

$$\sigma_g^2 = \left(\frac{\partial g}{\partial k_1}\right)^2 \sigma_{k_1}^2 + \left(\frac{\partial g}{\partial K_1}\right)^2 \sigma_{K_1}^2 + 2\left(\frac{\partial g}{\partial k_1}\right)\left(\frac{\partial g}{\partial K_1}\right)\sigma_{k_1 K_1}$$

where  $\sigma_{k_1 K_1}$  is the covariance of  $k_1$  and  $K_1$ . The three terms of this equation were numerically +0.06491, +0.06992, and -0.13472, so that the value of  $k_1/K_1$  (0.131) has a standard deviation of only 8%.

We feel that the use of a general procedure of this sort is much to be preferred over methods that rely on special characteristics of a particular problem or which obtain

a fit "by eye."<sup>96</sup> The present example is instructive in that the investigator would have been warned by the large standard deviations of  $k_1$  and  $K_1$  that the data did not adequately define these two constants even if the reason for this had not been recognized. This warning would not necessarily have been apparent with other more intuitive fitting methods.

(96) This type of rough fit (*e.g.*, with a CRT on-line to a digital computer) is a convenient way of obtaining the initial trial parameters for the least squares program.

## Stereochemistry of a Substrate for Pancreatic Ribonuclease. Crystal and Molecular Structure of the Triethylammonium Salt of Uridine 2',3'-O,O-Cyclophosphorothioate<sup>1</sup>

W. Saenger and F. Eckstein

Contribution from the Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, 34 Göttingen, Germany. Received December 31, 1970

**Abstract:** Uridine 2',3'-O,O-cyclophosphorothioate is a substrate for pancreatic ribonuclease. Knowledge of the absolute configuration of this substrate and of the reaction product obtained by enzymatic methanolysis is of interest from a mechanistic point of view. The title compound crystallizes in space group  $P2_12_12_1$ . The structure was solved from three-dimensional X-ray data and refined to an  $R$  value of 9.6%. The bicyclic system is "pseudo-mirror" symmetrical with respect to the O-P-S plane. The ribose exhibits the unusual O(1') *exo* conformation, the bonds C(1')-C(2'), C(3')-C(4') and C(2')-O(2'), C(3')-O(3') being pairwise coplanar. The heterocycle is in *anti* position with respect to the ribose and almost coplanar with the C(1')-O(1') bond. The conformation about the C(4')-C(5') bond, defined as  $\varphi_{oo}$  and  $\varphi_{oo}$ , is *trans,gauche*. The P-S bond seems to have double bond character, the negative charge being located at the free oxygen atom of the phosphorothioate group. The triethylammonium cation is disordered, its symmetry nearly  $C_{3v}$ .

Recently the uridine 2',3'-O,O-cyclophosphorothioate anion was synthesized<sup>2</sup> and one of the diastereomers isolated by fractional crystallization of the triethylammonium salt (Figure 1). This isomer is a substrate for pancreatic ribonuclease with the same  $K_m$  value as uridine 2',3'-O,O-cyclophosphate.<sup>3</sup> In the presence of methanol it is converted by the enzyme to uridine 3'-O-(O-methyl)phosphorothioate, which we were able to crystallize as well. Knowledge of the absolute configuration of substrate and reaction product would clarify the stereochemistry of the enzymatic methanolysis and would thus yield valuable information as to the stereochemistry of the transesterification and hydrolysis reaction of ribonuclease.<sup>4</sup> In this report the X-ray structural analysis of the triethylammonium salt of the crystalline isomer of uridine 2',3'-O,O-cyclophosphorothioate will be presented, which is interesting not only from a biochemical but also from a structural point of view since nucleoside 2',3'-O,O-cyclophosphates have not yet been investigated.

(1) Short communication published in *Angew. Chem. Int. Ed. Engl.*, 8, 595 (1969). Differences in some angles and bond distances in this and the present publication are due to the disorder of the triethylammonium cation which had not been accounted for at that time.

(2) F. Eckstein and H. Gindl, *Chem. Ber.*, 101, 1670 (1968).

(3) F. Eckstein, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, 2, 85 (1968);  $\delta$  values for uridine 2',3'-O,O-cyclophosphorothioate in this publication should be corrected to  $\delta = -68.5$  and  $-69.5$  ppm, respectively.

(4) M. R. Harris, D. A. Usher, H. P. Albrecht, G. H. Jones, and J. G. Moffatt, *Proc. Nat. Acad. Sci.*, 63, 246 (1969).

### Experimental Section

When an ethanolic solution of the triethylammonium salt of uridine 2',3'-O,O-cyclophosphorothioate was allowed to evaporate slowly at 4° stout prismatic crystals formed (mp 204–205°). The orthorhombic space group  $P2_12_12_1$  of these crystals was indicated by the mirror symmetries of the X-ray photographs and the systematic absence of reflections  $h00$ ,  $0k0$ ,  $00l$  when  $h,k,l$ , respectively, were odd. The unit cell dimensions were  $a = 12.495 \pm 0.005$  Å,  $b = 7.079 \pm 0.003$  Å,  $c = 22.574 \pm 0.008$  Å. The observed density of  $1.391 \pm 0.005$  g/cm<sup>3</sup> was in good agreement with the calculated value if four formula weights within the unit cell were assumed. We collected 1108 data on a four-circle automatic diffractometer using Zr-filtered Mo radiation and  $2\theta$  scan mode. In view of the small crystal dimensions of  $0.3 \times 0.1 \times 0.1$  mm and the linear absorption coefficient of  $2.83$  cm<sup>-1</sup> the data were corrected for geometrical factors, not for absorption.

The positions of the two heavy atoms, P and S, could be determined unambiguously from a sharpened Patterson map. Two successive Fourier syntheses, phased first with these two atoms and then with all but three atoms of the structure, revealed the geometry of the molecule. After two cycles of isotropic and four cycles of anisotropic full-matrix least-squares refinement using the 986 observed data and applying Hughes' weighting scheme<sup>5</sup> (Fomin = 4.2), the reliability index,  $R = \sum ||F_o| - |F_c|| / \sum |F_o|$ , dropped to 9.4%. A difference Fourier synthesis computed at this stage revealed a statistical disorder of the three  $\alpha$ -carbon atoms of the triethylammonium ion. In a further cycle of refinement the occupation and thermal parameters of the six "ordered" and "disordered"  $\alpha$ -carbon atoms of the ethyl groups were set at 0.5 and isotropic, respectively, and varied. Since the occupation parameters fluctuated around 0.5 but did not shift in a definite manner they were reset to 0.5, the isotropic thermal parameters converted

(5) E. W. Hughes, *J. Amer. Chem. Soc.*, 63, 1737 (1941).

