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The Crystal and Molecular Structure of 2-Thiouridine

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The 'minor' nucleoside 2-thiouridine, $C_9H_{12}O_5N_2S$, crystallizes in a monoclinic cell, space group $P2_1$ with $a = 5{\cdot}049$ (2), $b = 7{\cdot}526$ (2), $c = 14{\cdot}050$ (3) Å, $\beta = 90{\cdot}17$ (2)°, and $d = 1{\cdot}619$ g cm^{-3} (for $Z = 2$) at 22 ± 2°C. The structure was derived from 1334 unique intensities measured with an Oak Ridge computer-controlled diffractometer to a limit of $\sin\theta/\lambda = 0{\cdot}65$ Å^{-1} with Nb-filtered Mo $K\alpha$ radiation. Atomic parameters were obtained by a combination of Patterson and Fourier techniques and refined by full-matrix least squares to a final $R(F)$ value of 0·023 for all data. The bond lengths and angles in the molecule agree well with those of other thiopyrimidines [C(2)–S = 1·677 Å]. The conformation of the sugar ring relative to the base is *anti* with a torsion angle χ[O(1′)–C(1′) → N(1)–C(6)] of 17°. The sugar exists in the 3′-*endo* conformation. The O(5′)–C(5′) bond is *gauche* to C(4)–O(1′) and *trans* to C(4′)–C(3′) [torsion angles of 74 and −169° respectively]. The molecules are linked together in the crystal by hydrogen bonds in an intricate network which is identical to that inferred by Kojić-Prodić, Liminga, Šljukić & Ružić-Toroš [*Acta Cryst.* (1974), B**30**, 1550–1555] for the crystal structure of 5,6-dihydro-2-thiouridine.

Introduction

2-Thiouridine is a minor nucleoside of transfer RNA, derivatives of which have been isolated from several tRNA species (Baczynsky, Biemann & Hall, 1968; Carbon, David & Studier, 1968). A derivative of 2-thiouridine has also been found in the anticodon sequence of GAA-specific, glutamic acid tRNA from yeast (Yoshida, Takeishi & Ukita, 1970), where its role may be to prevent recognition of nonspecific codons. This study was undertaken to determine whether the 2-thio substitution on uridine confers any unusual conformational aspects to the nucleoside and to examine its potentialities for hydrogen bonding.

Since the beginning of this work, the structure of 5,6-dihydro-2-thiouridine has been determined (Kojić-Prodić, Liminga, Šljukić & Ružić-Toroš, 1974); the similarities of the structures as well as the details of the hydrogen bonding will be discussed.

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Experimental

The sample of 2-thiouridine used in this study was synthesized by Dr B. C. Pal (1971). Crystals were obtained as pale-yellow prisms from slowly evaporated aqueous solutions. The crystal used in the diffraction study was bounded by pairs of faces at distances from an internal origin of 0·125 mm for $\{01\bar{1}\}$ and $\{011\}$, 0·07 mm for $\{001\}$, and 0·21 mm for $\{10\bar{1}\}$ and by a single plane (104) at a distance of 0·10 mm. The space group and approximate cell dimensions were determined from precession and Weissenberg photographs. More accurate cell dimensions were obtained by least-squares refinement of the observed setting angles for ten Mo $K\alpha_1$ reflections measured with an Oak Ridge computer-controlled diffractometer (Busing, Ellison, Levy, King & Roseberry, 1968). The density of the crystals was measured by flotation in a mixture of bromoform and bromobenzene. Crystal data are summarized in Table 1.

Intensity data were collected on the diffractometer with Nb-filtered Mo $K\alpha$ radiation and the spindle axis roughly parallel to **a***. The intensities of 1334 unique reflections with $\sin\theta/\lambda \leq 0{\cdot}65$ Å^{-1} were measured by the θ–2θ step-scan technique. Of these, 1323 had net-positive intensities and 1311 had intensities greater than one estimated standard deviation. To minimize the errors introduced by inaccurate dead-time corrections, 25 reflections were remeasured at reduced tube currents. Several reflections, at low 2θ values, were also rescanned without the Nb filter to eliminate errors in the background measurements introduced by the proximity of the filter absorption edge. A single reference reflection was monitored throughout the data collection (at intervals of 25 reflections), and its intensity showed random fluctuations of less than 1%.

