

## STRUCTURAL AND SPECTRAL PROPERTIES OF 2,4-DITHIOURIDINE

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## 1. Introduction

In the past two years the chemical and enzymic syntheses of the 4-thio substituted pyrimidine polynucleotides  $s^4\text{Ups}^4\text{U}$  [1], poly  $s^4\text{U}$  [2, 3], poly  $d(\text{A}-s^4\text{T})$  [4] have been reported. The unusual physical properties of these polynucleotides [3–6] suggested the investigation of polynucleotides containing 2,4-dithiouridine. This communication reports the molecular and crystal structure and spectral properties of 2,4-dithiouridine (fig. 1).

## 2. Materials and methods

2,4-Dithiouridine, prepared according to the literature [22], crystallized as the monohydrate from an aqueous solution in the form of yellow needles. The orthorhombic space group  $P2_12_12_1$  and cell dimensions  $a = 8.445$ ,  $b = 5.257$ ,  $c = 27.988$  Å were derived from precession photographs and measurements on a Siemens four circle automatic diffractometer. We collected about 1400 reflections up to  $\theta = 70^\circ$  using Ni-filtered Cu-radiation ( $2\theta$ -w scan mode) and corrected them for geometrical factors but not for absorption. The positions of the two sulfur atoms were derived from a Patterson synthesis. A Fourier map phased with these two atoms revealed the whole structure. After two cycles of isotropic and two cycles of anisotropic full matrix least squares refinement a difference Fourier was computed from which we were able to pick out the positions of all hydrogen atoms except the one bound to atom  $\text{O5}'$ . The present crystallographic reliability factor  $R$  is 4.6%.

The P.M.R. spectrum of 2,4-dithiouridine was measured with a Varian AH 100 using *d*-DMSO as solvent and TMS as internal standard, the infrared absorption spectrum from a KBr pellet with a Perkin Elmer Infracord apparatus. The UV-absorption spectrum was measured with a Cary 14 spectrophotometer. The acidimetric titration was carried out in a cuvette by addition of small volumes of either 1N NaOH or 1N HCl without changing the total volume by more than 2%. The curves 1 to 6 (fig. 4) correspond

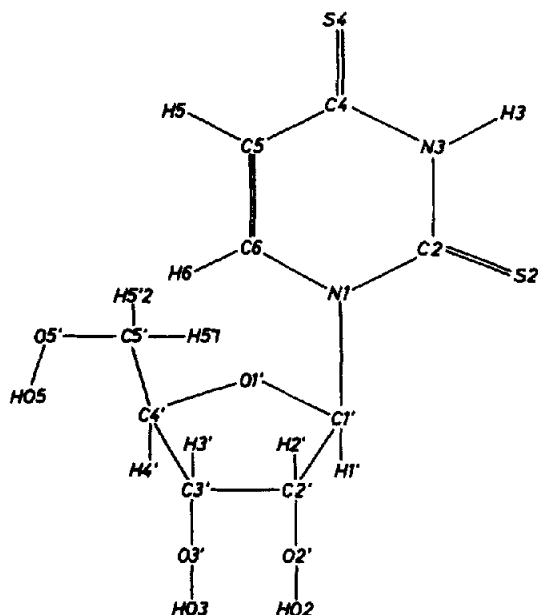


Fig. 1. 2,4-Dithiouridine.

to pH 5.74, 6.88, 7.48, 7.70, 8.30 and 10.04 respectively.

### 3. Results and discussion

#### 3.1. Crystal structure of 2,4-dithiouridine-mono hydrate

The bond distances and angles within the heterocyclic ring of 2,4-dithiouridine are similar to those published for 2,4-dithiouracil [7]. The heterocycle is almost planar, the greatest deviation from the least squares plane through the six atoms being 0.13 Å for atom S2.

