The Crystal Structure and Molecular Conformation of 7,2'-Anhydro-β-D-arabino-sylorotidine (Cyclo Ara-O)

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Abstract

The crystal structure of 7,2'-anhydro-β-D-arabinosylorotidine (cyclo ara-O) has been determined using three-dimensional intensities measured on a diffractometer. The crystals are of the space group $P1$ with $a = 8.653$ (2), $b = 5.927$ (1), $c = 5.564$ (1) Å, $\alpha = 77.44$ (2), $\beta = 105.52$ (3), $\gamma = 89.39$ (2)° and $Z = 1$. The complete structure was obtained by inspection of an $(E^2 - 1)$ Patterson map and was refined by full-matrix least-squares techniques to an R index of 0.032. The estimated standard deviations in bond distances and bond angles are in the range 0.003–0.005 Å and 0.2–0.3° respectively. The conformation of the molecule is constrained by the fused six-membered ring which assumes a sofa conformation with C(2') displaced by 0.54 Å from the ring plane. The glycosyl conformation is high-*anti* ($\chi = 92.7°$), the sugar pucker is C(1')-*exo*,C(2')-*endo* ($_1T^2$) ($P = 132.1°$, $\tau_m = 41.5°$), and the conformation about the exocyclic C(4')–C(5') bond is *gauche*$^+$ ($\psi = 48.8°$). These conformational parameters are compared with those for cycloarabinonucleosides with fused five-membered rings. The molecules are arranged in sheets in the (2$\bar{1}$$\bar{1}$) planes and are held together by both intra- and intersheet hydrogen bonds.

Introduction

The anhydronucleosides have served as important model compounds in spectroscopic studies because the structural constraints imposed by the formation of a fused ring between the base and the sugar limit the spatial disposition of the base (Ulbricht, 1972; Ikehara & Tada, 1972). These structures exhibit conformational features which are sterically allowed in common nucleosides, but are seldom observed. The structure of 7,2'-anhydro-β-D-arabinosylorotidine (cyclo ara-O,

$C_{10}H_{10}N_2O_7$) is unusual in that the cyclization results in a six-membered ring instead of a five-membered ring as was found in the 2,2'- and 6,2'-anhydro-β-D-arabinosylnucleosides, a number of whose X-ray structures have already been reported (see below). The size of the fused ring (six *vs* five atoms) seems to have a strong influence on the puckering parameters of the sugar ring and in this paper we report the structure of cyclo ara-O and compare some of its main conformational features with those of the 2,2'- and 6,2'-anhydronucleosides. In addition to their interesting conformational properties, orotic acid (uracil-6-carboxylic acid) and orotidylic acid are important as intermediates in the pyrimidine biosynthetic pathway.

Experimental

Cyclo ara-O was purchased from Terra-Marine Bioresearch, LaJolla, California, in a crystalline form and was used for diffraction studies without further recrystallization. The crystals are triclinic; the space group and the crystal parameters are listed in Table 1. An automated Picker FACS-I diffractometer equipped with a graphite-crystal monochromator was used to collect 848 unique reflections to a resolution limit of 127° in 2θ (Cu $K\alpha$ radiation, $\lambda = 1.5418$ Å). The 825 structure amplitudes (97.3%) which were greater than three times their standard deviations were used in the structure analysis. 220 pairs of equivalent reflections were also collected and they agreed to an R index ($\sum |F - \bar{F}|/\sum |\bar{F}|$) of 2.1%. No absorption correction was applied.

