(2*a*) and 1·468 Å (2*b*) are normal for a sulphoxide and agree closely with the value of 1·478 Å found for phenanthro-1,2,5-thiadiazole. The N–S–C angles of 86·4 and 86·9° are slightly smaller than the C–S–C angle of 87·0° in 1,3,4-thiadiazole (La Cour, 1974) and the C–S–C angle of 89·6° in mercaptothiadiazole (Bats, 1976). The S–C distances of 1·810 and 1·809 Å reflect single-bond character, like the values observed by Argay, Kálmán, Lazar, Ribár & Toth (1977) and Petrović, Ribár, Argay, Kálmán & Nowacki (1977) for various amino(imino)thiadiazoles (1·778 to 1·841 Å).

A comparison between the structures of 3-phenyl-2-phenylimino-1,3-thiazetidine and the 1,2,4-thiadiazolidines shows that the higher oxidation state of S puts that atom out of the plane. The degrees of conjugation, indicated by the C–N distances in the heterocycles, seem, however, to be similar. Further oxidation of 1-oxo-1-λ^4-1,2,4-thiadiazolidin-3-one is possible and the products obtained have been characterized as S double oxides by spectroscopic methods (Mösinger, 1977). No structure determination of these compounds has so far been carried out.

The computer calculations have been carried out at the Hochschulrechenzentrum der Universität Frankfurt. The support of the computer staff is gratefully acknowledged.

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Molecular and Crystal Structure of the Free Acid of Cytidine 2′,3′-Cyclophosphate

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(*Received* 5 *August* 1977; *accepted* 7 *November* 1977)

The crystal structure of cytidine 2′,3′-cyclophosphate (free acid) has been determined by X-ray diffraction techniques. The unit cell is orthorhombic, space group $P2_12_12_1$, with cell dimensions $a = 6{\cdot}680\ (5)$, $b = 18{\cdot}342\ (10)$, $c = 10{\cdot}128\ (7)$ Å and $Z = 4$. The data (1175 reflexions) were collected on a Stoe four-circle diffractometer using Cu $K\alpha$ radiation. The structure was solved by the tangent formula applying the *MULTAN* program and refined by a full-matrix least-squares procedure to a final R value of 0·064. All the H atoms were located. The ribose has C(2′)-*endo*, C(3′)-*exo* puckering and the conformation about the glycosidic bond is *anti* ($\chi_{CN} = 61{\cdot}6°$). This conformation for the cyclic phosphate is different from the earlier observations for similar molecules.

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Introduction

The pyrimidine nucleoside 2′,3′-cyclic phosphates are substrates for bovine pancreatic ribonuclease A and they occur as intermediates when polyribonucleotides are cleaved. Detailed NMR studies on these molecules (Lapper & Smith, 1973) suggest that in solution the furanose ring tends towards a 3′-*endo* (2′-*exo*) pucker. While these conclusions are in agreement with our knowledge about the preferred C(2′)-*endo*, C(3′)-*endo* or C(3′)-*exo* puckering modes in nucleosides, nucleotides and nucleic acids, crystallographic investigations on sodium cytidine 2′,3′-cyclophosphate monohydrate (Coulter, 1973; Na 2′,3′-CMP) and triethylammonium uridine 2′,3′-cyclothiophosphate (Saenger & Eckstein, 1970; 2′,3′-UMPS) showed that an O(1′)-*endo* envelope sugar puckering is obviously favoured for this class of molecules. A suggestion that this peculiar puckering should be responsible for the unusual *syn* conformation of Na 2′,3′-CMP was corroborated by a subsequent NMR analysis (Lavelle & Coulter, 1973). In view of these unusual results it appeared worthwhile to undertake another X-ray study of 2′,3′-CMP; this is described here.*

Experimental

Crystals of cytidine 2′,3′-cyclophosphate (free acid) were grown by slow diffusion of acetone into aqueous solutions of the sample (obtained from Boehringer & Co.) over a period of a few weeks. The crystals belong to the orthorhombic system with the space group $P2_12_12_1$. The crystal data were obtained from Weissenberg and precession photographs (Table 1). A crystal of approximate dimensions 0·5 × 0·15 × 0·1 mm was used to collect intensity data to $2\theta = 120°$ using a Stoe four-circle diffractometer and Cu $K\alpha$ radiation (Ni filter) with the $2\theta/\omega$ scanning method and stationary background counts on each side of the scans. Three check reflexions were monitored at intervals of 100 reflexions. The data were corrected for the geometrical factors but not for absorption. 1175 reflexions were collected, of which 1113 were considered observed ($|F_o| \geq 3\sigma|F_o|$).

