X-Ray Structure of 2′,3′-O-(2-Carboxyethyl)-ethylideneadenosine A Spacer Extended Ligand for Affinity Resins

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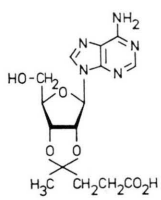
X-Ray Structure of 2′,3′-O-(2-Carboxyethyl)-ethylideneadenosine, Absolute Configuration and Conformation of a Spacer Extended Affinity Ligand

The title compound crystallizes in space group $P\,4_32_12$ with $a=b=9.558$ Å, $c=36.649$ Å. The structure has been determined by direct methods on the basis of 1624 X-ray counter data and refined by least squares methods to $R=6.7\%$. The acidic proton resides at the carboxyl group and not at adenine N(1) and the acetal methyl group is *exo* while the bulkier carboxyethyl group is *endo* relative to the ribose. The nucleoside occurs in the *syn* conformation, the conformation about C(4′)–C(5′) is *trans*, *gauche*, thus prohibiting an intramolecular O(5′)–H...N(3) hydrogen bond. The ribose is in a slightly puckered C(3′)-*exo* envelope conformation, the carboxyethyl group is all-*trans* rendering the molecule in an extended form with a distance 11 Å between the carboxyl group and amino group N(6). The acetal methyl group in *exo* displays a pmr signal at 1.35 ppm and can serve to distinguish the two methyl signals of O-2′,3′-isopropylideneadenosine at 1.38 ppm and at 1.65 ppm.

Introduction

The immobilization of ribonucleosides by attachment to polymers has a special importance because of their widespread occurence as substrates and cofactors for certain classes of enzymes [1–3]. Recently we developed a new method for immobilizing ribonucleosides in which the binding to the polymer is effected through HO-2′,3′ [4, 5]. The general utility of the immobilization via 2′,3′-cyclic acetal derivatives has promted us to investigate the crystal and molecular structure of 2′,3′-O-(2-carboxyethyl)-ethylideneadenosine (**1**).



1

This spacer extended adenosine binds strongly to the active centre of the enzyme adenosine deaminase [5], where it is converted to the corresponding inosine derivative. Condensation of **1** with polymers *e.g.* aminohexyl agarose derivatives led to affinity resins with high specificity towards adenosine deaminase [5].

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Experimental

The ethyl ester of **1** was prepared by treatment of adenosine with ethyl levulinate in the presence of hydrogen chloride and triethyl orthoformate [6]. After saponification with aqueous sodium hydroxide the free acid was precipitated at pH 5. Tetragonal bipyramids with well defined faces were obtained when a concentrated aqueous solution of **1** was cooled down slowly.

Crystallographic data derived by photographic methods and from measurements using an automated STOE four circle diffractometer are presented in Table I. Reflection data were collected with Ni-

Table I. Crystal data.

Chemical formula:	$C_{15}H_{19}N_5O_6$
	$a=b=9.558(3)$ Å; $c=36.649(6)$ Å
Space group	$P\,4_32_12$
	$z=8$
	$d_{calc.}=1.45$ g/cm³; $d_{obs}=1.44$ g/cm³
	$Cu_k=1.5418$ Å
Number of reflections	1624
Final R value	6.7%

filtered $Cu_{k\alpha}$ radiation in the $\omega/2\,\Theta$ scan mode and corrected for geometrical factors but not for absorption. Three check reflections monitored periodically after 100 measurements showed no significant loss of intensity.

According to Wilson's method [7] an overall temperature factor ($B = 3.6$ Å) and scale factor were evaluated and used to compute normalized structure amplitudes, E's. The structure was solved in space group $P\,4_12_12$ by the multisolution method MULTAN [8] with a starting set consisting of four reflections. 64 different phase sets were obtained for the 237 normalized structure amplitudes with $E > 1.4$, one of which was clearly more consistent than the others. With the phase angles of this set an E-map was computed which revealed the whole structure but obviously in its wrong enantiomorph so that the space group had to be changed to $P\,4_32_12$. The structure was refined by full matrix least squares methods. The hydrogen atom positions were calculated from the heavy atom skeleton and included in the structure factor calculations during the last cycles of refinement with assignment of the anisotropic temperature factors of the attached atoms; the methyl group C(5″) was refined as a rigid body. The data were corrected for secondary extinction according to the expression $F^* = (1 - 0.0001 \cdot x \cdot F^2)/\sin\Theta$ and the weighting scheme used was $\omega = K/[\mathrm{sig}^2(F_0) + g(F_0)^2]$. Parameters k, x and g were subjected to refinement. A final difference Fourier synthesis did not reveal the hydrogen atoms bonded to O(5′), O(1″1) and N(6). In the last refinement cycle average parameter changes were less than 1/3 the standard deviations derived from the correlation matrix. The final R factor is 6.7% including all reflections.

