92	−0.10	0.41	−0.03
C(52)	0.15778	0.4413	0.2865	2.68	2.21	3.16	0.20	1.02	0.66
C(62)	0.15673	0.3086	0.2117	3.06	3.16	2.18	0.95	0.64	0.21
H(31)	0.048	0.504	0.647	3.6					
H(51)	0.0854	0.471	0.117	2.2					
H(61)	0.0790	0.252	0.154	2.5					
H(32)	0.229	0.409	0.722	7.5					
H(52)	0.1725	0.499	0.212	3.2					
H(62)	0.1581	0.307	0.073	3.4					
S(13)	0.32031	0.50021	0.2552	3.03	3.27	4.09	0.99	−0.12	−1.29
O(23)	0.25037	0.4428	−0.0163	3.12	5.95	4.60	1.42	−0.87	−1.13
C(23)	0.29042	0.4065	0.0697	2.83	3.76	3.19	0.21	0.44	−0.67
N(33)	0.31102	0.2967	0.0229	2.63	3.69	3.82	0.02	−0.44	−1.48
O(43)	0.36953	0.1503	0.0207	3.30	4.10	5.80	0.42	−0.20	−2.96
C(43)	0.35504	0.2386	0.1022	2.26	2.52	3.62	−0.25	0.51	−0.77
C(53)	0.38251	0.2832	0.2917	2.41	2.03	2.50	−0.06	0.70	−0.04
C(63)	0.37798	0.4199	0.3380	2.33	2.77	2.25	0.12	0.13	−0.51
S(14)	0.41811	0.49310	0.0060	4.00	2.33	3.14	0.48	0.50	0.81
O(24)	0.46056	0.4163	−0.2791	5.00	4.22	1.98	−0.31	0.41	0.83
C(24)	0.45420	0.3915	−0.1144	2.70	2.48	2.18	−0.61	−0.21	0.06
N(34)	0.47412	0.2863	−0.0252	3.58	2.53	2.10	0.37	0.82	0.08
O(44)	0.48337	0.1309	0.1883	3.46	2.63	3.08	1.17	0.87	0.75
C(44)	0.46663	0.2331	0.1485	1.91	2.70	1.62	−0.19	0.11	0.09
C(54)	0.43983	0.3066	0.2843	2.38	2.11	1.58	0.11	−0.04	−0.05
C(64)	0.42815	0.4448	0.2565	2.70	2.14	2.13	−0.29	−0.05	−0.45
H(33)	0.293	0.262	−0.094	6.3					
H(53)	0.3746	0.230	0.387	2.4					
H(63)	0.3825	0.435	0.476	2.9					
H(34)	0.488	0.240	−0.098	4.2					
H(54)	0.4583	0.292	0.415	1.9					
H(64)	0.4526	0.501	0.324	2.4					
Standard Deviations[b]									
S	0.00003	0.00006	0.0001	0.04	0.03	0.03	0.03	0.03	0.03
O	0.00007	0.0002	0.0003	0.10	0.10	0.09	0.08	0.07	0.08
N	0.00008	0.0002	0.0003	0.10	0.10	0.10	0.08	0.08	0.08
C	0.00009	0.0002	0.0004	0.12	0.12	0.12	0.10	0.09	0.10
H(C)	0.0008	0.002	0.003	0.5					
H(N)	0.001	0.003	0.004	0.8					

[a] Thermal parameters are in the form: $\exp(-B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)$. [b] Standard deviations are the root mean square for each type of atom. Differences within each group from its mean are no greater than the error made in rounding to one significant digit.

Comparison of the heterocyclic ring bond lengths with those of other pyrimidine photodimers (Table II) shows that the conjugation through the C(═O)NHC(═O) system has changed; C(2)–N(3) and N(3)–C(4) are more nearly equal as in uracil. The unequal S–C bonds are analogous to those found in a derivative of thiazoline[11] in which the carbons of the C–S–C group are also nonequivalent. Similar unequal lengths involving N(1) are observed in the usual pyrimidine dimers.