**Table I.** Positional and Thermal Parameters in the Form  $\exp[-\Sigma_i \Sigma_j h_i h_j B_{ij}]$  and Their Estimated Standard Deviations

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> <sub>11</sub>	<i>B</i> <sub>22</sub>	<i>B</i> <sub>33</sub>	<i>B</i> <sub>12</sub>	<i>B</i> <sub>13</sub>	<i>B</i> <sub>23</sub>
P	0.1071 (3)	0.0735 (6)	-0.0844 (2)	0.0049 (3)	0.0108 (9)	0.0030 (1)	-0.0004 (5)	-0.0013 (2)	0.0007 (3)
S	0.1280 (4)	0.0960 (8)	0.0008 (2)	0.0092 (4)	0.0298 (13)	0.0034 (1)	0.0030 (7)	-0.0012 (2)	-0.0036 (4)
O	0.1676 (8)	-0.0769 (19)	-0.1148 (5)	0.0041 (7)	0.034 (3)	0.0029 (3)	0.0044 (15)	-0.0004 (4)	-0.0046 (9)
O(1')	-0.0927 (9)	0.488 (1)	-0.1011 (4)	0.0084 (10)	0.011 (3)	0.0007 (2)	0.000 (1)	-0.0013 (4)	-0.0014 (6)
O(2')	-0.0171 (8)	0.062 (2)	-0.1025 (6)	0.0041 (9)	0.016 (3)	0.0041 (4)	0.002 (2)	-0.0010 (5)	0.0025 (10)
O(3')	0.1309 (9)	0.269 (2)	-0.1148 (7)	0.0053 (10)	0.012 (3)	0.0048 (5)	-0.002 (2)	-0.0018 (6)	0.0037 (10)
O(5')	0.0784 (11)	0.725 (2)	-0.2094 (5)	0.0167 (11)	0.022 (3)	0.0016 (3)	-0.001 (2)	-0.0002 (5)	-0.0001 (8)
C(1')	-0.1414 (13)	0.314 (3)	-0.1153 (7)	0.0023 (11)	0.017 (5)	0.0026 (4)	0.002 (2)	-0.0007 (6)	0.0008 (13)
C(2')	-0.0503 (14)	0.193 (2)	-0.1460 (7)	0.0055 (12)	0.017 (5)	0.0013 (3)	-0.002 (2)	-0.0002 (6)	0.0015 (2)
C(3')	0.0430 (15)	0.334 (2)	-0.1554 (9)	0.0038 (13)	0.009 (4)	0.0036 (5)	-0.004 (2)	-0.0024 (8)	0.0011 (13)
C(4')	0.0028 (13)	0.528 (3)	-0.1348 (7)	0.0061 (14)	0.026 (6)	0.0022 (4)	0.000 (3)	-0.0011 (7)	0.0041 (15)
C(5')	-0.0195 (12)	0.660 (2)	-0.1852 (7)	0.0025 (11)	0.018 (4)	0.0020 (4)	-0.002 (2)	-0.0001 (6)	0.0001 (11)
N(1)	-0.2298 (9)	0.348 (2)	-0.1580 (5)	0.0054 (11)	0.007 (3)	0.0014 (3)	-0.002 (2)	-0.0016 (5)	-0.0007 (8)
C(2)	-0.2911 (11)	0.201 (2)	-0.1747 (7)	0.0029 (12)	0.008 (4)	0.0017 (4)	0.002 (2)	0.0003 (6)	0.0010 (11)
O(2)	-0.2637 (8)	0.036 (2)	-0.1614 (5)	0.0043 (10)	0.017 (3)	0.0026 (3)	-0.001 (1)	-0.0011 (5)	0.0024 (8)
N(3)	-0.3750 (10)	0.229 (2)	-0.2099 (5)	0.0042 (9)	0.009 (3)	0.0018 (3)	0.002 (2)	0.0001 (5)	0.0017 (9)
C(4)	-0.4090 (15)	0.405 (3)	-0.2270 (6)	0.0061 (15)	0.025 (5)	0.0018 (4)	-0.004 (3)	-0.0006 (7)	-0.0002 (13)
O(4)	-0.4863 (10)	0.417 (2)	-0.2614 (5)	0.0085 (11)	0.013 (3)	0.0029 (3)	0.006 (2)	-0.0028 (5)	-0.0011 (9)
C(5)	-0.3531 (13)	0.562 (3)	-0.2054 (7)	0.0072 (14)	0.022 (5)	0.0027 (5)	0.005 (3)	-0.0031 (7)	-0.0024 (14)
C(6)	-0.2692 (15)	0.530 (2)	-0.1697 (8)	0.0079 (18)	0.003 (4)	0.0029 (5)	0.001 (2)	-0.0013 (8)	-0.0006 (11)
NA	0.3433 (10)	-0.273 (2)	-0.0661 (6)	0.0034 (9)	0.021 (4)	0.0013 (3)	-0.000 (2)	-0.0001 (4)	-0.0006 (9)
CA (2)	0.264 (2)	0.382 (4)	0.5170 (8)	0.017 (2)	0.042 (7)	0.0022 (4)	0.012 (4)	-0.0010 (9)	-0.0019 (16)
CA (4)	0.053 (3)	-0.031 (4)	0.425 (2)	0.026 (4)	0.015 (7)	0.0070 (11)	-0.004 (4)	0.0034 (18)	-0.0011 (22)
CA (6)	0.160 (2)	0.473 (5)	0.341 (1)	0.013 (2)	0.055 (10)	0.0048 (8)	0.002 (5)	0.0004 (12)	0.0034 (26)
CA (11)	0.155 (2)	0.303 (4)	0.501 (1)	0.003 (2)	0.022 (8)	0.0036 (7)	-0.005 (3)	0.0021 (10)	0.0011 (23)
CA (12)	0.177 (2)	0.444 (4)	0.474 (1)	0.014 (3)	0.087 (14)	0.0043 (10)	-0.003 (7)	0.0009 (15)	-0.0052 (33)
CA (31)	0.052 (2)	0.161 (4)	0.414 (2)	0.007 (2)	0.046 (10)	0.0022 (8)	0.008 (4)	-0.0002 (12)	-0.0039 (26)
CA (32)	0.061 (2)	0.164 (4)	0.456 (2)	0.010 (3)	0.022 (7)	0.0065 (13)	0.002 (4)	0.0004 (16)	0.0025 (28)
CA (51)	0.159 (2)	0.477 (4)	0.411 (1)	0.012 (3)	0.028 (9)	0.0005 (6)	-0.004 (4)	0.0007 (10)	0.0018 (18)
CA (52)	0.116 (2)	0.385 (4)	0.380 (1)	0.024 (4)	0.050 (11)	0.0005 (6)	-0.006 (6)	0.0029 (13)	0.0009 (21)