*This work was presented at the Third European Crystallographic Meeting, Zürich (1976), Collected Abstracts, pp. 305–306.

Table 1. *Crystal data of cytidine 2′,3′-cyclophosphate*

Molecular formula $C_{10}H_{11}N_2O_7P$, $M_r = 302{\cdot}2$
Space group $P2_12_12_1$

$a = 6{\cdot}680$ (5) Å	$Z = 4$
$b = 18{\cdot}342$ (10)	$d_o = 1{\cdot}616$ g cm^{-3}
$c = 10{\cdot}128$ (7)	μ(Cu $K\alpha$) = 23·23 cm^{-1}

Structure determination and refinement

The structure was solved by direct methods using the *MULTAN* program (Main, Germain & Woolfson, 1971). A set of 200 E's greater than 1·34 was used in the initial phasing. Origin fixing and the selection of three symbols (including one for the enantiomorph) were done automatically. An E map computed on the basis of the phases generated by the program showed part of the phosphate group, ribose and cytosine ring (15 atoms out of 20). The remaining nonhydrogen atoms were found from a subsequent difference Fourier map phased with these 15 atoms. The structure was refined by a full-matrix least-squares procedure using the *ORFLS* program (Busing, Martin & Levy, 1962). All the H atoms were located from a difference Fourier synthesis. The refinement converged after refining the positional parameters of the H atoms to a final R value of 0·064. The scattering factors were taken from *International Tables for X-ray Crystallography* (1974). The weighting scheme applied was based on counter statistics (Stout & Jensen, 1968).

Results and discussion

Tables 2 and 3 give the positional parameters of nonhydrogen and H atoms respectively. Fig. 1 shows the bond lengths and angles not involving H atoms. All bond lengths and angles to H atoms are normal and they are not tabulated.*

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33219 (9 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 2. *Fractional atomic positional parameters* ($\times 10^4$) *for the non-hydrogen atoms of cytidine 2′,3′-cyclophosphate*

	x	y	z
P	4329 (3)	3221 (1)	7225 (2)
C(1′)	2688 (12)	4686 (4)	5504 (9)
C(2′)	1825 (12)	4189 (4)	6575 (8)
C(3′)	1382 (12)	3500 (4)	5788 (8)
C(4′)	971 (13)	3746 (4)	4385 (8)
O(1′)	1930 (9)	4464 (3)	4256 (6)
O(2′)	3259 (8)	3994 (2)	7546 (5)
O(3′)	3264 (8)	3090 (3)	5833 (5)
O(7)	3721 (9)	2677 (3)	8232 (6)
O(6)	6533 (8)	3312 (3)	7020 (6)
C(5′)	−1264 (15)	3828 (4)	4086 (10)
O(5′)	−2255 (9)	4235 (3)	5078 (7)
C(2)	3661 (13)	5963 (4)	5825 (11)
N(3)	3050 (9)	6673 (3)	5115 (7)
C(4)	1112 (11)	6885 (4)	6159 (8)
C(5)	−399 (11)	6360 (4)	5883 (9)
C(6)	176 (11)	5669 (4)	5680 (10)
O(2)	5416 (9)	5820 (3)	5743 (9)
N(4)	665 (11)	7568 (3)	6483 (7)
N(1)	2134 (10)	5463 (3)	5692 (8)

Table 3. *Hydrogen-atom positions and isotropic temperature factors*

	x	*y*	*z*	*B* (Å²)
H(C1')	0·435 (11)	0·460 (3)	0·544 (7)	5·2 (2)
H(C2')	0·088 (11)	0·445 (3)	0·713 (7)	5·9 (2)
H(C3')	0·019 (12)	0·318 (4)	0·625 (7)	6·3 (2)
H(C4')	0·183 (12)	0·340 (4)	0·359 (7)	6·7 (2)
H(C5')1	−0·206 (12)	0·334 (4)	0·394 (7)	5·9 (2)
H(C5')2	−0·128 (11)	0·408 (4)	0·325 (7)	6·9 (2)
N(3)	0·407 (14)	0·696 (5)	0·627 (8)	8·8 (3)
H(C5)	−0·160 (15)	0·644 (5)	0·591 (9)	11·7 (3)
H(C6)	−0·095 (11)	0·521 (3)	0·554 (6)	4·8 (2)
H(N4)1	0·156 (13)	0·784 (4)	0·713 (8)	8·9 (2)
H(N4)2	−0·088 (12)	0·771 (4)	0·674 (6)	5·3 (2)
H(O5')	−0·240 (11)	0·406 (4)	0·577 (7)	5·9 (2)