Results and Discussion

a) X-ray analysis

Fig. 1 gives bond angles and distances with standard deviations *. The molecular conformation is displayed in Fig. 2 with ellipsoids indicating thermal motion of the second row atoms.

Within the standard deviations bond distances and angles are in satisfactory agreement with data obtained from crystal structures containing adenine or ribose [10, 11]. From bond lengths N(1) – C(2), N(1) – C(6) and the angle C(2) – N(1) – C(6) there is no evidence for N(1) to be protonated [13]. The acidic proton must be attached to O(1″1) as suggested by the asymmetry of the carboxylic group.

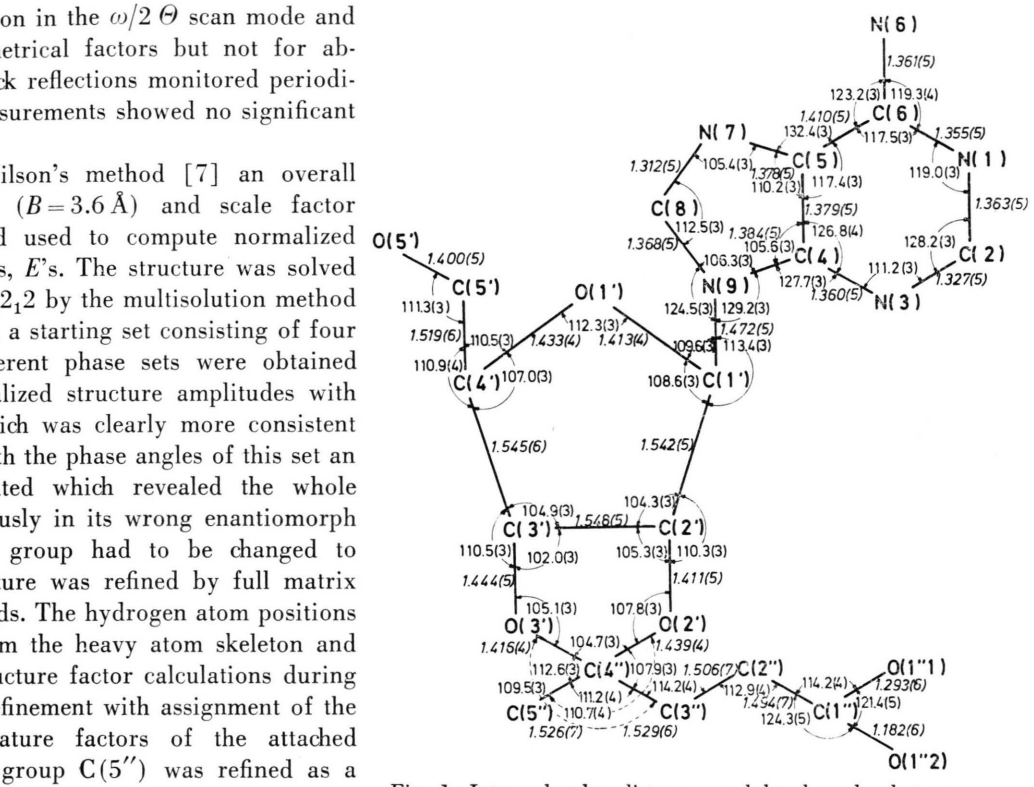

Fig. 1. Intramolecular distances and bond angles between nonhydrogen atoms; standard deviations in brackets.

The purine heterocycle is fairly planar (Table IV) and in *syn* conformation relative to the ribose [$\chi_{CN} = O(1') - C(1') - N(9) - C(4) = 77.5°$, Table III]. The orientation of the C(5′) – O(5′) bond is *trans*, *gauche* [O(5′) – C(5′) – C(4′) – O(1′) = 173.5°] and therefore no intramolecular hydrogen bond O(5′) – H···N(3) can form. The best four atoms least squares plane involving ribose atoms (Table IV) indicates that the five membered ring

Table II. Intramolecular hydrogen bonds.

Donor	Acceptor	Distance (Å)	Symmetry-Operation (on the first atom)
O(1″1)	N(1)	2.703	$-1-x, \; -1-y, \; .5+z$
N(6)	O(1″2)	2.839	$1-y, \; 1-x, \; 1.5-z$
N(6)	O(5′)	2.907	$.5+y, \; .5-x, \; -.75+z$
O(5′)	N(7)	2.775	$-1+y, \; x, \; -z$

* The atomic parameters are available on request.