to anisotropic mode, and all the atoms of the structure refined in three further cycles. In the last cycle of refinement the parameter shifts were no more than one-third the estimated standard deviation. The *R* factor was still 9.6% but the interatomic distances and angles had improved with respect to those values calculated before the disorder of the cation had been accounted for. The *R* factor of the improved structure was 0.02% higher than the *R* factor of the "ordered" structure. The reason might be that the  $\alpha$ -carbon atoms of the "ordered" triethylammonium cation simulated the two disordered  $\alpha$ -carbon atoms. They were located almost between the corresponding disordered  $\alpha$ -carbon atoms and had isotropic thermal vibration parameters of about 10 Å<sup>2</sup> (average isotropic thermal vibration parameters 3.8 Å<sup>2</sup>). A difference Fourier synthesis computed with the final atomic parameters did not reveal any significant peaks. In view of the disorder of the cation we did not attempt to locate the hydrogen atoms.

## Results

The final coordinates of the atoms in fractions of the crystallographic axes and the anisotropic temperature factors are listed in Table I. We have summarized intramolecular bond distances, angles, and dihedral angles in Figure 5 and Table II, respectively. The least-squares planes through some parts of the molecule were calculated and are listed in Table III. In Figures 7 and 8 a packing diagram of the unit cell contents is presented. A list of the observed and calculated structure factors is available on request.

## Discussion

**(a) The Bicyclic System.** In all the nucleosides investigated by X-ray analysis so far the ribose moiety has been found to be in the envelope conformation as has been predicted by Spencer.<sup>6</sup> Usually atoms C(2') or C(3') are out of the plane through the other four atoms of the five-membered ribose ring by  $\pm 0.5$ – $0.7$  Å, the conformation being called *endo* or *exo*, respectively,

(6) M. Spencer, *Acta Crystallogr.*, **12**, 59 (1959).

**Table II.** Selected Dihedral Angles within the Uridine 2',3'-O,O-Cyclophosphorothioate Anion<sup>a</sup>

O(1')-C(1')-C(2')-C(3')	-9.4°
C(1')-C(2')-C(3')-C(4')	-3.6°
C(2')-C(3')-C(4')-O(1')	14.9°
C(3')-C(4')-O(1')-C(1')	-23.0°
C(4')-O(1')-C(1')-C(2')	20.7°
C(2')-O(2')-P-O(3')	12.9°
O(2')-P-O(3')-C(3')	-11.7°
P-O(3')-C(3')-C(2')	7.5°
O(3')-C(3')-C(2')-O(2')	-1.2°
C(3')-C(2')-O(2')-P	-9.5°
N(1)-C(1')-C(2')-C(3')	107.9°
N(1)-C(1')-O(1')-C(4')	-97.1°
N(1)-C(1')-C(2')-O(2')	-139.1°
C(2')-C(1')-N(1)-C(6)	-127.7°
O(1')-C(1')-N(1)-C(6)	12.9°
O(1')-C(1')-C(2')-O(2')	103.6°
C(1')-C(2')-O(2')-P	-120.5°
C(1')-C(2')-C(3')-O(3')	112.6°
C(2')-O(2')-P-S	128.6°
C(2')-O(2')-P-O	-100.2
C(3')-O(3')-P-S	-126.2
C(3')-O(3')-P-O	102.7°
C(2')-C(3')-C(4')-C(5')	-106.7°
O(3')-C(3')-C(4')-C(5')	139.6°
C(1')-O(1')-C(4')-C(5')	99.3°
C(3')-C(4')-C(5')-O(5')	74.1°
O(1')-C(4')-C(5')-O(5')	168.3°

<sup>a</sup> These four atom angles are defined as zero if the bond between the first two atoms is colinear with the bond between the last two atoms looking down the central bond. The angle is measured positive if the far bond is rotated clockwise.

if the out-of-plane atom is on the same or opposite side of the plane as C(5'). The dihedral angle O(2')-C(2')-C(3')-O(3'), *i.e.*, the angle between the two exocyclic C-O bonds viewed along the central C(2')-C(3') bond, is predominantly  $\pm 45$ – $65^\circ$ . In the uridine 2',3'-O,O-cyclophosphorothioate anion this angle has

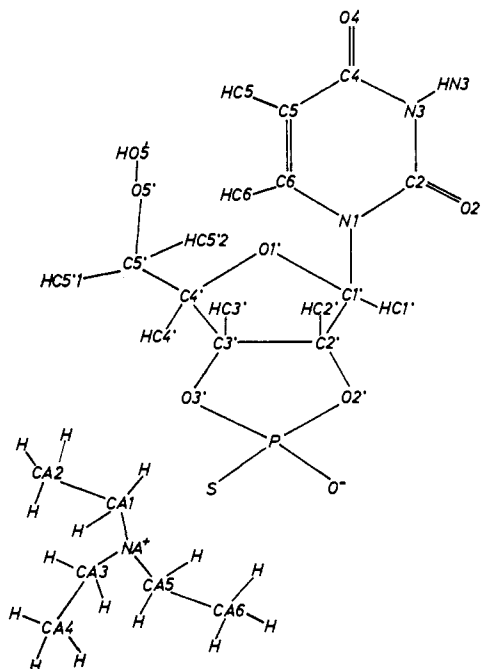


Figure 1.