In table 1 the C-S distances of some thio-substituted pyrimidines and pyrimidine nucleosides, thiourea and the theoretical values for the C-S single and double bonds are listed. The C-S bond distances of the given thio-substituted pyrimidine nucleosides are greater than the theoretical value for a C-S double bond but shorter than the C-S bond observed in thiourea whose length is halfway between a theoretical C-S double and single bond. It follows that the C-S bonds in the thio-substituted pyrimidine nucleosides listed in table 1 have double bond character and thus are in the keto form.

The geometry of the ribose moiety is not unusual [8]. The ribose is in the C3'-endo conformation, i.e. atom C3' is out of the plane through atoms C4', O1', C1', C2' and on the same side as C5'. The conforma-

tion about the C4'-C5' bond, *gauche, gauche*, is quite common in nucleosides [9, 10].

2,4-Dithiouridine is in the anti-conformation [11]; the dihedral angles O1'-C1'-N1-C6 [12] and C2'-C1'-N1-C6 [13] are 18.0° and -101.5° respectively.

The packing of the molecules is best described in terms of hydrophobic and hydrophilic bands which extend parallel to the *a,b*-plane around  $c = 0$  and  $c = 1/4$  (fig. 2).

The intermolecular forces within the hydrophobic region are base stacking (perpendicular distance between heterocycles = 3.51 Å) and the hydrogen bonds S2-O5' (3.221 Å) [14] and S4-N3 (3.328 Å) [15].

Within the hydrophilic region the water molecules, being situated near a twofold screw axis, form a zig-zag chain parallel to the crystallographic *b*-axis. There are four hydrogen bonds emanating tetrahedrally from a water oxygen OW in the direction of two symmetry related water oxygen atoms (2.980 Å) and atoms O2' (2.733 Å) and O5' (2.958 Å). Another hydrogen bond connects atoms O2' and O3' intermolecularly (2.708 Å).

#### 3.2. Spectral properties of 2,4-dithiouridine

In accordance with observations on substituted thioformamides [16] the magnetic resonance signals of the 2,4-dithiouridine protons H6 and H1' show significant downfield shifts with respect to the corresponding uridine signals.

Table 1  
Comparison of C-S distances in thio-substituted pyrimidine bases and nucleosides with the corresponding distance in thiourea and theoretical values.

Compound	Bond lengths (Å)	
	C2-S2	C4-S4
2,4-Dithiouracil [7]	1.645	1.684 (± 0.006)
2,4-Dithiouridine	1.657	1.674 (± 0.003)
4-Thiouridine [13]		1.662 (± 0.003)
3'-Acetyl-4-thiothymidine [18]		1.673 (± 0.003)
1-Methyl-4-thiouracil (complex with 9-methyladenine) [19]		1.649 (± 0.005)
Thiourea [20]	1.720	(± 0.009)
C-S single bond [21]	1.812	
C-S double bond [21]	1.607	