A study of the bond lengths of the cyclobutane rings leads to interesting comparisons (see Figure 2). The interheterocyclic ring bonds show the same feature as the uracil dimer: the C(6)–C(6) bond is shorter than the C(5)–C(5) bond (1.563 and 1.572 Å, respectively, for the uracil dimer). This was ascribed to the fact that the bonds are between carbon atoms with different substituents[10] (NH and C(═O)) as is presently the case. However, now the difference between the two C(5)–C(6) bonds (see Table II) is also significant (whereas the observed difference is not significant in the uracil photodimer, although the sense is the same). This observation may be correlated with the following: the two halves of the dimer differ according to the axial or equatorial character of the substituents of the cyclobutane ring. Smaller angles are made by axial substituents with the arms of the cyclobutane ring permitting characterization of the nature of the substituents. For example, the angles S(11)–C(61)–C(62) and S(11)–C(61)–C(51) are markedly smaller than the angles

---

[11] J. L. Flippen and I. L. Karle, *J. Phys. Chem.*, 74, 769 (1970).

Table II. Comparison of Heterocyclic Ring Bond Lengths in Pyrimidine Photodimers with Thiauracil Photodimers and Uracil

Pyrimidine	N(1)-C(2)	C(2)-N(3)	N(3)-C(4)	C(4)-C(5)	C(5)-C(6)	C(6)-N(1)	Ref
Cis-syn	1.330	1.398	1.364	1.497	1.533	1.435	*a*
uracil dimer	1.336	1.390	1.360	1.498	1.540	1.441	
Trans-anti thymine dimer	1.334	1.390	1.357	1.508	1.547	1.440	*b*
Cis-anti	1.342	1.426	1.378	1.509	1.533	1.431	*c*
dimethyl thymine dimer	1.344	1.406	1.377	1.503	1.529	1.449	
1,1′-Trimethylene-	1.341	1.395	1.365	1.513	1.555	1.444	*d*
linked cis-syn thymidine dimer	1.339	1.388	1.374	1.508	1.548	1.455	
Cis-syn[f] 1-thiaura-	1.759	1.364	1.388	1.498	1.540	1.803	This
cil dimer	1.748	1.375	1.377	1.489	1.530	1.801	work
	1.747	1.370	1.381	1.497	1.527	1.795	
	1.752	1.372	1.378	1.492	1.539	1.803	
Uracil	1.371	1.376	1.371	1.430	1.340	1.358	*e*

[a] Reference 10. [b] N. Camerman and S. C. Nyburg, *Acta Crystallogr., Sect. B*, **25**, 388 (1969). [c] N. Camerman, D. Weinblum, and S. C. Nyburg, *J. Amer. Chem. Soc.*, **91**, 982 (1969). [d] N. J. Leonard, K. Golankiewic, R. S. McCredie, S. M. Johnson, and I. C. Paul, *ibid.*, **91**, 5855 (1969). [e] R. F. Stewart and L. H. Jensen, *Acta Crystallogr.*, **23**, 1102 (1967). [f] Read S(1) for N(1).