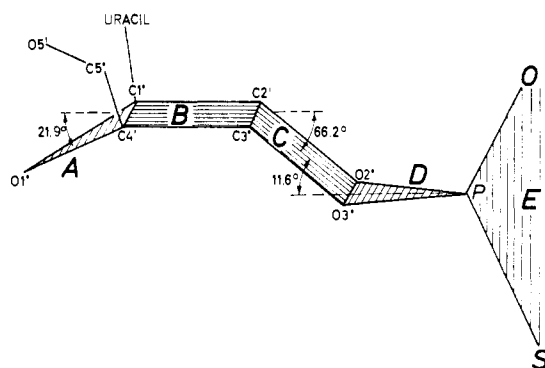


Figure 2. Schematic representation of the bicyclic system. The ribose and phosphodiester five-membered rings are formed by the planes A, B and C, D, respectively.

been reduced by the cyclization to only  $1.2^\circ$ ; the diol group is *cis* planar. As a consequence of this atomic arrangement the bonds  $C(1')-C(2')$  and  $C(3')-C(4')$  are also coplanar and the pucker of the ribose moiety is such that atom  $O(1')$  is out of the plane of these four atoms by  $0.29 \text{ \AA}$  (Table III, plane B) on the opposite side as  $C(5')$ ; the ribose moiety in this molecule is in the  $O(1')$  *exo* conformation.

The pucker of the cyclic phosphodiester group is similar to that of the ribose moiety. The phosphorus atom is  $0.21 \text{ \AA}$  out of the best four atoms plane (Table III, plane C) on the opposite side from  $C(1')$  and  $C(4')$ .

The angle between the two mentioned ribose and phosphodiester four-atom planes,  $66.2^\circ$ , is noted in Figure 2 which shows schematically the geometry of the bicyclic system. The equations of the five different planes (A, B, C, D, E) and distances of some atoms from these planes are listed in Table III.

In Table IV we have collected the angles between plane O-P-S (plane E) and the four planes comprising the bicyclic system. Plane O-P-S is perpendicular to these four planes and the distances of pairs of atoms

Table III. Least-Squares Planes through Parts of the Molecule and Distances of Some Atoms from These Planes<sup>a</sup>

Plane equation	Distances of atoms comprising the plane, $\text{\AA}$	Distances of other atoms from this plane, $\text{\AA}$
Heterocycle		
$a = 6.533$	N(1), 0.064	O(1'), -0.021
$b = -0.192$	C(2), -0.040	C(1'), -0.075
$c = -16.621$	N(3), -0.005	C(2'), 1.173
	C(4), 0.025	O(2), -0.052
	C(5), 0.000	O(4), 0.096
	C(6), -0.044	
Bicyclic system		
Plane A		
$a = -1.818$	C(4')	C(2'), -0.541
$b = 0.848$	O(1')	C(3'), -0.582
$c = -4.131$	C(1')	O(2'), -1.875
		O(3'), -2.040
		N(1), 1.391
		C(5'), 1.370
Plane B		
$a = -1.339$	C(1')	O(1'), -0.289
$b = 0.402$	C(2')	O(2'), -1.205
$c = -5.901$	C(3')	O(3'), -1.352
	C(4')	N(1), 1.317
		C(5'), 1.331
		P, -2.137
Plane C		
$a = 3.394$	O(2')	P, 0.212
$b = -3.315$	C(2')	C(1'), -1.412
$c = -12.197$	C(3')	C(4'), -1.336
	O(3')	
Plane D		
$a = 1.843$	O(2')	C(2'), 0.284
$b = -1.998$	P	C(3'), 0.279
$c = -11.281$	O(3')	O, 1.274
		S, -1.625
Plane E		
$a = 5.722$	O	O(2'), -1.141
$b = 2.809$	P	O(3'), 1.227
$c = -2.176$	S	C(2'), -0.698
		C(3'), 0.850
		C(1'), -1.101
		C(4'), 1.293
		O(1'), 0.098
		N(1), -1.618
		C(5'), -1.621

<sup>a</sup> The plane equations are in the form  $ax + by + cz - 1 = 0$ ;  $x, y, z$  refer to atomic positions in fractions of the crystallographic axes.

Table IV. Angles between the Normals of Some of the Planes Listed in Table III

Plane 1	Plane 2	Angle, deg
Heterocycle	B	58.7
E	D	89.4
E	C	89.9
E	B	90.4
E	A	90.5
A	B	21.9
B	C	66.2
C	D	11.6

( $O(2')$  and  $O(3')$ ,  $C(2')$  and  $C(3')$ ,  $C(1')$  and  $C(4')$ ) from plane O-P-S are almost equal (Figure 3). Furthermore the angles having atoms  $C(2')$  and  $C(3')$  as the vertex are symmetrically distributed with respect to this plane as will be discussed in the next paragraph. Thus the plane O-P-S can be called a "pseudo-mirror plane" for the bicyclic system.

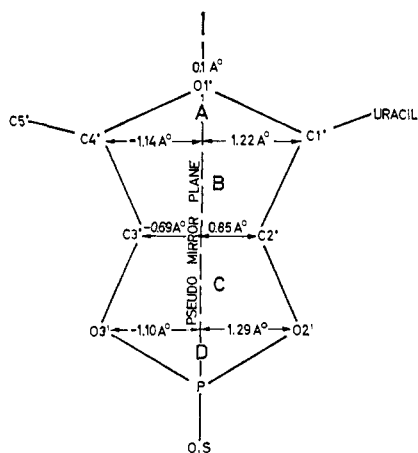


Figure 3. See caption to Figure 2. The "pseudo-mirror plane" O-P-S is indicated by the broken line.

**(b) Bond Lengths and Angles within the Bicyclic System.** In order to facilitate the discussion of the geometry of the uridine 2',3'-cyclophosphorothioate anion we have collected in Figure 4 the averaged data which were reported for the ribose moieties of nucleosides. Some general features of the data which are necessary for the discussion shall be described first although they have already been mentioned<sup>7</sup> in part.