Lorentz and polarization factors were applied to the data in the usual manner. Absorption corrections, in the range 0·92 to 0·96, were applied to the data with the program *ORABS*-2 (Busing & Levy, 1957). Each intensity was assigned a variance $\sigma^2(I)$, based on counting statistics, plus a term $(0{\cdot}03\ I)^2$, where I is the net intensity, to allow for a lack-of-confidence in the experiment (Peterson & Levy, 1957).

Structure solution and refinement

A trial structure for the nonhydrogen atoms was derived from a sharpened, three-dimensional Patterson function (program *FORDAPER* of A. Zalkin, modified by G. Brunton), calculated with (E^2-1) coefficients (program *FAME* of R. B. K. Dewar & A. Stone). The two centrosymmetrically related images of the molecule (arising from sulfur-to-light-atom vectors) in the Patterson function were sorted out on the basis of chemical criteria. The image with the known absolute configuration for D(+)-ribose was selected for refinement.

This trial structure was refined using the 1323 nonzero intensities by the full-matrix least-squares method [program *XFLS*-3, a modification of *ORFLS* (Busing, Martin & Levy, 1962)], in which the function minimized was $\Sigma\, w(F_o^2-F_c^2)^2$, with weights w equal to the reciprocals of the estimated variances. With isotropic thermal parameters, the refinement converged to $R(F)=\Sigma\big||F_o|-|F_c|\big|/\Sigma|F_o|=0{\cdot}075$. The H atoms were then located from a three-dimensional difference Fourier synthesis, and refinement was continued with anisotropic thermal parameters for the nonhydrogen atoms and isotropic thermal parameters for the H atoms. The final values for $R(F)$ and $R_w(F^2)=[\Sigma\, w|F_o^2-F_c^2|^2/\Sigma|F_o^4|]^{1/2}$ were 0·023 and 0·059 respectively. The standard deviation of an observation of unit weight, defined as $[\Sigma\, w|F_o^2-F_c^2|^2/(n-p)]^{1/2}$, where n is the number of observations and p is the number of adjusted parameters, was 1·52. The largest parameter shift during the last cycle of refinement was less than 10% of its estimated standard deviation. A final difference map calculated without H atom contributions, showed no nonhydrogen peaks larger than $\frac{1}{4}$ the height of a H atom (0·6 to 1·0 e Å^{-3}).

Scattering factors for the nonhydrogen atoms were taken from *International Tables for X-ray Crystallography* (1962) and for H from Stewart, Davidson & Simpson (1965). Corrections for anomalous scattering due to S were deemed unnecessary [$\Delta f'=0{\cdot}13$, $\Delta f''=0{\cdot}16$; Cromer (1965)]. The final values of the atomic parameters and their estimated standard deviations are given in Table 2. The observed and calculated structure factors are given in Table 3.*

Discussion of the 2-thiouridine structure

Fig. 1 is a perspective drawing of the molecule showing the atom labeling. Bond distances and angles within the molecule are given in Table 4.

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* Table 3 has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32022 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1. *Crystal data for* 2-*thiouridine*

Formula:	$C_9H_{12}O_5N_2S$	F.W.	260·3
a	5·049 (2) Å	$F(000)$	272
b	7·526 (2)	d_c	1·619 g cm^{-3} (for $Z=2$)
c	14·050 (3)	d_o	1·62 (1) (by flotation)
β	90·17 (2)°	T	22 ± 2°C

Space group: $P2_1$, absences $0k0$, $k=2n+1$
Crystal dimensions: 0·12 × 0·25 × 0·40 mm
Linear absorption, μ(Mo $K\alpha$) = 0·32 mm^{-1}

The bond distances and angles in the uracil moiety are unremarkable. The C(2)—S distance, 1·677 (3) Å, is normal for a thiopyrimidine in the 'diketo' form [see, for example, the reviews in Saenger & Suck (1971*a*) and Lin, Sundaralingam & Arora (1971)]. The pyrimidine ring is planar to within ±0·02 Å (Table 5), while C(1′) and O(4) are displaced from this plane by 0·16 and 0·11 Å respectively. The S substituent, at position 2, is in the plane.