Table 1. *Crystal parameters for cyclo ara-O*

Chemical formula	$C_{10}H_{10}N_2O_7$		
Molecular weight	270.2		
Space group	$P1$		
a	8.653 (2) Å	V	267.7 Å^3
b	5.927 (1)	Z	1
c	5.564 (1)	D_c	1.676 Mg m^{-3}
α	77.44 (2)°	D_m	1.656
β	105.52 (3)		
γ	89.39 (2)		

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The structure was solved by a visual inspection of the three-dimensional $E^2 - 1$ Patterson map which revealed virtually the entire structure with the plane of the base lying along the $(2\bar{1}\bar{1})$ plane in the unit cell. The structure was refined using full-matrix least-squares techniques employing a weighting scheme based on counting statistics where $w = 1/[\sigma_{F_o}^2 + (0.01F_o)^2]$. All of the H atoms were located in difference electron density maps and their positional parameters only were refined. The final R factor was 0.032 ($R = \sum \|F_o\| - k|F_c\|/\sum |F_o|$). Atomic scattering factors used for O, N and C were those of Cromer & Waber (1965) and for H, those of Stewart, Davidson & Simpson (1965).

Results

The positional parameters for the atoms of cyclo ara-O and their estimated standard deviations are listed in Table 2.* Drawings of the molecular conformation are

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34946 (5 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Positional parameters for the atoms of cyclo ara-O*

Standard deviations are given in parentheses and refer to the least significant digits. The positional parameters of the atom N(1) were not refined in order to fix the origin of the space group $P1$.

|	x	y	z
N(1)	0.2231	0.1578	0.2636
C(2)	0.1160 (3)	0.1631 (5)	0.0253 (5)
O(2)	0.1202 (2)	0.3040 (4)	−0.1663 (3)
N(3)	0.0088 (3)	−0.0058 (5)	0.0266 (4)
C(4)	−0.0007 (3)	−0.1762 (6)	0.2320 (6)
O(4)	−0.1026 (3)	−0.3155 (4)	0.2057 (5)
C(5)	0.1182 (3)	−0.1739 (5)	0.4711 (6)
C(6)	0.2248 (3)	−0.0130 (5)	0.4772 (5)
C(7)	0.3578 (3)	−0.0217 (5)	0.7173 (5)
O(7)	0.3739 (2)	−0.1804 (4)	0.9020 (3)
C(1')	0.3400 (3)	0.3273 (5)	0.2717 (5)
O(4')	0.4710 (2)	0.2480 (4)	0.1958 (3)
C(2')	0.4175 (3)	0.3607 (5)	0.5383 (5)
O(2')	0.4625 (2)	0.1389 (3)	0.7200 (3)
C(3')	0.5718 (3)	0.4688 (5)	0.5080 (5)
O(3')	0.5443 (2)	0.7158 (3)	0.4412 (4)
C(4')	0.6093 (3)	0.3745 (5)	0.2874 (5)
C(5')	0.7575 (3)	0.2116 (6)	0.3605 (6)
O(5')	0.7692 (2)	0.0307 (3)	0.5778 (4)
HN(3)	−0.064 (4)	0.016 (6)	−0.117 (6)
HC(5)	0.116 (3)	−0.307 (6)	0.607 (5)
HC(1')	0.281 (3)	0.477 (5)	0.159 (5)
HC(2')	0.341 (4)	0.447 (7)	0.600 (7)
HC(3')	0.669 (3)	0.428 (5)	0.676 (5)
HO(3')	0.507 (4)	0.778 (8)	0.281 (7)
HC(4')	0.619 (3)	0.512 (5)	0.134 (5)
H1C(5')	0.855 (4)	0.304 (6)	0.397 (5)
H2C(5')	0.755 (4)	0.159 (7)	0.195 (6)
HO(5')	0.702 (4)	−0.070 (6)	0.550 (6)
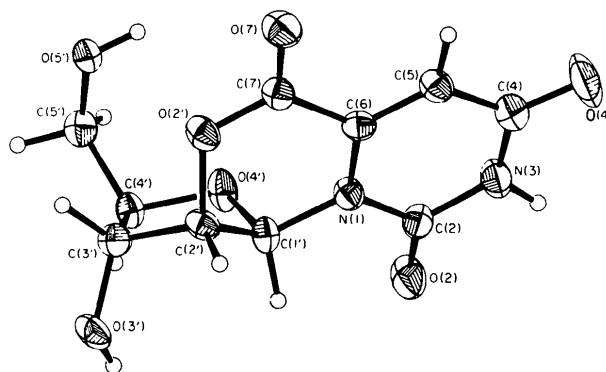
Fig. 1. Molecular conformation and atom numbering of cyclo ara-O shown in an *ORTEP* plot (Johnson, 1965) with thermal ellipsoids for the non-hydrogen atoms drawn at 50% probability. Note the C(1') puckering in the arabinose ring.
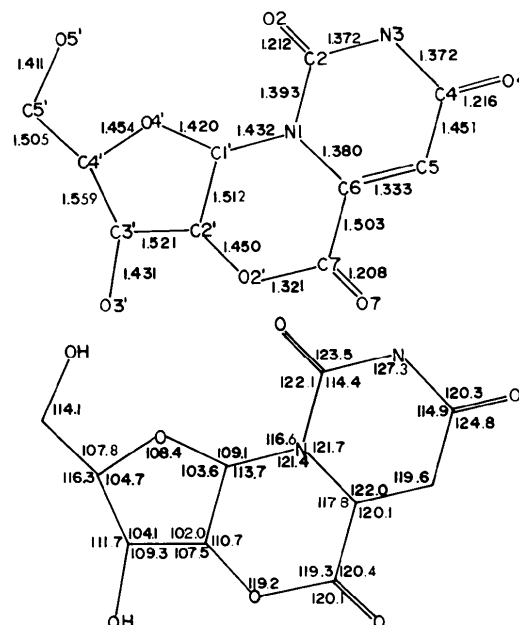