From the data so far available one might conclude that the conformation of the sugar residues has no marked influence on the bond distances within the five-membered ring. The maximum discrepancy between C(2') *endo* and C(3') *endo* data is 0.02 Å, *i.e.*, no more than twice the estimated standard deviations. However the exocyclic C-O bond lengths seem about "normal" at the in-plane carbon atom but shortened at the *endo* atom (exceptions are the reported data for 5-methyluridine).<sup>8</sup>

In C(3') *endo* ribose units the angle at C(1'), 107.5°, is greater than the angle at C(4'), 103.8°. In C(2') *endo* nucleosides these angles seem to be almost equal. The exocyclic angles at C(2') and C(3') are different for the two conformations; they are about 113° at the out-of-plane (*endo*) carbon atom but about 109° at the in-plane carbon atom.

The bond distances and angles within the bicyclus of the anion (Figure 5) will now be compared with the data sampled in Figure 4.

In the bicyclus the bond distances C(1')-C(2'), C(3')-O(3'), and P-O(2') are significantly increased with respect to the average values in Figure 4 and the data found in cyclic methyl ethylene phosphate.<sup>9</sup> We attribute this increase in bond distance to the steric strain induced by the fusion of the two five-membered rings. It is unlikely that intermolecular forces are responsible for this distortion since intermolecular distances between the bicyclus and other atoms of the structure are greater than or equal to the interatomic distances reported in similar structures.<sup>10</sup>

The angles within the phosphodiester part of the bicyclus are similar to those reported by Steitz and

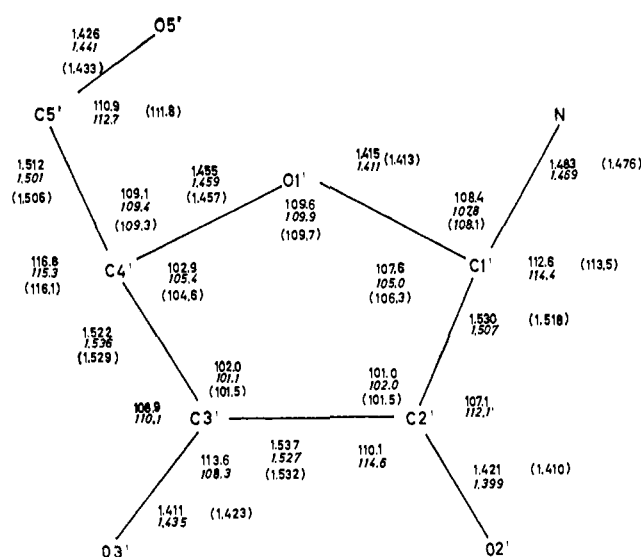


Figure 4. Average bond angles and distances in the ribose unit of nucleosides. Normal numbers are average values for the C(3') *endo* nucleosides 5-methyluridine,<sup>7</sup> inosine (P. Tollin and A. R. I. Munns, *Nature*, **222**, 1170 (1969)), adenosine 3'-phosphate,<sup>21</sup> adenosine 5'-phosphate (J. Kraut and L. H. Jensen, *Acta Crystallogr.*, **16**, 79 (1963)), the uridine part in  $\beta$ -adenosine-2'- $\beta$ -uridine-5'-phosphoric acid,<sup>12</sup> 4-thiouridine,<sup>18</sup> 2,4-dithiouridine (W. Saenger and D. Suck, to be published). The average values for the C(2') *endo* nucleosides cytidine 3'-phosphate [monoclinic (C. E. Bugg and R. E. Marsh, *J. Mol. Biol.*, **25**, 67 (1967)) and orthorhombic<sup>17</sup>] and the adenosine part in  $\beta$ -adenosine-2'- $\beta$ -uridine-5'-phosphoric acid<sup>12</sup> are given in italics. The data for the angle N-C(1')-C(2') in 4-thiouridine and the distances C-O when the oxygen is part of a phosphate group were not considered for reasons discussed elsewhere.<sup>18,6</sup> The average values for C(2') and C(3') *endo* nucleosides together are presented by numbers in parentheses except for exocyclic angles at C(2') and C(3'). The estimated standard deviations of the structures used for the averaging were about 0.01 Å for bond distances and 1° for bond angles.

Lipscomb.<sup>9</sup> It is of interest that in both cases the angle O(2')-P-O(3') is about 10° smaller than would be required for a tetrahedral arrangement of substituents around the phosphorus atom.

Within the ribose part of the anion the angles at O(1'), C(2'), and C(3') are 3-4° greater than the average values. The other two endocyclic angles at C(1') and C(4') are about equal but are different in C(3') *endo* nucleosides as mentioned above.

The exocyclic angles at atoms C(2') and C(3') are similar to each other in the anion since the bonds C(1')-C(2'), C(3')-C(4') and C(2')-O(2'), C(3')-O(3') are pairwise coplanar. It should be noted that all angles around atoms C(2') and C(3'), exocyclic and endocyclic, are similar to C-C-O angles at the in-plane carbon atom in C(2') *endo* and C(3') *endo* nucleosides.

**(c) Phosphorothioate Group.** The sulfur atom is situated on the same side of the phosphodiester five-membered ring as C(1') and C(4') or, in a chemist's terms, in *endo* position with respect to the bicyclic system.

To our knowledge no structure analysis of a phosphorothioate diester has been reported so far. One should assume the negative charge to be located both on sulfur and oxygen atom but the P-S distance of 1.946 Å is characteristic for a double bond ( $1.91 \pm 0.06$  Å)<sup>11</sup> between these atoms whereas the P-O dis-

(11) D. E. C. Corbridge in "Phosphorus Chemistry," Vol. III, D. E. C. Corbridge, M. S. Pearson, C. Walling, and E. J. Griffith, Ed., Intersci-

(7) M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 930 (1965).

(8) D. J. Hunt and E. Subramanian, *Acta Crystallogr.*, **B**, **35**, 2144 (1969).

(9) T. A. Steitz and W. N. Lipscomb, *J. Amer. Chem. Soc.*, **81**, 2488 (1965).

(10) M. Sunaralingam, *Acta Crystallogr.*, **21**, 495 (1966).