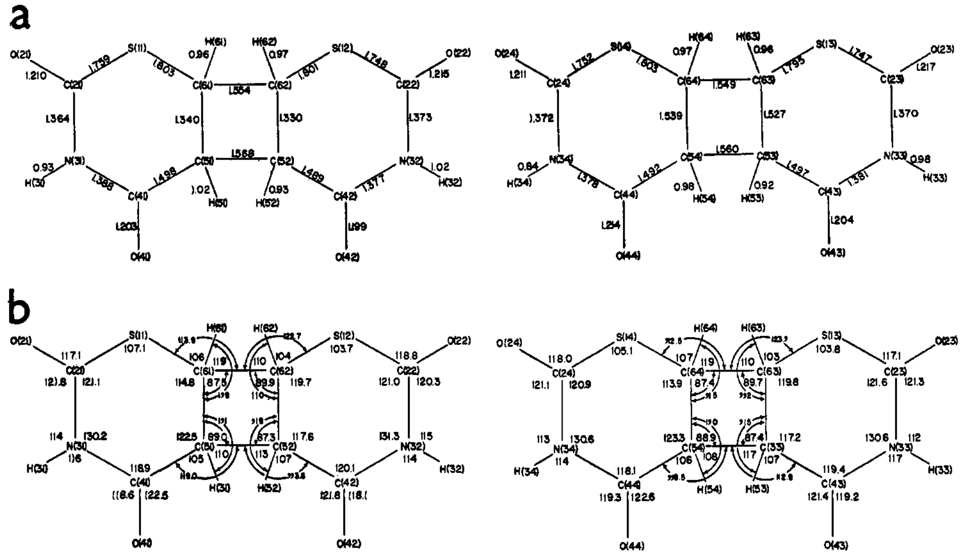


Figure 2. Bond distances (a) and angles (b) for the cis-syn 1-thiauracil dimer. Standard deviations in bond lengths and bond angles are 0.003 Å and 0.10° for nonhydrogen atoms and 0.03 Å and 1.00° for bonds and angles involving hydrogen atoms.

S(12)-C(62)-C(61) and S(12)-C(62)-C(52), indicating that S(11) is axial and S(12) equatorial. Thus the shorter C(5)-C(6) bond may be seen to be between those atoms to which axial carbonyl and equatorial sulfur are bound. In the case of the "anti" dimers, carbonyl groups are both axial, and the C(5)-C(6) bonds are identical (see Table II).[12,13]

The conformations of the heterocyclic rings are schematically illustrated in Figure 3. Three of the four rings are essentially equivalent to each other and to the uracil dimer, while the fourth differs—the primary difference between the two molecules of the crystallographic asymmetric unit. The conformation effectively establishes the direction of the carbonyl groups so, if the energy lost due to a change in conformation is less than that gained by forming hydrogen bonds, the packing and hydrogen bonding arrangement is probably sufficient to determine the conformation. The conformations of the heterocyclic portions of other pyrimidine photodimers are usually a minor variation of a twist-chair conformation consistent with alternative packing and hydrogen bonding modes. For example, the cis-anti dimethyl thymine dimer[13] (not involved in hydrogen bonding) has a conformation such that five of the six atoms in the heterocyclic ring are nearly coplanar; the trans-anti dimer[12] has an essentially flat ring with carbonyl groups pointed toward positions intermediate to those observed in the present compound.

In contrast to the uracil photodimer which has more donor sites for hydrogen bonding, not all the potential receptor sites of the 1-thiauracil can be utilized in such bonding. Of eight carbonyl oxygens, five act as receptors, including O(21), O(22), O(23), O(41), and O(44). It is interesting that ring 4, which has the different conformation, has only one carbonyl involved in hydrogen bonding, whereas ring 1 has two. However, atom N(34) in ring 4 appears to participate in a bifurcated hydrogen bond, sharing its hydrogen with carbonyl oxygens of two different neighboring molecules. The geometry of this arrangement is shown in Figure 4. The bifurcated hydrogen bond parameters are similar

(12) See Table II, footnote *b*.
(13) See Table II, footnote *c*.

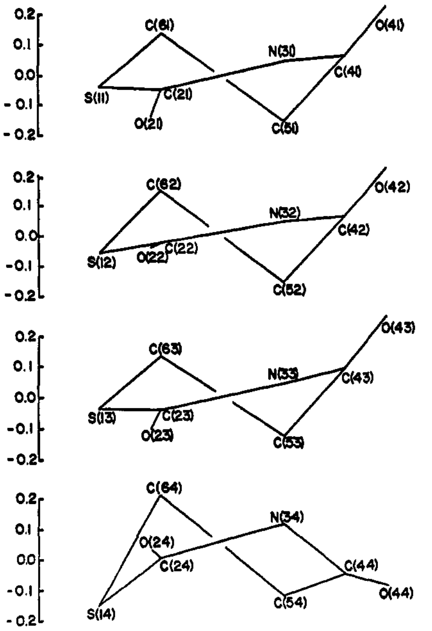


Figure 3. Schematic drawing of conformations of heterocyclic rings of the 1-thiauracil dimer. The ordinate is the distance in ångströms from a least-squares plane of the 6-ring atoms in each case.

to those of α-glycine.[14] It is at best a weak linkage; using accepted values of van der Waals contact radii[15] of each of the atoms (C, 1.7 Å; N, 1.5 Å; O, 1.4 Å; H, 1.2 Å) only the O···H distances appear to be significantly less than mere contact distances. The other hydrogen bonds (data in Table III) are typical NH···O hydrogen bonds,[16] somewhat weaker than those in the uracil photodimer.