Fig. 2. Bond lengths (Å) (top) and bond angles (°) (bottom) for cyclo ara-O. Estimated standard deviations are between 0.003 and 0.005 Å for bond lengths and between 0.2 and 0.3° for bond angles.
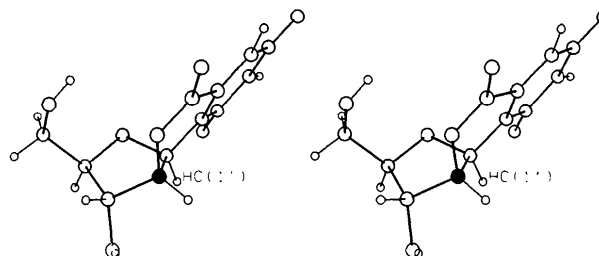

Fig. 3. A stereodiagram showing the conformation of the molecule with the sofa conformation for the six-membered lactone ring with C(2') (dark circle) puckered.
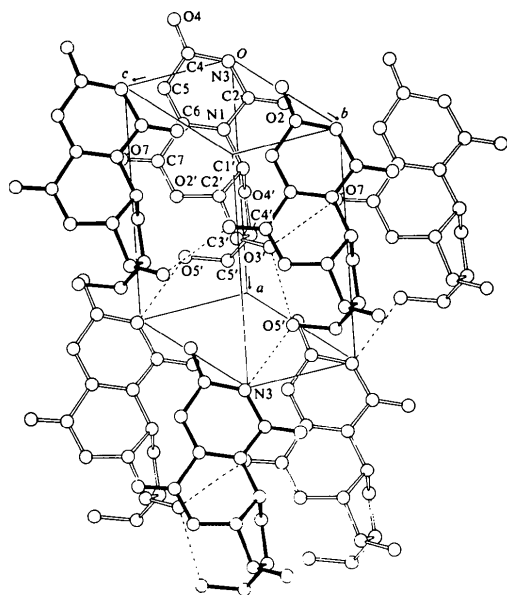
Fig. 4. Crystal-packing diagram of cyclo ara-O viewed normal to the (2Ī1) planes. Dashed lines represent hydrogen bonds.

shown in Figs. 1 and 3, the bond lengths and angles are shown in Fig. 2 and the molecular packing and hydrogen-bonding scheme are illustrated in Fig. 4.