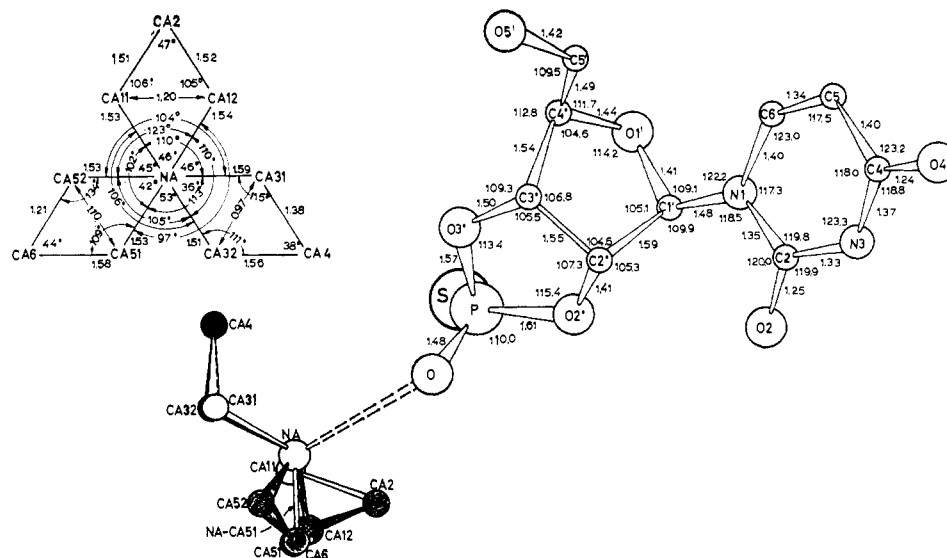


Figure 5. Bond distances and angles for the triethylammonium salt of uridine 2',3'-O,O-cyclophosphorothioate. Distances and angles not shown in the figure are: P-S = 1.946 Å; O(2')-P-O(3') = 96.7°; O(2')-P-S = 112.6°; O-P-S = 116.7°; O-P-O(3') = 109.6°; S-P-O(3') = 109.5°; O-NA-CA(11) = 118°; O-NA-CA(32) = 120°; O-NA-CA(12) = 119°; O-NA-CA(51) = 109°; O-NA-CA(31) = 107°; O-NA-CA(52) = 102°. The estimated standard deviations obtained from the variance-covariance matrix are 0.01 Å and 0.7°, respectively, for the phosphorothioate group, 0.02 Å and 1°, respectively, for the rest of the anion, and 0.05 Å and 5°, respectively, for the cation.

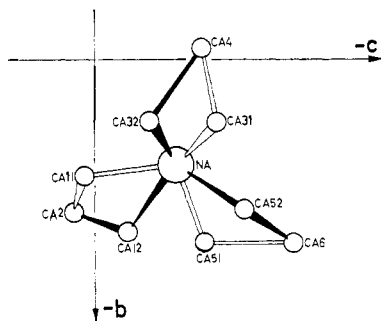
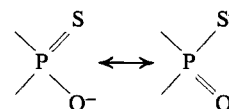


Figure 6. Projection of the cation on 100. White and shadowed bonds indicate the two superimposed triethylammonium structures.

tance of 1.48 Å length is indicative of P-O single bond ( $1.51 \pm 0.02$  Å)<sup>11</sup> with a negative charge located at the oxygen atom. It might well be that the P=S double

angles O...NA- $\alpha$ -C are all about equal,  $111 \pm 9^\circ$  (legend to Figure 5), and the O...NA distance of 2.78 Å is typical for a hydrogen bond.



(d) **Uracil Residue.** The geometry of this part of the 2',3'-O,O-cyclophosphorothioate anion is not unusual. All bond angles and lengths are in reasonable agreement with the values published for 1-methyluracil,<sup>12a</sup> uridine 5'-phosphate,<sup>12b</sup> and the uracil residue in  $\beta$ -adenosine-2'- $\beta$ -uridine-5'-phosphoric acid.<sup>13</sup> It should be noted that the atoms C(1') and O(1'), *i.e.*, the bond C(1')-O(1'), are only 0.08 and 0.02 Å, respectively, off the plane through the six atoms comprising the heterocycle. Thus the C(1')-O(1') bond is almost within

Table V. A Comparison of Exocyclic Angles (in Degrees) at C(1') and C(4') in Nucleosides of Different Ribose Conformation

Nucleoside	N(1)-C(1')-O(1')	N(1)-C(1')-C(2')	C(5')-C(4')-C(3')	C(5')-C(4')-O(1')	Ribose conformation
Uridine 2',3'-O,O-cyclophosphorothioate	109.1	109.9	112.8	111.7	O(1') <i>exo</i>
Deoxyadenosine <sup>a</sup>	108.4	110.5	113.7	112.2	C(3') <i>exo</i>
Thymidine <sup>b</sup>	108.2	114.5	113.3	110.5	C(3') <i>exo</i>
Average values for nucleosides	108.4	112.6	117.0	109.1	C(3') <i>endo</i>
	107.8	114.4	115.3	109.4	C(2') <i>endo</i>

<sup>a</sup> Reference 15. <sup>b</sup> Reference 16.

bond is polarized to some extent, but a mesomeric state can be ruled out. It is in agreement with this charge distribution that the triethylammonium cation is situated directly opposite the oxygen atom O. The

ence. New York, N. Y., 1966, pp 211, 293. The covalent radii of double bonded P and S atoms according to L. Pauling (Die Natur der chemischen Bindung, Verlag Chemie, Weinheim/Bergstr., Germany, 1962, p 213) are 1.00 and 0.94 Å and thus add up to the observed P-S bond length.

this plane and a steric interaction between atom O(1') and the hydrogen atom attached to atom C(6) should be negligible, which we attribute to the unusual pucker of the ribose moiety. It was calculated by Haschemeyer

(12) (a) F. S. Mathews and A. Rich, *J. Mol. Biol.* 8, 89 (1964); (b) E. Shefter and K. N. Trueblood, *Acta Crystallogr.*, 18, 1067 (1964).

(13) E. Shefter, M. Barlow, R. A. Sparks, and K. N. Trueblood, *ibid.*, B, 25, 895 (1969).