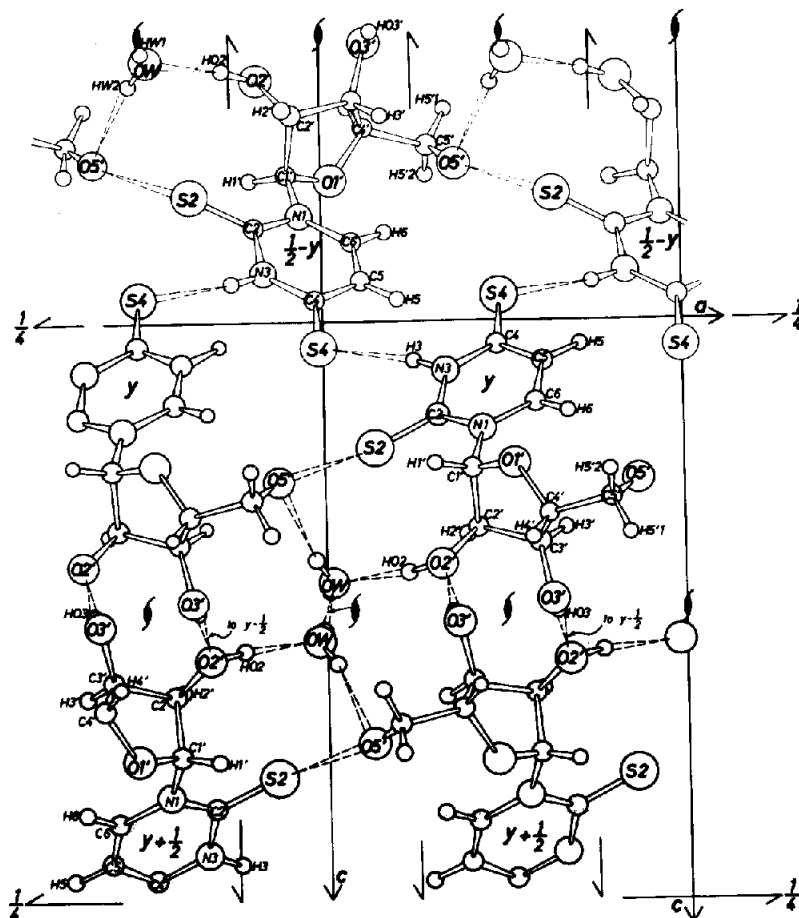


Fig. 2. Crystal structure of 2,4-dithiouridine-mono-hydrate projected down the *b* axis.

Table 2  
Proton magnetic resonance signals of 2,4-dithiouridine.

Proton	Chemical shift $\delta$ (ppm)
H5	6.58 (doublet, $J = 7.5$ Hz)
H6	8.10 (doublet, $J = 7.5$ Hz)
H1'	6.45 (doublet, $J = 3$ Hz)
H5', H2', H3'	3.2–4.2 (not well resolved)
C5'-OH	5.0
C3'-OH, C2'-OH	5.4

The infrared absorption spectrum of 2,4-dithiouridine was rather difficult to interpret. Rao and Venkataraghavan [17] suggested for thioamides a nomenclature similar to that used for amides and assigned the thioamide bands I ( $1570\text{--}1395\text{ cm}^{-1}$ ), II ( $1420\text{--}1260\text{ cm}^{-1}$ ) and III ( $1140\text{--}940\text{ cm}^{-1}$ ) to the C=S vibration. The infrared spectrum of 2,4-dithiouridine displays three strong bands in these regions:  $1610\text{ cm}^{-1}$ ,  $1500\text{ cm}^{-1}$  and  $1210\text{ cm}^{-1}$  which should be interpreted as thioamide bands I, II and III (fig. 3). Only the band at  $1610\text{ cm}^{-1}$ , however, can be identified as thioamide band I by comparison of the infrared absorption spectra of uridine, 4-thiouridine,