The furanose ring is in the 3′-*endo* conformation, with C(3′) displaced by 0·63 Å from the plane of the other four atoms (Table 5). The bond distances and angles associated with the ribose moiety are normal for this 3′-*endo* conformation, with the exception of the C(2′)—O(2′) and C(3′)—O(3′) distances. In the present study these distances are equal, while several reviews (Saenger & Eckstein, 1970; Sundaralingam & Jensen, 1965) have indicated that the C—O distance involving the *endo* atom may be shortened.

The conformation about the C(4′)—C(5′) bond is *gauche–trans* [O(5′)—C(5′) → C(4′)—O(1′) = 74, O(5′)—C(5′) → C(4′)—C(3′) = −169°], a conformation observed less frequently than *gauche–gauche* in pyrimidine nucleosides having the 3′-*endo* conformation (Sundaralingam, 1973). This may be due to the hydrogen bond involving the O(5′)—H group (see below).

The conformation of the base relative to the sugar is *anti* [O(1′)—C(1′) → N(1)—C(6) = 17°], the low value of the torsion angle being in agreement with values usually found for 3′-*endo* pyrimidines (Sundaralingam, 1973). This conformation appears to be controlled by several short intramolecular contacts [*e.g.* HC(1′)—S = 2·64, HC(6)—O(1′) = 2·35 Å].

The hydrogen-bonding network in the crystal struc-

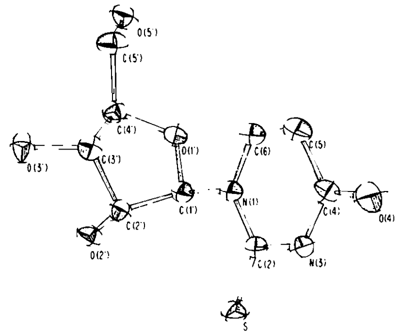

Fig. 1. Perspective view of the molecule with thermal ellipsoids drawn at 50% probability (Johnson, 1965).

Table 2. *Positional and thermal parameters*

Standard deviations in the least significant figures are given in parentheses.

(a) Nonhydrogen atoms (all values are × 10^4)

The form of the temperature factor is $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S	16 (1)	2500*	1793 (1)	486 (2)	72 (1)	42 (1)	−5 (1)	−51 (1)	−4 (1)
O(4)	−5202 (3)	−1822 (3)	−7 (1)	361 (6)	123 (2)	51 (1)	−16 (3)	−54 (2)	−8 (1)
N(1)	743 (3)	−1033 (2)	1845 (1)	279 (6)	75 (2)	25 (1)	10 (3)	11 (1)	2 (1)
N(3)	−2519 (3)	6 (2)	828 (1)	309 (6)	79 (2)	31 (1)	14 (3)	−24 (2)	2 (1)
C(2)	−604 (4)	390 (2)	1479 (1)	297 (6)	79 (3)	24 (1)	0 (3)	−6 (2)	0 (1)
C(4)	−3294 (4)	−1675 (3)	531 (1)	289 (7)	97 (3)	29 (1)	−18 (4)	−8 (2)	−3 (1)
C(5)	−1718 (4)	−3098 (3)	909 (1)	344 (7)	80 (3)	34 (1)	−9 (4)	−9 (2)	−10 (1)
C(6)	200 (4)	−2737 (2)	1537 (1)	332 (7)	80 (3)	30 (1)	15 (4)	−7 (2)	−2 (1)
O(1′)	4332 (2)	−2203 (2)	2743 (1)	249 (4)	118 (3)	30 (1)	46 (3)	7 (1)	1 (1)
O(2′)	2979 (3)	710 (2)	4141 (1)	291 (5)	105 (2)	35 (1)	4 (3)	−11 (1)	−19 (1)
O(3′)	688 (3)	−2324 (2)	4955 (1)	304 (5)	146 (3)	29 (1)	−65 (3)	5 (1)	3 (1)
O(5′)	6775 (3)	−5517 (2)	3579 (1)	351 (6)	114 (3)	45 (1)	52 (3)	−19 (2)	−14 (1)
C(1′)	2722 (3)	−687 (3)	2622 (1)	237 (6)	88 (3)	27 (1)	2 (3)	−5 (2)	−2 (1)
C(2′)	1317 (3)	−358 (2)	3568 (1)	214 (6)	95 (3)	26 (1)	19 (3)	−5 (2)	−5 (1)
C(3′)	1223 (3)	−2239 (3)	3972 (1)	205 (5)	108 (3)	28 (1)	−20 (3)	−6 (1)	0 (1)
C(4′)	3933 (3)	−2944 (2)	3687 (1)	211 (5)	83 (3)	30 (1)	−7 (3)	−13 (2)	0 (1)
C(5′)	4102 (4)	−4932 (2)	3679 (2)	282 (7)	85 (3)	49 (1)	−8 (4)	−22 (2)	−4 (1)