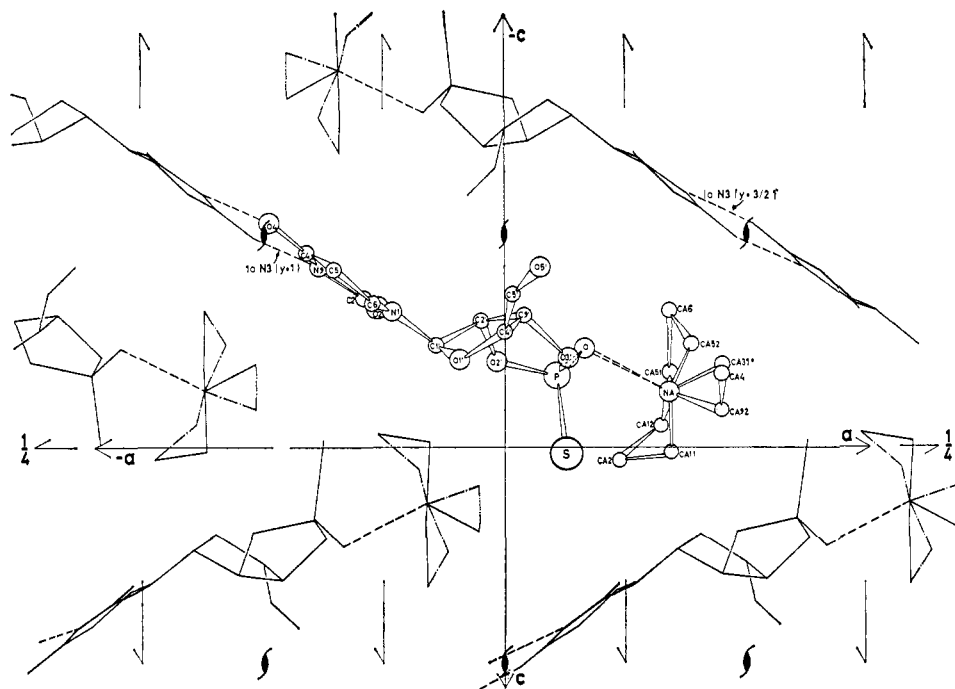


Figure 7. Projection of the unit cell content on 010.

and Rich<sup>14</sup> that in pyrimidine nucleosides during rotation about the glycosidic C(1')-N(1) bond at least some steric interaction between atom O(1') and the hydrogen atom in position 6 of the heterocycle occurs.

The angles O(1')-C(1')-N(1) and C(2')-C(1')-N(1) are almost equal as are C(3')-C(4')-C(5') and O(1')-C(4')-C(5'). Similar results have been obtained for deoxyadenosine<sup>15</sup> and the angles C(3')-C(4')-C(5'), O(1')-C(4')-C(5') in thymidine,<sup>16</sup> Table V. These nucleosides are both in C(3') *exo* configuration. In contrast, in nucleosides exhibiting C(2') or C(3') *endo* configuration the angles C(2')-C(1')-N(1) and C(3')-C(4')-C(5') have been found to be about 5 and 7° (Figure 4) greater than O(1')-C(1')-N(1) and O(1')-C(4')-C(5'). One is tempted to correlate angles around C(1') and C(4'), respectively, with *endo* and *exo* puckering of the sugar moiety but more data should be available to support these conclusions.

**(e) Conformation about the C(1')-N(1) and C(4')-C(5') Bonds.** The heterocycle of a nucleoside cannot rotate freely about the glycosidic C(1')-N(1) bond with respect to the ribose moiety but assumes two positions which were called *syn* or *anti*<sup>17</sup> if the oxygen atom O(2) is close to or away from the sugar moiety. A quantitative measure of this torsion is the dihedral angle O(1')-C(1')-N(1)-C(6)<sup>18</sup> which in the uridine 2',3'-O,O-cyclophosphorothioate ion is 12.9°. The anion is in the *anti* conformation as are all the other pyrimidine nucleosides investigated so far except 4-thiouridine.<sup>19</sup>

(14) A. E. V. Haschemeyer and A. Rich, *J. Mol. Biol.*, **27**, 369 (1967).  
 (15) D. C. Watson, D. J. Sutor, and P. Tollin, *Acta Crystallogr.*, **19**, 111 (1965).

(16) D. W. Young, P. Tollin, and H. R. Wilson, *ibid.*, **B**, **25**, 1423 (1969).

(17) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).

(18) M. Sundaralingam and L. H. Jensen, *ibid.*, **25**, 67 (1967).

(19) W. Saenger and K. H. Scheit, *ibid.*, in press. In this paper the torsion angle about the glycosidic bond has been redefined for stereochemical reasons as C(2')-C(1')-N(1)-C(6) (Table II), the *syn* and *anti* ranges being 0-180° and 0-180°, respectively.

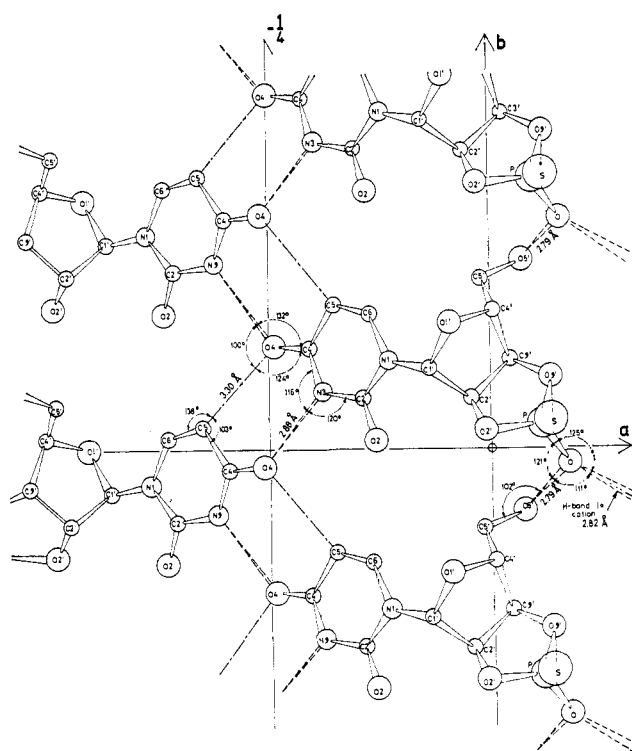


Figure 8. Projection of the structure on 001, representing the zigzag band of molecules along the screw axis at  $a = -1/2$ ,  $c = 1/4$ . The hydrogen bonding scheme and the short intermolecular contact of 3.3 Å between O(4) and O(5) are indicated.