Discussion

Cyclo ara-O is the only available structure of a cycloarabinoside with a six-membered fused ring involving the base and the 2' position of the sugar. However, numerous crystal structures of cyclo-arabinosides consisting of five-membered fused rings have been reported: 6,2'-cyclo ara-C (Yamagata, Fujii, Kanai, Ogawa & Tomita, 1979), 2,2'-cyclo ara-C (Brennan & Sundaralingam, 1973; Kartha, Phillips & Ambady, 1975), 2,2'-cyclo ara-U (Delbaere & James, 1973; Suck & Saenger, 1973), 2,2'-cyclo ara-3-deaza-U (Hutcheon & James, 1977), 2,2'-anhydro-1-β-D-arabinofuranosyl-5-dimethylsulfonio-6-oxocytosine chloride, 2,2'-anhydro-5-chloro-1-(3',5'-di-O-acetyl-β-D-arabinofuranosyl)-6-oxocytosine (Yamagata, Ko-shibe, Tokuoka, Fujii, Fujiwara, Kanai & Tomita, 1979), and 2,2'-cyclo ara-C-3',5'-diphosphate (Yamagata, Suzuki, Fujii, Fujiwara & Tomita, 1979). Whenever relevant, the molecular parameters of cyclo ara-O will be compared with those of the five-membered fused-ring systems.

Dimensions of the orotic acid base (uracil-6-carboxylic acid)

The pyrimidine base (orotic acid) of cyclo ara-O has some bond lengths and angles which differ signifi-

cantly from those found in the crystal structure of the base orotic acid monohydrate (Takusagawa & Shimada, 1973). C(4)—O(4) is shortened in cyclo ara-O (1·216 Å) compared with orotic acid (1·237 Å), and N(1)—C(2) is lengthened in cyclo ara-O (1·393 Å) compared with orotic acid (1·363 Å). The angle N(1)—C(6)—C(7) is increased to 117·8° in cyclo ara-O compared to 114·1° in orotic acid. To compensate for the above increase, the angles C(5)—C(6)—C(7) and O(7)—C(7)—O(2') are contracted in cyclo ara-O. The exocyclic angles around C(2) in cyclo ara-O are 122·1 and 123·5° while the trend is reversed in orotic acid (124·1 and 121·2°). The major differences in bond lengths and bond angles between cyclo ara-O and orotic acid itself are a result of the cyclization in the former. The remaining bond lengths and angles in the two crystal structures agree within three times their estimated standard deviations.

Geometry of the arabinose sugar

The sugar bond lengths of cyclo ara-O are typical for arabinose rings with the exception of C(3')—C(4') in cyclo ara-O which is rather long (1·559 Å). This is probably due to the small (5·9°) torsion angle C(2')—C(3')—C(4')—O(4') (Table 3) resulting from the puckering at the C(1') atom and thereby enhancing the eclipsing interactions between the substituents at C(3') and C(4'). However, the arabinose rings of the cycloarabinosides with five-membered fused rings, which are puckered at C(4'), have small values for the endocyclic torsion angle τ_1, and this enhances the

Table 3. Conformational parameters for cyclo ara-O

Torsion-angle definitions are after Sundaralingam (1969) and pseudorotation parameters are after Altona & Sundaralingam (1972). E.s.d.'s for torsion angles are about 0·5°.