Molecular geometry and conformation

Glycosidic bond. The conformation about the β-glycosidic bond is *anti* [$\chi_{CN} = 61{\cdot}6°$, Sundaralingam, 1969; O(1')–C(1')–N(1)–C(2) = −118·4°]. While most of the nucleosides and 3'- or 5'-nucleotides show a preference for the *anti* conformation about the glycosidic bond in the crystalline state, many cyclic nucleotides (for example Na 2',3'-CMP, adenosine 3',5'-cyclophosphate, and guanosine 3',5'-cyclophosphate) occur in the *syn* conformation (Sundaralingam, 1969). It is noteworthy that the same compound (*viz* 2',3'-CMP) crystallizes in different conformations about the glycosidic bond. The behaviour must be attributed to the crystal-packing energies which also forced 4-thiouridine to adopt the *syn* conformation (Saenger & Scheit, 1970; Scheit & Saenger, 1969).

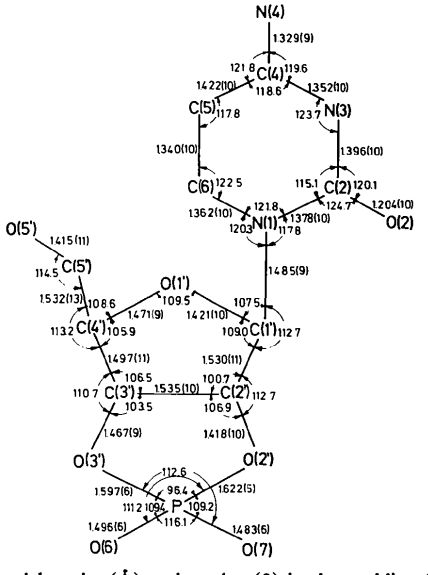

Fig. 1. Bond lengths (Å) and angles (°) in the cytidine 2',3-CMP free acid; average e.s.d.'s in bond angles are 0·6°.

Ribose. Sugar rings in nucleosides and nucleotides are usually puckered with either C(2') or C(3') out of the plane formed by the other four atoms (Spencer, 1959; Sundaralingam, 1969). An analysis of the ribose moiety in the present structure shows that the pucker is best described as a C(2')-*endo*, C(3')-*exo* twist with C(2') being farther away (0·321 Å) than C(3') (−0·151 Å) from the C(4')–O(1')–C(1') plane. This sugar conformation is in agreement with NMR data obtained for nucleoside 2',3'-cyclophosphates (Lapper & Smith, 1973; Lapper, Mantsch & Smith, 1972) but contrasts with the X-ray results for the other two comparable cyclic nucleotides (Na 2',3'-CMP, 2',3'-UMPS) which both display O(1') puckering with the other four atoms of the ribose essentially coplanar. Fig. 2 describes the ribose puckering in Na 2',3'-CMP and in the present structure. The torsion angles in the ribose moiety are presented in Table 4.

C(4')–C(5') *bond.* The conformation about the C(4')–C(5') bond is *gauche–gauche* with the angles O(1')–C(4')–C(5')–O(5') and C(3')–C(4')–C(5')–O(5') −48·7 and 68·5° respectively. This is again a commonly observed conformation but differs from that observed in the other two nucleotide 2',3'-cyclophosphates (*i.e.* Na 2',3'-CMP; 2',3'-UMPS), where *gauche–trans* and *trans–gauche* conformations were observed.