* Position fixed to define y coordinates in this polar space group.

(*b*) Hydrogen atoms

	x	y	z	B (Å^2)		x	y	z	B (Å^2)
H(N3)	−0·328 (5)	0·089 (4)	0·061 (2)	2·87 (46)	H(C4′)	0·529 (4)	−0·245 (4)	0·410 (1)	1·88 (34)
H(C5)	−0·210 (5)	−0·424 (4)	0·074 (2)	3·27 (49)	H(C5′)$_a$	0·352 (5)	−0·538 (4)	0·425 (2)	3·48 (53)
H(C6)	0·124 (5)	−0·359 (4)	0·174 (2)	3·72 (57)	H(C5′)$_b$	0·299 (6)	−0·545 (5)	0·317 (2)	5·07 (69)
H(C1′)	0·374 (4)	0·033 (3)	0·243 (1)	1·82 (38)	H(O2′)	0·209 (6)	0·132 (4)	0·443 (2)	3·53 (57)
H(C2′)	−0·030 (4)	0·014 (3)	0·347 (1)	2·08 (41)	H(O3′)	0·166 (5)	−0·176 (4)	0·524 (2)	3·14 (51)
H(C3′)	−0·019 (4)	−0·293 (3)	0·363 (1)	1·67 (36)	H(O5′)	0·689 (6)	−0·603 (5)	0·317 (2)	4·30 (70)

ture is shown in Fig. 2, and relevant distances and angles are given in Table 6. The base residues which are more hydrophobic are hydrogen bonded together [*via* N(3)–H–O(4)] to form ribbons parallel to the twofold screw axis. This arrangement is similar to that found in 5-chlorouridine (Hawkinson & Coulter, 1971), in which the bases are tilted by 34° from the twofold screw axis, the controlling contact being Cl–O(4) = 3·30 Å. In the present case the bases are tilted only 5° from the twofold screw axis, while the HC(5)–O(4) distance is 2·58 Å.

The sugar residues are linked together by a network of hydrogen bonds involving the 2′-, 3′- and 5′-hydroxyl groups. In addition, there is possibly a weak

Table 4. *Bond distances* (Å) *and bond angles* (°) *in the molecule*

The estimated standard deviations are approximately 0·003 Å for bond distances and 0·3° for bond angles involving nonhydrogen atoms, and 0·1 Å and 1° for those involving hydrogen atoms.

S–C(2)	1·677	C(5′)–O(5′)	1·427
O(4)–C(4)	1·228	C(2′)–O(2′)	1·412
N(1)–C(2)	1·368	C(3′)–O(3′)	1·410
C(2)–N(3)	1·360	N(3)–H(N3)	0·83
N(3)–C(4)	1·388	C(5)–H(C5)	0·92
C(4)–C(5)	1·435	C(6)–H(C6)	0·87
C(5)–C(6)	1·337	C(1′)–H(C1′)	0·97
C(6)–N(1)	1·381	C(2′)–H(C2′)	0·91
N(1)–C(1′)	1·500	C(3′)–H(C3′)	1·01
C(1′)–C(2′)	1·528	C(4′)–H(C4′)	0·97
C(2′)–C(3′)	1·526	C(5′)–H(C5′)$_a$	0·92
C(3′)–C(4′)	1·522	C(5′)–H(C5′)$_b$	0·98
C(4′)–O(1′)	1·453	O(2′)–H(O2′)	0·76
O(1′)–C(1′)	1·411	O(3′)–H(O3′)	0·76
C(4′)–C(5′)	1·498	O(5′)–H(O5′)	0·70