The conformation about the C(4')-C(5') bond is defined by the dihedral angles  $\varphi_{oo}$  and  $\varphi_{oc}$ , i.e., O(5')-C(5')-C(4')-O(1') and O(5')-C(5')-C(4')-C(3').<sup>12</sup> These angles are *gauche*, *gauche* in most of the nucleosides investigated in crystalline state but are *trans*, *gauche* in this molecule (Table II). This has only been observed in 5-fluoro-2'-deoxyuridine<sup>20</sup> and the

(20) D. R. Harris and W. M. Macintyre, *Biophys. J.*, **4**, 203 (1964).

uridine 3',5'-O,O-cyclophosphate anion;<sup>21</sup> in the latter case the conformation *trans,gauche* is required by the six-membered phosphodiester ring.

**(f) The Cation.** In the initial stages of this structure analysis the triethylammonium cation appeared to be ordered and displayed almost C(3) symmetry. When a difference Fourier synthesis was computed at the end of the refinement procedure three more atoms showed up in chemically reasonable positions. These atoms correspond to disordered  $\alpha$ -carbon atoms and render the symmetry of the cation nearly C<sub>3v</sub>, Figure 6. Apart from the disorder this result is not too surprising since a triethylammonium ion has two ways to arrange its  $\alpha$ -carbon atoms even if the locations of the nitrogen and  $\beta$ -carbon atoms remain fixed. This disorder of the triethylammonium cation has also been observed by Schwalbe and Lipscomb<sup>22</sup> but is not a general phenomenon; recently an ordered structure with this cation has been examined.<sup>21</sup>

**Packing Arrangement.** The packing of the molecules within the unit cell is indicated in two schematic projections down the shortest axis (on 010, Figure 7) and down the *c* axis (on 001, Figure 8). The molecules are

(21) C. L. Coulter, *Acta Crystallogr.*, **B**, **25**, 2055 (1969).

(22) C. H. Schwalbe and W. N. Lipscomb, *J. Amer. Chem. Soc.*, **91**, 194 (1969).

arranged in fishbone manner. They are linked by hydrogen bonds which are indicated by short intermolecular contacts in the direction of the *b* axis but not in the *a*, *c* plane. The hydrogen bonds are formed between translation equivalent molecules along the *b* axis and between molecules which are symmetry related by the twofold screw axes parallel to *b* at  $a = 0, c = +1/4$  and  $a = 1/2, c = 3/4$ . Along these symmetry elements extend zigzag bands of triethylammonium uridine 2',3'-O,O-cyclophosphorothioate molecules (Figure 7). These zigzag bands are symmetry related to each other by the twofold screw axes at  $a = 1/2, c = 1/4$ , and  $a = 0, c = 3/4$ .

The hydrogen bonds between atoms O(5')-O and O(4)-N(3) are indicated in Figure 8. It should be noted that the distance O(4)-C(5) is only 3.3 Å long. Similar short contacts between aromatic C-H groups and proton acceptors have been observed by several authors<sup>10,21,23</sup> and were correlated with hydrogen bonding forces.

**Acknowledgment.** The authors thank Professor F. Cramer for support, Dr. B. Krebs for stimulating discussions, Miss H. Schmidt for skillful technical assistance, and the Deutsche Forschungsgemeinschaft for financial assistance for parts of this work.

(23) D. J. Sutor, *J. Chem. Soc.*, 1105 (1963).

## Nucleoside Phosphorothioates

F. Eckstein<sup>1</sup>

*Contribution from the Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, 34 Göttingen, Germany. Received February 2, 1970*

**Abstract:** The synthesis of some nucleoside 5'-phosphorothioates is described. They are resistant to alkaline phosphatase. On reaction with acetic anhydride the formation of only one mixed anhydride can be detected by <sup>31</sup>P nmr. It is hydrolyzed by aqueous pyridine to a mixture of nucleoside 5'-phosphate and nucleoside 5'-phosphorothioate. Adenosine 5'-phosphorothioate can be cyclized to adenosine 3',5'-cyclic phosphorothioate. 3',5'-Dithymidine phosphorothioate is resistant to snake venom and spleen phosphodiesterase and is a competitive inhibitor for these enzymes. Uridine 2',3'-O,O-cyclic phosphorodithioate is obtained by reaction of 5'-acetyluridine and P<sub>2</sub>S<sub>5</sub> and is a substrate for pancreatic ribonuclease.

Although there exist a great number of nucleotide analogs in which either the sugar or the base is modified, only a small number of analogs are known in which the phosphate group is altered. Among these are nucleoside phosphites,<sup>2</sup> nucleoside phosphonates,<sup>3</sup> homonucleoside phosphonates,<sup>4</sup> and dialky esters of nucleoside phosphorothioates.<sup>5</sup> We have recently<sup>1</sup> synthesized nucleoside phosphorothioates, analogs in

which the phosphate is modified to a thiophosphate group. We hoped that such nucleotide analogs would be hydrolyzed by phosphatases and phosphodiesterases, respectively, with slower rates than the parent compounds and therefore be of interest particularly in biochemical investigations where an increased stability of the phosphate ester bond is desired.

A number of recent publications show that nucleoside phosphorothioates<sup>6</sup> as well as phosphorothioate analogs of polynucleotides<sup>7</sup> do indeed possess properties which make them suitable tools for studies of enzymes involved in nucleic acid metabolism.

The synthesis of nucleoside 5'-phosphorothioates was conveniently carried out by thiophosphorylation

(1) Habilitationsschrift, Mathematisch-Naturwissenschaftliche Fakultät Braunschweig, 1968; a preliminary report of a portion of this work has been published: F. Eckstein, *J. Amer. Chem. Soc.*, **88**, 4292 (1966); part of this work was supported by the Deutsche Forschungsgemeinschaft.

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(5) M. E. Wolff and A. Burger, *ibid.*, **79**, 1970 (1957).

(6) A. W. Murray and M. R. Atkinson, *Biochemistry*, **7**, 4023 (1968); A. Hampton, L. W. Brox, and M. Bayer, *ibid.*, **8**, 2303 (1969).

(7) E. DeClercq, F. Eckstein, and T. Merigan, *Science*, **165**, 1137 (1969).