Table III. Hydrogen Bond Geometry[a] of Thiauracil Dimer

Type	Atoms in bond	O–N	O–H	C–O–N	O–H–N, deg
I	N(31)–H(31)···O(44)[b]	2.968	2.044	124.5	173
II	N(32)–H(32)···O(23)[c]	2.874	1.867	123.7	171
III	N(33)–H(33)···O(22)[d]	2.956	2.00	125.4	171
IV	N(34)–H(34)···O(21)[e]	3.05	2.34	136.1	143.8
IV	···O(41)[f]	2.98	2.41	142.1	126.4

[a] Superscripts refer to symmetry-related positions. [b] $-\frac{1}{2} - x$, $\frac{1}{2} + y$, $1 - z$. [c] $-x$, y, $1 + z$. [d] $-x$, y, $-1 + z$. [e] $-\frac{1}{2} + x$, $\frac{1}{2} - y$, $-1 + z$. [f] $-\frac{1}{2} - x$, $-\frac{1}{2} + y$, $-z$.

Hydrogen bonds are prominent in the overall arrangement of molecules in the crystal (see Figure 5). Two molecules of the asymmetric unit are joined together by pairs of hydrogen bonds: N(32)···O(23); N(33)···O(22) (II and III,[17] oriented vertically in Figure 5). Pairs of molecules are in turn joined to screw-related pairs by single hydrogen bonds N(31)···O(44) (I) forming ribbons of molecules extended in the *b* direction. The bifurcated H bond then both joins ribbons in the *c* direction [N(34)···O(41)] forming sheets extended normal to *a**, and joins the sheets together [N(34)···O(21)]. The *a** direction is also the direction of least rapid growth in the development of the crystals, consistent with the presumption that the bifurcated hydrogen bond in that direction is at best a weak linkage, but also indicating that additional forces are extant during crystallization which promotes growth in the *c* direction where there is apparently an equally weak bond.

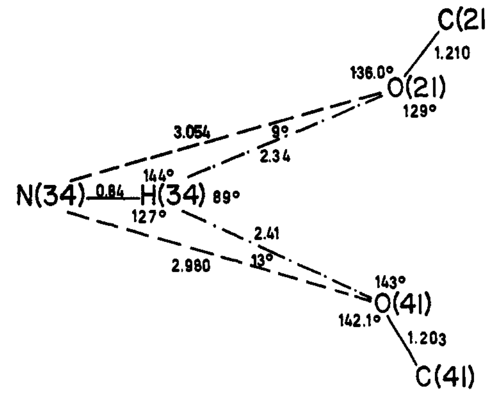


Figure 4. Geometry of the bifurcated hydrogen bond.

The molecules, in fact, are quite tightly packed as indicated by the number of intermolecular close approaches less than 3.4 Å listed in Table IV. Most of these involve carbonyl oxygens. Six of the distances involving the three oxygen atoms O(42), O(43), and O(44) approach the lower limit of van der Waals contact distances. Of these, O(43) is closely packed with the back side of the cyclobutane rings [O(43)–H(51) = 2.54, O(43)–H(52) = 2.45 Å] while O(44) and O(42) are contiguous with C(5)–C(4) bonds of rings 1 and 3, respectively.