C(2)–N(1)–C(6)	120·8	C(2)–N(3)–H(N3)	114
C(2)–N(1)–C(1′)	117·8	C(4)–N(3)–H(N3)	120
C(6)–N(1)–C(1′)	121·3	C(6)–C(5)–H(C5)	121
C(2)–N(3)–C(4)	126·6	C(4)–C(5)–H(C5)	120
N(3)–C(2)–N(1)	116·0	C(5)–C(6)–H(C6)	120
N(3)–C(2)–S	120·7	N(1)–C(6)–H(C6)	118
N(1)–C(2)–S	123·4	O(1′)–C(1′)–H(C1′)	112
O(4)–C(4)–N(3)	119·2	N(1)–C(1′)–H(C1′)	107
O(4)–C(4)–C(5)	126·4	C(2′)–C(1′)–H(C1′)	112
N(3)–C(4)–C(5)	114·4	O(2′)–C(2′)–H(C2′)	112
C(6)–C(5)–C(4)	119·5	C(3′)–C(2′)–H(C2′)	114
N(1)–C(6)–C(5)	122·6	C(1′)–C(2′)–H(C2′)	111
O(1′)–C(1′)–N(1)	109·3	O(3′)–C(3′)–H(C3′)	108
O(1′)–C(1′)–C(2′)	107·1	C(4′)–C(3′)–H(C3′)	110
N(1)–C(1′)–C(2′)	110·6	C(2′)–C(3′)–H(C3′)	109
O(2′)–C(2′)–C(3′)	109·6	O(1′)–C(4′)–H(C4′)	108
O(2′)–C(2′)–C(1′)	108·2	C(5′)–C(4′)–H(C4′)	110
C(3′)–C(2′)–C(1′)	100·8	C(3′)–C(4′)–H(C4′)	110
O(3′)–C(3′)–C(4′)	114·6	O(5′)–C(5′)–H(C5′)$_a$	106
O(3′)–C(3′)–C(2′)	114·4	C(4′)–C(5′)–H(C5′)$_a$	110
C(4′)–C(3′)–C(2′)	101·4	O(5′)–C(5′)–H(C5′)$_b$	110
O(1′)–C(4′)–C(5′)	111·6	C(4′)–C(5′)–H(C5′)$_b$	112
O(1′)–C(4′)–C(3′)	103·5	H(C5′)$_a$–C(5′)–H(C5′)$_b$	108
C(5′)–C(4′)–C(3′)	113·7	C(2′)–O(2′)–H(O2′)	107
C(1′)–O(1′)–C(4′)	109·8	C(3′)–O(3′)–H(O3′)	111
O(5′)–C(5′)–C(4′)	111·3	C(5′)–O(5′)–H(O5′)	110

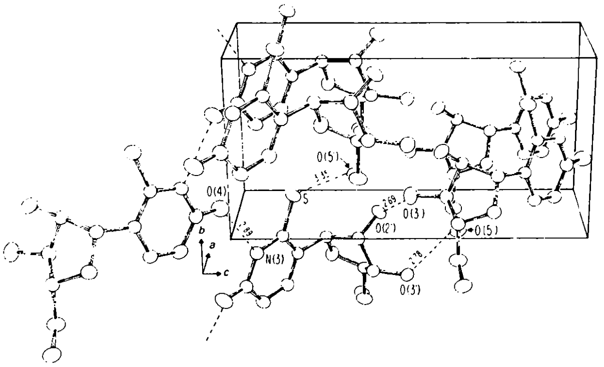

Fig. 2. The packing of 2-thiouridine molecules in the crystal, with hydrogen bonds indicated by dashed lines.