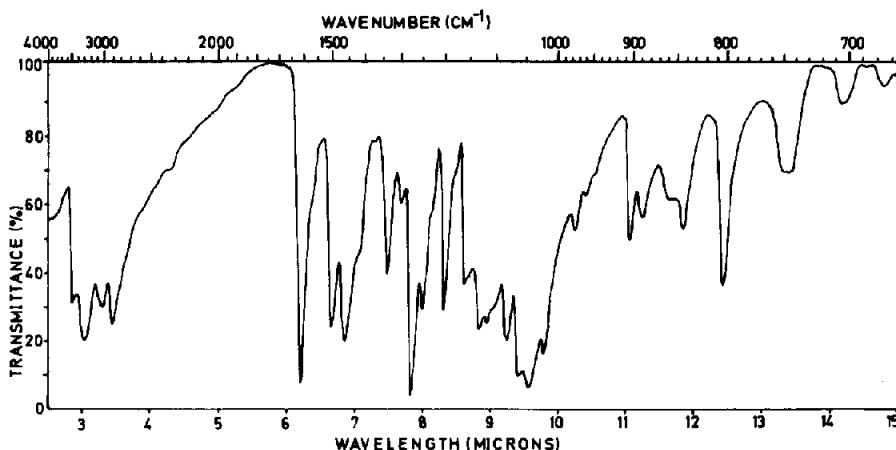
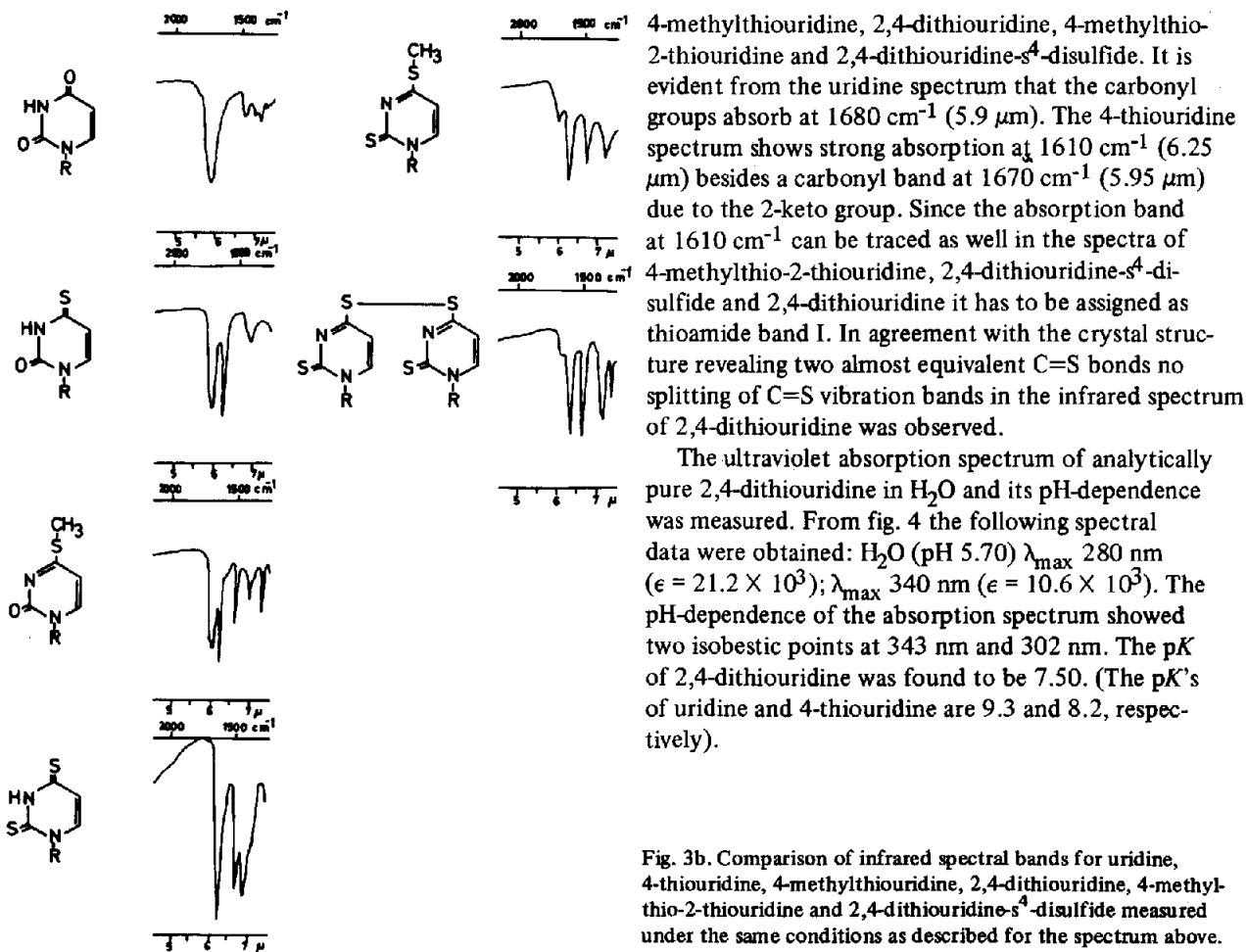


Fig. 3a. Infrared absorption spectrum of 2,4-dithiouridine.