Table IV. Contact Distances Less than 3.4 Å[a]

$O(21)^{\mathrm{I}}$–C(61)	3.240	O(43)–$C(51)^{\mathrm{V}}$	3.132
$O(21)^{\mathrm{II}}$–O(41)	3.314	O(43)–$C(52)^{\mathrm{V}}$	3.175
$O(41)^{\mathrm{IV}}$–$N(31)^{\mathrm{II}}$	3.312	O(24)–$C(54)^{\mathrm{I}}$	3.236
$O(41)^{\mathrm{IV}}$–C(54)	3.289	O(24)–$C(64)^{\mathrm{I}}$	3.240
$O(41)^{\mathrm{IV}}$–C(53)	3.360	O(24)–$C(63)^{\mathrm{I}}$	3.214
O(41)–$O(44)^{\mathrm{III}}$	3.353		
		$O(44)^{\mathrm{III}}$–C(51)	3.137
O(22)–$O(42)^{\mathrm{IV}}$	3.100	$O(44)^{\mathrm{III}}$–C(61)	3.214
O(22)–$C(42)^{\mathrm{IV}}$	3.313	$O(44)^{\mathrm{III}}$–C(41)	3.934
		$O(44)^{\mathrm{III}}$–N(31)	3.204
$O(42)^{\mathrm{IV}}$–C(53)	3.048		
$O(42)^{\mathrm{IV}}$–C(43)	3.124		
$O(42)^{\mathrm{IV}}$–O(43)	3.264		

[a] Roman numeral superscripts refer to atom positions generated by the following symmetry relations: I, $x, y, z - 1$; II, $-x, 1 - y, 1 - z$; III, $\frac{1}{2} + x, \frac{1}{2} - y, z$; IV, $\frac{1}{2} - x, -\frac{1}{2} + y, 1 - z$; V, $\frac{1}{2} - x, -\frac{1}{2} + y, -z$.

In summary, the structure of the 1-thiauracil photodimer illustrates several points: (1) the molecule is the same isomeric form as the cis–syn photodimer of uracil with essentially the same features including a puckered cyclobutane ring; (2) two presumably identical bonds in the cyclobutane ring are, in fact, significantly different due to the conformation of substituents; (3) a bifurcated hydrogen bond appears to exist and to be an important factor in the overall crystal structure.

---

(14) R. E. Marsh, *Acta Crystallogr.*, 11, 654 (1958).

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

(16) J. Donohue in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968, p 458.

(17) Roman numerals refer to those in Table IV.

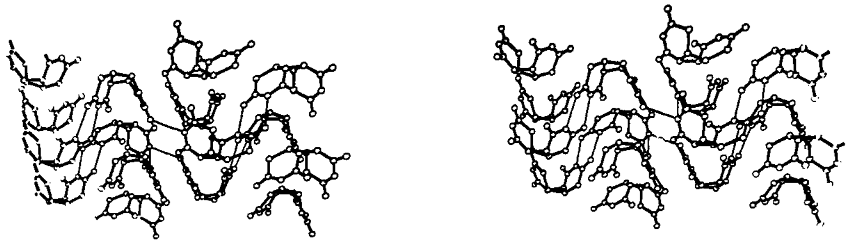


Figure 5. Stereodrawing of the contents of two unit cells. In the *a* axis, positive direction goes to the right. The *c** direction goes from bottom to top (from 0 to 2*c*) and *b* goes into the page. Hydrogen bonds are represented by narrow lines.

Acknowledgments. All the programs used were part of "XRAY67 Program System for X-ray Crystallography," Technical Report 67-58, Computer Science Center, University of Maryland. The authors acknowledge the work of Keith Watenpaugh in making these programs usable on the University of Washington IBM 7090–7094 computer. The plots in Figures 1 and 5 were done with Carroll Johnson's ORTEP program, ORNL-3794, and absorption corrections were done using a 1620 program[18a,b] adapted to the IBM 7090–7094 by Sue Eilers and D. Cullen and modified by one of the authors (E. A.). This work was supported by U. S. Public Health Service Grant No. GM-10828 from the National Institutes of Health.

(18) A. H. Tompa, "The Absorption Correction in Crystal Structure Analysis," 1620 General Program Library, 8.4.014; J. de Meulenaer and H. Tompa, *Acta Crystallogr.*, **19**, 1014 (1965).