Table 5. *Least-squares planes*

Atoms included in the calculation are marked with an asterisk. X', Y', Z' are orthogonal coordinates (in Å) along the **a**, **b**, **c*** directions respectively.

Pyrimidine ring equation:

$$0\cdot661\,X' + 0\cdot083\,Y' - 0\cdot746\,Z' = -1\cdot733$$

	Deviation (Å)		Deviation (Å)
N(1)*	−0·022	S	0·012
C(2)*	0·003	O(4)	−0·110
N(3)*	0·023	C(1′)	−0·156
C(4)*	−0·029		
C(5)*	0·011		
C(6)*	0·014		
r.m.s.(Δ)	0·019		

Furanose ring equation [C(3′) excluded]:

$$-0\cdot762\,X' - 0\cdot558\,Y' - 0\cdot328\,Z' = -1\cdot980$$

	Deviation (Å)		Deviation (Å)
O(1′)*	−0·020	C(3′)	0·628
C(1′)*	0·020	C(5′)	0·785
C(2′)*	−0·012		
C(4′)*	0·012		
r.m.s.(Δ)	0·017		

The dihedral angle between these planes is 78°.

Table 6. *Distances and angles in the hydrogen bonds D*–H–*A*

Donor (D)	Acceptor (A)	In molecule at	D–A (Å)	H–A (Å)	D–H–A (°)
N(3)	O(4)	$-1-x, \frac{1}{2}+y, -z$	2·889	2·07	174
O(2′)	O(3′)	$-x, \frac{1}{2}+y, 1-z$	2·691	1·94	170
O(3′)	O(5′)	$1-x, \frac{1}{2}+y, 1-z$	2·779	2·06	157
O(5′)	S	$1+x, -1+y, z$	3·350	2·73	149

hydrogen bond from O(5′) to S [O(5′)–S = 3·35 Å]. This type of O(5′)–H–S hydrogen bond has also been noted in the structures of 2,4-dithiouridine [Lin & Sundaralingam (1971), O(5′)–S = 3·22; Saenger & Suck (1971*a*), O(5′)–S = 3·23 Å] and 3′-*O*-acetyl-4-thiothymidine [Saenger & Suck (1971*b*), O(5′)–S = 3·23 Å]. The formation of the hydrogen bonds O(3′)–H–O(5′) and O(5′)–H–S is related to a *gauche–trans* conformation about the C(5′)–C(4′) bond in this structure.

The general features of crystal packing in pyrimidine nucleosides have been discussed by Motherwell, Riva di Sanseverino & Kennard (1973). The packing in 2-thiouridine generally follows their type I classification (translational base stacking) with the pyrimidine planes separated by 3·47 Å (due to the *a*-axis translation).

A general conclusion concerning the effect of substituting S for O at the 2 position in uridine is that no conformational parameters of the nucleoside are modified to any significant extent from those usually found in pyrimidines. However, the 2-thio substitution may play a significant role in modifying the hydrogen-bonding specificity of uracil, especially with regard to the more unusual uracil–guanine base pair postulated by Crick (1966). Specifically, the added distortion provided by the longer hydrogen bond involving S (Bugg & Thewalt, 1970; Thewalt & Bugg, 1972) may prevent 2-thiouridine from pairing with guanine in the way suggested by Yoshida *et al.* (1970).