4-methylthiouridine, 2,4-dithiouridine, 4-methylthio-2-thiouridine and 2,4-dithiouridine-*s*<sup>4</sup>-disulfide. It is evident from the uridine spectrum that the carbonyl groups absorb at 1680  $\text{cm}^{-1}$  (5.9  $\mu\text{m}$ ). The 4-thiouridine spectrum shows strong absorption at 1610  $\text{cm}^{-1}$  (6.25  $\mu\text{m}$ ) besides a carbonyl band at 1670  $\text{cm}^{-1}$  (5.95  $\mu\text{m}$ ) due to the 2-keto group. Since the absorption band at 1610  $\text{cm}^{-1}$  can be traced as well in the spectra of 4-methylthio-2-thiouridine, 2,4-dithiouridine-*s*<sup>4</sup>-disulfide and 2,4-dithiouridine it has to be assigned as thioamide band I. In agreement with the crystal structure revealing two almost equivalent C=S bonds no splitting of C=S vibration bands in the infrared spectrum of 2,4-dithiouridine was observed.

The ultraviolet absorption spectrum of analytically pure 2,4-dithiouridine in  $\text{H}_2\text{O}$  and its pH-dependence was measured. From fig. 4 the following spectral data were obtained:  $\text{H}_2\text{O}$  (pH 5.70)  $\lambda_{\text{max}}$  280 nm ( $\epsilon = 21.2 \times 10^3$ );  $\lambda_{\text{max}}$  340 nm ( $\epsilon = 10.6 \times 10^3$ ). The pH-dependence of the absorption spectrum showed two isobestic points at 343 nm and 302 nm. The  $\text{pK}$ 's of 2,4-dithiouridine was found to be 7.50. (The  $\text{pK}$ 's of uridine and 4-thiouridine are 9.3 and 8.2, respectively).

Fig. 3b. Comparison of infrared spectral bands for uridine, 4-thiouridine, 4-methylthiouridine, 2,4-dithiouridine, 4-methylthio-2-thiouridine and 2,4-dithiouridine-*s*<sup>4</sup>-disulfide measured under the same conditions as described for the spectrum above.

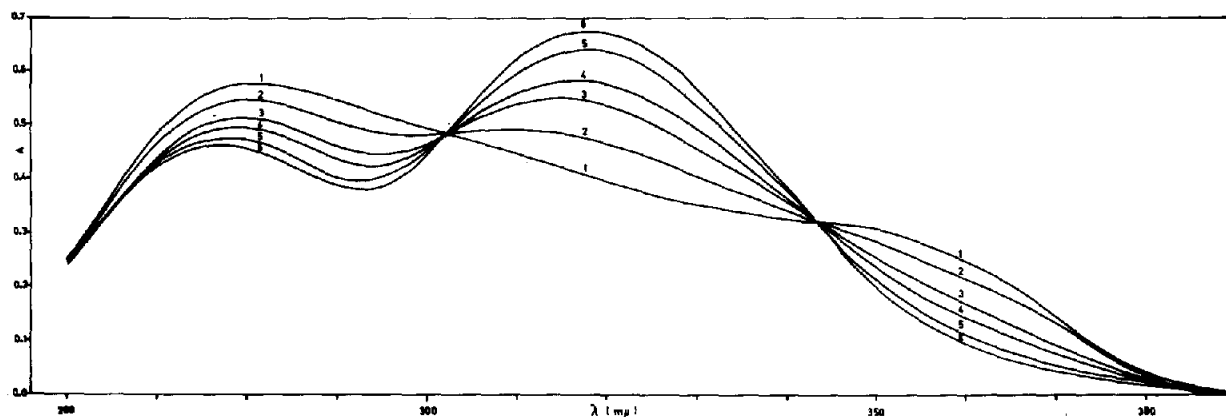


Fig. 4. pH-dependence of the UV-absorption spectrum of 2,4-dithiouridine.

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