Glycosyl conformation	χ	92·7°	'high anti'
Arabinose pucker	$_1T^2$		
Pseudorotation parameters			
phase angle	P	132·1	
puckering amplitude	τ_m	−41·4	
Arabinose endocyclic	τ_0	−39·0	
torsion angles	τ_1	41·0	
	τ_2	−27·8	
	τ_3	5·9	
	τ_4	20·5	
Exocyclic C(4')—C(5')	ψ	48·8	gauche⁺
conformation			

Endocyclic torsion angles of the fused six-membered ring
C(6)—N(1)—C(1')—C(2')	−22·4°
N(1)—C(1')—C(2')—O(2')	45·1
C(1')—C(2')—O(2')—C(7)	−46·8
C(2')—O(2')—C(7)—C(6)	22·9
O(2')—C(7)—C(6)—N(1)	3·3
C(7)—C(6)—N(1)—C(1')	−2·4

C(1')–C(2') bond distance by virtue of similar eclipsing interactions. Thus, the variability in the arabinose ring conformation is reflected in its bond distances. The severity of the ring strain present in the anhydroarabinosides with five-membered fused rings is somewhat relaxed in the six-membered fused ring of cyclo ara-O. Thus, the differences in molecular dimensions of cyclo ara-O from the noncyclized arabinosides (Sundaralingam, 1965) are less than those of the five-membered fused-ring arabinosides.

The planarity of the fused-ring system

In cyclo ara-O, the pyrimidine ring and fused ring are essentially planar with the exception of the atom C(2'). The atomic deviations are given in Table 4. C(2') is displaced by 0·567 Å on the HC(1') side (plane 1), thus conferring a sofa conformation on the six-membered lactone ring (Fig. 3).

Molecular conformation

The glycosyl conformation

The glycosyl torsion angle χ [C(6)–N(1)–C(1')–O(4')] (Sundaralingam, 1969) is obviously restricted in

cyclonucleosides since the base is fused to the sugar. In cyclo ara-O, its value is 92·7°, within the familiar 'high-*anti*' range (Prusiner, Brennan & Sundaralingam, 1973). The bases in the 2,2'-anhydroarabinosides are rotated 180° with respect to cyclo ara-O and their χ values are in the negative *anti* domain (−60 to −70°). However, the χ value in 6,2'-cyclo ara-C (111·4°) is similar to that of cyclo ara-O, as would be expected by the fusion to the C(6) atom of the base. The conformational parameters for cyclo ara-O are listed in Table 3.

The C–N glycosyl bond is extremely short in both cyclo ara-O (1·432 Å) and 6,2'-cyclo ara-C (1·437 Å) when compared to the average value for pyrimidine nucleosides of 1·491 Å (Lin, Sundaralingam & Arora, 1971). It has been found that as χ increases from 0° to about 90°, the C–N distance decreases (Prusiner *et al.*, 1973). A similar correlation has also been found between the C–N bond length and the torsion angle N(1)–C(1')–O(4')–C(4') (Altona & Sundaralingam, 1965; Lo, Shefter & Cochran, 1975). The C–N bond length decreases as the N–C–O–C torsion approaches 180° and the O(4') lone pair of orbitals are oriented *gauche* to the N(1) atom. In cyclo ara-O this torsion is −160·4° and it is 142·3° in 6,2'-cyclo ara-C. Thus, both the χ values and the C(1')–O(4') bond orientation predict short glycosyl bonds for these two structures.

Table 4. *Least-squares planes for cyclo ara-O*

The equations to the planes are

1    $-0·6985X + 0·6690Y + 0·2540Z = 0·038$
2    $0·7112X - 0·6723Y - 0·2054Z = 0·007$
3    $-0·6904X + 0·6589Y + 0·2986Z = 0·186$,

where X,Y,Z are in Å referred to the orthogonal system **a**, **c*** × **a**, **c***.

Displacements from the planes (Å) (e.s.d.'s ~0·003 Å)

	Plane 1	Plane 2	Plane 3
N(1)	0·972†	−0·034†	−0·014†
C(2)	−0·019†	−0·010†	−0·163
O(2)	−0·103†	0·027†	−0·294
N(3)	−0·030†	−0·008†	−0·170
C(4)	0·014†	−0·002†	−0·074
O(4)	0·000†	−0·003†	−0·093
C(5)	0·006†	0·014†	0·036
C(6)	0·072†	0·018†	0·041†
C(7)	−0·001†	0·161	0·028†
O(7)	−0·132†	0·340	−0·055†
C(1')	0·056†	−0·007	−0·031†
O(2')	0·005†	0·165	0·031†
C(2')	0·567	−0·449	0·540
HC(1')	0·64	−0·63	0·52

The dihedral angles between the planes are: (1,2) = 2·9, (1,3) = 2·7, (2,3) = 5·5°.