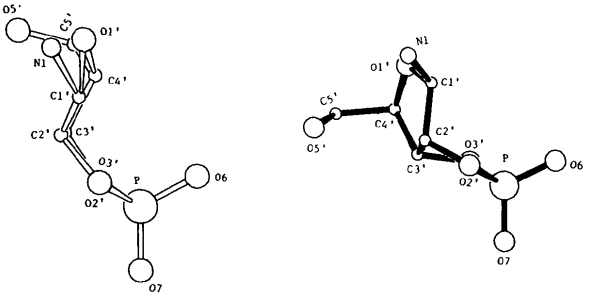

Fig. 2. A comparison of the puckering of ribose in (*a*) Na 2',3'-CMP [O(1')-*endo*] and (*b*) 2',3'-CMP free acid [C(2')-*endo*].

Table 4. *Torsion angles* (°) *in the ribose moiety*

C(1')–C(2')–C(3')–C(4')	−28·5
C(1')–C(2')–C(3')–O(3')	88·2
C(2')–C(3')–C(4')–O(1')	22·3
C(2')–C(3')–C(4')–C(5')	−96·5
C(3')–C(4')–O(1')–C(1')	−6·0
C(4')–O(1')–C(1')–C(2')	−12·8
O(1')–C(1')–C(2')–C(3')	25·4
O(1')–C(1')–C(2')–O(2')	139·0
H(1')—H(2')	146·9
H(2')—H(3')	−31·3
H(3')—H(4')	−96·8
H(4')—H(5')	52·4
H(4')—H'(5')	−68·1

Base. The cytosine base occurs as a cation, N(3) being the site of protonation. It has been shown earlier that cytidylic acid exists as a zwitterionic structure (Sundaralingam & Jensen, 1965). The major differences in bond distances and angles between the cytosine cation and the neutral base are confined to the site of protonation (Viswamitra, Reddy, Lin & Sundaralingam, 1971). The base ring atoms are essentially coplanar with an r.m.s. deviation of 0·0284 Å, but the exocyclic atoms of the cation are significantly displaced from the mean plane.

Molecular packing and hydrogen bonding

Fig. 3 shows a view of the packing of the molecules along the *c* axis and the hydrogen-bonding scheme. The hydrogen-bond distances and angles are given in Table 5. The protonated site of the base N(3) and the amino group N(4) are acting as hydrogen-bond donors to the phosphate O(7)($1-x$, $\frac{1}{2}+y$, $\frac{1}{2}-z$). The phosphate O(6)($-x$, $\frac{1}{2}+y$, $\frac{1}{2}-z$) also forms two hydrogen bonds with N(4) and the hydroxyl O(5′) as donors. The molecules related by these four hydrogen bonds form a zigzag chain pattern running along the *b* axis and the crystal packing may be viewed as an arrangement of these chains into sheets along the *ab* plane (Fig. 3). The carbonyl O(2) does not participate in any hydrogen bonding, similar to most of the protonated cytosine nucleosides and nucleotides [for example: 3′-cytidylic acid (3′-CMP, monoclinic, Bugg & Marsh, 1967; 3′-CMP, orthorhombic, Sundaralingam & Jensen, 1965); and deoxycytidine.HCl (Subramanian & Hunt, 1970)], the only exception being deoxycytidine 5′-phosphate (Viswamitra *et al.*, 1971).

Table 5. *Details of the hydrogen bonds*

A—H···*B*	*A*···*B*	*A*—H	H···*B*	∠*A*—H···*B*
N(4)—H(4)···O(6)	2·77 Å	1·02 Å	1·76 Å	169·9°
N(4)—H′(4)···O(7)	2·95	1·09	1·90	160·5
N(3)—H(3)···O(7)	2·91	0·87	2·04	180·0
O(5′)—H(O5′)···O(6)	2·72	0·78	2·00	153·4

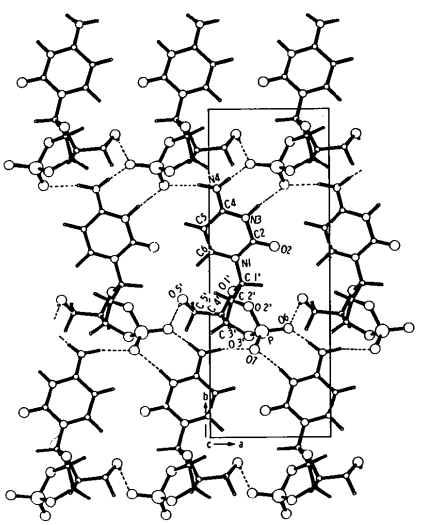

Fig. 3. Hydrogen-bonding scheme in 2′,3′-CMP viewed along the *c* axis; for clarity, not all the molecules are shown in the unit cell.