A Nuclear Magnetic Resonance Study of Lysozyme Inhibition. Effects of Dimerization and pH on Saccharide Binding

Joel F. Studebaker,[1a,b] Brian D. Sykes,*[1a] and Richard Wien[1b,c]

Contribution from the Department of Chemistry,
Harvard University, Cambridge, Massachusetts 02138,
and the Department of Chemistry, Stanford University,
Stanford, California 94305. *Received September 14, 1970*

Abstract: Nuclear magnetic resonance methods were used to study the interaction of *N*-acetyl-α- and -β-D-glucosamine with lysozyme. The association constant and bound chemical shift of the acetyl protons of each anomer have been determined as a function of pH. These results are interpreted in terms of the amino acid residues in the active site of lysozyme. In addition, several models for lysozyme dimerization are tested.

Important clues in elucidating the mechanism of action of an enzyme can be gained from a study of the pH dependence of substrate and inhibitor binding to the enzyme. Ribonuclease,[2a] α-chymotrypsin,[2b,c] and lysozyme,[3–6] for example, have received detailed study. In the case of lysozyme, both the rates of cleavage of polysaccharide substrates and the inhibition by saccharides are pH dependent.

Several workers have used ultraviolet spectroscopy methods to study the pH dependence of the inhibition of lysozyme by *N*-acetyl-α-D-glucosamine (α-NAG)[3] and tri-*N*-acetyl-D-glucosamine.[4–6] Their results show that a plot of the association constant *vs.* pH is bell shaped, indicating that at least two ionizable groups are involved in the lysozyme mechanism.

Nuclear magnetic resonance methods have also been used to study the interaction of inhibitors with lysozyme,[7–13] and Dahlquist and Raftery have studied the pH dependence of the β-methyl-*N*-acetyl-D-glucosaminide (β-MNAG) inhibition of lysozyme by these methods.[9] The advantage of nmr methods is that a study of the observed chemical shift or relaxation times of nuclei on the inhibitor molecule as a function of inhibitor and enzyme concentration at a given pH yields not only the association constant at that pH, but also the chemical shift or relaxation times of those nuclei in the enzyme–inhibitor complex. These parameters char-

(1) (a) Harvard University; (b) National Science Foundation Predoctoral Fellow; (c) Stanford University.

(2) (a) T. C. French and G. G. Hammes, *J. Amer. Chem. Soc.*, **87**, 4669 (1965); (b) J. R. Garel and B. Labouesse, *J. Mol. Biol.*, 47, 41 (1970), and references therein; (c) J. McConn, G. D. Fasman, and G. P. Hess, *ibid.*, **39**, 551 (1969), and references therein.

(3) C. J. Kowalski and P. R. Schimmel, *J. Biol. Chem.*, 244, 3643 (1969).

(4) S. S. Lehrer and G. D. Fasman, *Biochem. Biophys. Res. Commun.*, 23, 133 (1966).

(5) J. A. Rupley, L. Butler, M. Gerring, F. J. Hartdegen, and R. Pecoraro, *Proc. Nat. Acad. Sci. U. S.*, 57, 1088 (1967).

(6) F. W. Dahlquist, L. Jao, and M. Raftery, *ibid.*, 56, 26 (1966).

(7) M. A. Raftery, F. W. Dahlquist, S. I. Chan, and S. M. Parsons, *J. Biol. Chem.*, 243, 4175 (1968).

(8) F. W. Dahlquist and M. A. Raftery, *Biochemistry*, **7**, 3269 (1968).

(9) F. W. Dahlquist and M. A. Raftery, *ibid.*, 7, 3277 (1968).

(10) F. W. Dahlquist and M. A. Raftery, *ibid.*, **8**, 713 (1969).

(11) M. A. Raftery, F. W. Dahlquist, S. M. Parson, and R. G. Wolcott, *Proc. Nat. Acad. Sci. U. S.*, **62**, 44 (1969).

(12) B. D. Sykes, *Biochemistry*, **8**, 1110 (1969).

(13) B. D. Sykes and C. Parravano, *J. Biol. Chem.*, **244**, 3900 (1969).