Comparison with the 5,6-dihydro-2-thiouridine structure

Recently, the structure of 5,6-dihydro-2-thiouridine was determined from Weissenberg film data (Kojić-Prodić *et al.*, 1974) and refined to $R = 0{\cdot}098$. Their reported cell dimensions ($a = 15{\cdot}330$, $b = 7{\cdot}517$, $c = 5{\cdot}070$ Å, and $\beta = 107{\cdot}89°$) can be transformed by $\mathbf{a}' = -\mathbf{c}$, $\mathbf{b}' = \mathbf{b}$, $\mathbf{c}' = \mathbf{c} + \mathbf{a}$ to give $a' = 5{\cdot}070$, $b' = 7{\cdot}517$, $c' = 14{\cdot}593$ Å, and $\beta' = 91{\cdot}35°$. These cell dimensions are then very similar to those for 2-thiouridine. A comparison of the structures reveals that the two molecules have crystallized in identical arrangements. When the atomic coordinates for 2-thiouridine are rotated and translated to provide the best molecular overlap with those of 5,6-dihydro-2-thiouridine,* the r.m.s. displacement between atoms of the two molecules is only 0·09 Å. This overlap was calculated by a least-squares fitting procedure (A. D. Rae, 1975, unpublished) in which the S was given twice the weight of the other atoms, and C(5) and C(6), the atoms which are displaced from the plane of the uracil ring in the dihydro derivative, were given zero weight. The maximum displacement among the fitted atoms is 0·18 Å for O(4), while the saturated atoms C(5) and C(6) deviate by 0·44 and 0·26 Å respectively. Some feeling for the significance of these values can be had by comparing chemically identical bond lengths in the two molecules. The r.m.s. difference in the nine bond lengths of the ribose moiety is only 0·03 Å.

Since the crystal structures are so nearly identical, the hydrogen-bonding networks must be the same. Although Kojić-Prodić *et al.* (1974) could only speculate as to the location of the H atoms in 5,6-dihydro-2-thiouridine, they are clearly in similar environments to those in 2-thiouridine. The hydrogen bond which may be inferred for 5,6-dihydro-2-thiouridine is O(5′)–H–S, the O(5′)–S distance of 3·35 Å being identical to that in 2-thiouridine.

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* The coordinates given by Kojić-Prodić *et al.* (1974) are, unfortunately, for the incorrect enantiomer and must be inverted to be compared with those of 2-thiouridine.

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X-ray Study of 1,5,*N*(4),*N*(4)-Tetramethylcytosine – an Overcrowded Molecule with Planar Structure

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1,5,*N*(4),*N*(4)-Tetramethylcytosine ($C_8H_{13}N_3O$) is monoclinic, space group $P2_1/c$, with $a = 15{\cdot}362$, $b = 10{\cdot}240$, $c = 12{\cdot}452$ Å, $\beta = 114{\cdot}98°$, $Z = 8$. The structure was solved by direct methods and refined to an R of 8·0% for 1984 diffractometer data. Average standard deviations are 0·006 Å and 0·4°. The dimethylamino group is not rotated, to avoid steric hindrance with the *ortho* methyl group at C(5), but is coplanar with the pyrimidine ring. The sp^2 configuration of N(4) is retained and steric strain is relieved by spreading of the angles at C(4), C(5) and N(4).

Introduction

Recently, two papers on the 'barrier to rotation and conformation of the $-NR_2$ group in cytosine and its derivatives' have been published (Geller & Lesyng, 1975; Kulakowska, Geller, Lesyng, Bolewska & Wierzchowski, 1975). Among other cytosine derivatives, 1,5,*N*(4),*N*(4)-tetramethylcytosine (TMC, Fig. 1) was investigated with CNDO/2 and INDO calculations and experimental dipole moments were evaluated. One of the authors' suggestions was that the dimethylamino group should be rotated by about 30° relative to the pyrimidine plane. Further, the configuration of the amino N atom should be sp^3 rather than sp^2. These results prompted us to carry out an X-ray analysis to establish the geometrical details of TMC.

Experimental

A prismatic single crystal 0·35 × 0·5 × 0·15 mm was sealed in a capillary because of its hygroscopic properties and used for all subsequent X-ray measurements. Crystallographic data are given in Table 1. X-ray intensities were collected in the $2\theta/\omega$ scan mode on an automated Stoe diffractometer equipped with a Cu tube (Ni filter, Cu $K\alpha = 1{\cdot}54182$ Å) and corrected for geometrical factors but not for absorption. Three check reflexions monitored every 100 measurements showed no significant change in intensity.

Wilson's (1942) method was used to obtain overall temperature ($B = 3{\cdot}6$ Å^2) and scale factors and normalized structure amplitudes were evaluated (Karle & Hauptman, 1956). The structure was solved with *MULTAN* (Main, Germain & Woolfson, 1970) on the

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