Conclusions

The free acid of 2′,3′-CMP exhibits a conformation preferred by most of the nucleosides and nucleotides. It is *anti* about the glycosidic bond and shows C(2′)-*endo*, C(3′)-*exo* ribose puckering while the Na salt of this nucleotide displays rather unusual conformational features [*syn* and O(1′)-*endo* puckering respectively]. It would be interesting to study whether this unusual change in the conformation is due to the presence of Na ions and hydration and, therefore, due to a different packing environment. It may be noted that cyclic 2′,3′-CMP crystallizes in various forms (for example, the ammonium salt of 2′,3′-CMP crystallizes in the monoclinic system with two molecules in the unit cell; Reddy & Viswamitra, 1971).

Thus it seems probable that the furanose ring in cyclic nucleotides does not correspond strictly to any possible rigid conformation, either O(1′) buckled or planar, in agreement with the conclusions drawn from the NMR study that the furanose ring here is also flexible as in most other nucleotides. The occurrence of O(1′)-*exo* and C(2′)-*endo* puckering modes in the ratio 3:1, however, suggests that unlike normal nucleotides the 2′,3′-cyclic species have different preferred sugar conformations.

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Acta Cryst. (1978). B**34**, 1524–1528

Crystal and Molecular Structure of (−)-(5*S*)-5-Hydroxy-5,6-dihydrothymidine

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(*Received* 14 *September* 1977; *accepted* 12 *November* 1977)

$C_{10}H_{16}N_2O_6$ is monoclinic, space group $P2_1$, with $a = 5{\cdot}534\ (2)$, $b = 12{\cdot}023\ (3)$, $c = 9{\cdot}242\ (3)$ Å, $\beta = 97{\cdot}91\ (3)°$. The structure was refined to an R of 0·070 for 1138 significant reflexions. The pyrimidine ring displays a twisted half-chair conformation with C(5) and C(6) displaced by 0·286 and 0·362 Å on opposite sides of the plane. The conformation about the glycosyl linkage is *anti* with $\chi = 68{\cdot}7°$. The conformation of the hydroxymethyl exocyclic group about C(4')–C(5') is *gauche–gauche*. The deoxyribose ring has the O(1')-*endo* envelope conformation with O(1') situated 0·519 Å from the plane through C(1'), C(2'), C(3') and C(4').

Introduction

Saturation of the heterocyclic ring of the pyrimidine bases represents a major type of radiation-induced damage in nucleic-acid *in vitro* models (Téoule, Bonicel, Bert, Cadet & Polverelli, 1974; Scholes, 1976), and in living cells (Hariharan & Cerutti, 1974). It was shown that ring-saturation pyrimidine radiation products could disrupt the ordered conformation of double-stranded deoxyribonucleic acids and interfere with their biological functions (Swinehart, Bobst & Cerutti, 1972). Important conformational changes take place in modified thymine derivatives, as demonstrated by the X-ray analyses of (−)-(5*S*)-5,6-dihydrothymidine (Konnert, Karle & Karle, 1970) and *cis*-5,6-dihydroxy-5,6-dihydrothymine (Flippen, 1973). We describe here the crystal structure and the sterically allowed conformations of a diastereoisomer of a pyrimidine ring-saturation nucleoside, (−)-(5*S*)-5-hydroxy-5,6-dihydrothymidine, which was obtained by γ irradiation of oxygen-free aqueous solutions of thymidine (Cadet, Ducolomb, Grand & Téoule, 1976).

Experimental

The compound was recrystallized from ethanol and mounted along **c**. The space group was determined from photographs with Cu $K\alpha$ radiation. From the extinctions obtained ($0k0$ absent for k odd), space groups $P2_1$ or $P2_1/m$ were indicated; however, as $Z = 2$, it was determined to be $P2_1$. Table 1 records the crystallographic data. Intensities were collected on a Siemens four-circle automatic diffractometer at the Laue–Langevin Institute (Grenoble) by the five-points method (Troughton, 1969) with Ni-filtered Cu $K\alpha$ radiation. 1290 independent reflexions were recorded in the range $3 < \theta < 70°$. A standard reflexion, checked periodically, showed no significant deviation. The data

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