† Atoms included in the calculations.

Conformation of the arabinosyl furanose ring

The major conformational difference between cyclo ara-O and the anhydroarabinosides with five-membered fused rings resides in the arabinose sugar. In the anhydroarabinosides the five-membered fused rings are nearly planar, with the C(1')–C(2') endocyclic torsion angles close to zero. In the arabinose rings the O(4')–C(1')–C(2')–C(3') (τ_1) torsion angles range from 2·9 to 12·9°. The planarity of the fused ring restricts the arabinose puckering to the atom C(4'), yielding either C(4')-*endo* or C(4')-*exo* puckering.

The conformation of the arabinose ring in cyclo ara-O is markedly different from that of the anhydroarabinosides and is caused by the presence of the additional atom in the fused ring which results in a reduction of ring strain. Because of the conjugation between the pyrimidine ring and the C(7)–O(7) carbonyl function, the fused ring is planar at all atoms except C(2'), and the endocyclic C(1')–C(2') torsions in both the arabinose and fused rings are significantly non-zero. This causes the arabinose ring to assume C(1')-*exo*,C(2')-*endo* ($_1T^2$) puckering with a pseudorotation phase angle P of 132·1° (Altona & Sundaralingam, 1972). Thus, the size and the conformation of the fused rings are correlated with the conformation of the arabinose ring.

The exocyclic C(4')—C(5') conformation

The rotation around the exocyclic C(4')—C(5') bond is described by the torsion angle ψ [C(3')—C(4')—C(5')—O(5')] (Sundaralingam, 1969), where O(5') may occupy one of three non-eclipsed orientations: gauche⁺ (g⁺), gauche⁻ (g⁻) and trans (t) (Shefter & Trueblood, 1965; Sundaralingam, 1965). In cyclo ara-O, the exocyclic C(4')—C(5') conformation is g⁺, but the $_1T^2$ sugar pucker prevents any short contacts to the base as was found, for example, in cyclo ara-C (Brennan & Sundaralingam, 1973).

Hydrogen bonding and crystal packing

Hydrogen-bonding geometries for cyclo ara-O are listed in Table 5, and the packing in the triclinic unit cell of the crystal is shown in Fig. 4. All three potential hydrogen-bonding protons are involved in hydrogen bonds. The hydroxyl oxygens O(3') and O(5') are both donors and acceptors, so that only four atoms are involved in the three hydrogen bonds. There is a chain of hydrogen bonds running from N(3) to O(5') to O(3') and terminating at O(7). Neither of the carbonyl oxygens [O(2) and O(4)] is hydrogen bonded in cyclo ara-O; an unusual occurrence in uracil compounds. This may be due to the presence of only translational symmetry operations in the cyclo ara-O crystal.

The molecules lie approximately on the (21 1̄) planes with an interplanar spacing of 3·09 Å. The pyrimidine and fused rings are thus not directly stacked on one another. The N(3)—O(5')—O(3')—O(7) hydrogen-bon-

ding chain involves molecules both between and within the (21 1̄) planes. The N(3)···O(5') and O(3')···O(7) hydrogen bonds are intraplanar, and the O(5')···O(3') hydrogen bond links the adjacent planes. Several close intermolecular contacts are observed in the crystal and they mainly occur between molecules on adjacent (21 1̄) planes. These are listed in Table 6. It is notable that the closest contact (2·841 Å) is between the ring oxygen atoms O(2') and O(4') of atoms on adjacent (21 1̄) planes.

References

ALTONA, C. & SUNDARALINGAM, M. (1965). Unpublished work.

ALTONA, C. & SUNDARALINGAM, M. (1972). J. Am. Chem. Soc. 94, 8205–8212.

BRENNAN, T. & SUNDARALINGAM, M. (1973). Biochem. Biophys. Res. Commun. 52, 1348–1353.

CROMER, D. T. & WABER, J. T. (1965). Acta Cryst. 18, 104–109.

DELBAERE, L. T. J. & JAMES, M. N. G. (1973). Acta Cryst. B29, 2905–2912.

HUTCHEON, W. L. B. & JAMES, M. N. G. (1977). Acta Cryst. B33, 2228–2232.

IKEHARA, M. & TADA, H. (1972). Jerusalem Symp. Quantum Chem. Biochem. 4, 455–468.

JOHNSON, C. K. (1965). ORTEP. Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.

KARTHA, G., PHILLIPS, T. & AMBADY, G. (1975). Acta Cryst. A31, S52–S53.

LIN, H. Y., SUNDARALINGAM, M. & ARORA, S. K. (1971). J. Am. Chem. Soc. 93, 1235–1241.

LO, A., SHEFTER, E. & COCHRAN, T. G. (1975). J. Pharm. Sci. 64, 1707–1710.

PRUSINER, P., BRENNAN, T. & SUNDARALINGAM, M. (1973). Biochemistry, 12, 1196–1202.

SHEFTER, E. & TRUEBLOOD, K. N. (1965). Acta Cryst. 18, 1067–1077.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). J. Chem. Phys. 42, 3175–3187.

SUCK, D. & SAENGER, W. (1973). Acta Cryst. B29, 1323–1330.

SUNDARALINGAM, M. (1965). J. Am. Chem. Soc. 87, 599–606.

SUNDARALINGAM, M. (1969). Biopolymers, 7, 821–860.

TAKUSAGAWA, F. & SHIMADA, A. (1973). Bull. Chem. Soc. Jpn, 46, 2011–2019.

ULBRICHT, T. L. V. (1972). Jerusalem Symp. Quantum Chem. Biochem. 4, 170–177.

YAMAGATA, Y., FUJII, S., KANAI, T., OGAWA, K. & TOMITA, K. (1979). Acta Cryst. B35, 378–382.

YAMAGATA, Y., KOSHIBE, M., TOKUOKA, R., FUJII, S., FUJIWARA, T., KANAI, T. & TOMITA, K. (1979). Acta Cryst. B35, 382–389.

YAMAGATA, Y., SUZUKI, Y., FUJII, S., FUJIWARA, T. & TOMITA, K. (1979). Acta Cryst. B35, 1136–1140.

Table 5. *Hydrogen-bonding geometries for cyclo ara-O*

E.s.d.'s are about 0·003 Å and 0·3° for distances and angles.

Donor (D)	Acceptor (A)	Symmetry for acceptor	D···A (Å)	D—H (Å)	H···A (Å)	D—H···A (°)
N(3)	O(5')	$x-1, y, z-1$	2·751	0·86	1·90	174
O(3')	O(7)	$x, y+1, z-1$	2·892	0·86	2·07	159
O(5')	O(3')	$x, y-1, z$	2·784	0·85	1·94	173

Table 6. *Short intermolecular contacts in cyclo ara-O*

E.s.d.'s are about 0·003 Å.

Atom A	Atom B		Symmetry for atom B
Between (21 1̄) planes			
O(4')	O(2')	2·841 Å	$x, y, z-1$
O(2)	C(7)	3·089	$x, y, z-1$
C(6)	O(3')	3·257	$x, y-1, z$
C(7)	O(3')	3·114	$x, y-1, z$
O(4')	O(3')	3·136	$x, y-1, z$
Within a (21 1̄) plane			
O(4)	C(4')	3·184	$x-1, y-1, z$
O(7)	C(1')	3·250	$x, y-1, z+1$
O(7)	C(4')	3·286	$x, y-1, z+1$