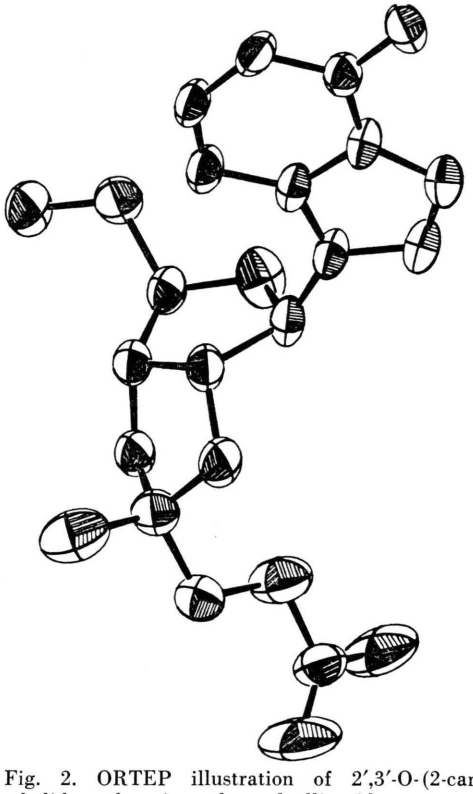

Fig. 2. ORTEP illustration of 2′,3′-O-(2-carboxyethyl)-ethylideneadenosine; thermal ellipsoides are represented at the 50% probability level.

Table III. Selected torsion angles. These four-atom angles A−B−C−D are defined as zero if, when looking from B to C, bonds A−B and C−D are cis-planar. The angle is counted positive if the far bond C−D is rotated clockwise with respect to the near bond A−B.

A−B−C−D				Angle in degrees (mean error: ±0.5°)
C(8)	−N(9)	−C(1′)	−O(1′)	−101.3
C(4)	−N(9)	−C(1′)	−O(1′)	77.5
C(1′)	−C(2′)	−C(3′)	−C(4′)	−16.0
C(2′)	−C(3′)	−C(4′)	−O(1′)	16.3
C(3′)	−C(4′)	−O(1′)	−C(1′)	−10.2
C(4′)	−O(1′)	−C(1′)	−C(2′)	− .4
O(1′)	−C(1′)	−C(2′)	−C(3′)	10.6
O(1′)	−C(4′)	−C(5′)	−O(5′)	173.5
O(3′)	−C(3′)	−C(4′)	−O(1′)	−92.9
O(5′)	−C(5′)	−C(4′)	−C(3′)	−68.0
O(5′)	−C(5′)	−C(4′)	−O(1′)	173.5
O(2′)	−C(2′)	−C(3′)	−O(3′)	−16.9
C(2′)	−C(3′)	−O(3′)	−C(4″)	34.4
C(3′)	−O(3′)	−C(4″)	−O(2′)	−39.7
O(3′)	−C(4″)	−O(2′)	−C(2′)	28.4
C(4″)	−O(2′)	−C(2′)	−C(3′)	− 6.6
C(3′)	−O(3′)	−C(4″)	−C(5″)	80.6
C(3′)	−O(3′)	−C(4″)	−C(3″)	−155.1
C(4″)	−C(3″)	−C(2″)	−C(1″)	−176.5
C(3″)	−C(2″)	−C(1″)	−O(1″1)	169.9

Table IV. Least squares planes through the heterocyclic rings, the sugar ring and adjacent ring. The equations of the planes are of the form $l\,x+m\,y+n\,z+p=0$ were x, y, z are along a, b, c. Minimized distances are marked by *.

A. Heterocycles

$l=.6799$ $m=-.5152$ $n=.5218$ $p=-7.9326$

Atoms	Displacement [Å]
N(1)	−.007 *
C(2)	−.002 *
H(2)	.007 *
N(3)	−.006 *
C(4)	−.010 *
C(5)	−.016 *
C(6)	.009 *
N(6)	.010 *
N(7)	−.009 *
C(8)	.004 *
H(8)	−.002 *
N(9)	.021 *
C(1′)	.043
C(1″)	1.941

B. Sugar

$l=.7470$ $m=.6648$ $n=.0103$ $p=-4.4891$

Atoms	Displacement [Å]
C(1′)	.002 *
O(1′)	−.002 *
C(4′)	.001 *
C(3′)	0.270
C(2′)	−.001 *

C. Adjacent cyclic acetal

$l=-.3789$ $m=.8931$ $n=.2423$ $p=-5.0764$

Atoms	Displacement [Å]
C(6)	.920
C(3′)	−.094 *
C(2′)	.097 *
O(2′)	−.066 *
O(3′)	.064 *
C(4″)	−.488

is only slightly puckered, with C(3′) in *exo* position by only 0.27 Å compared with the 0.5 Å observed for unmodified nucleosides. This finding confirms pmr studies on isopropylidene nucleosides proposing a C(3′)-*exo* conformation constrained by the attached five membered acetal ring [13]. In this crystal structure the latter is puckered as well with C(4″) in *exo* orientation with respect to the ribose.

The acetal methyl group is oriented *exo* while the bulkier carboxyethyl chain is *endo* and so the absolute configuration at C(4″) is *R*. The chain occurs in an all *trans* conformation and is located nearly

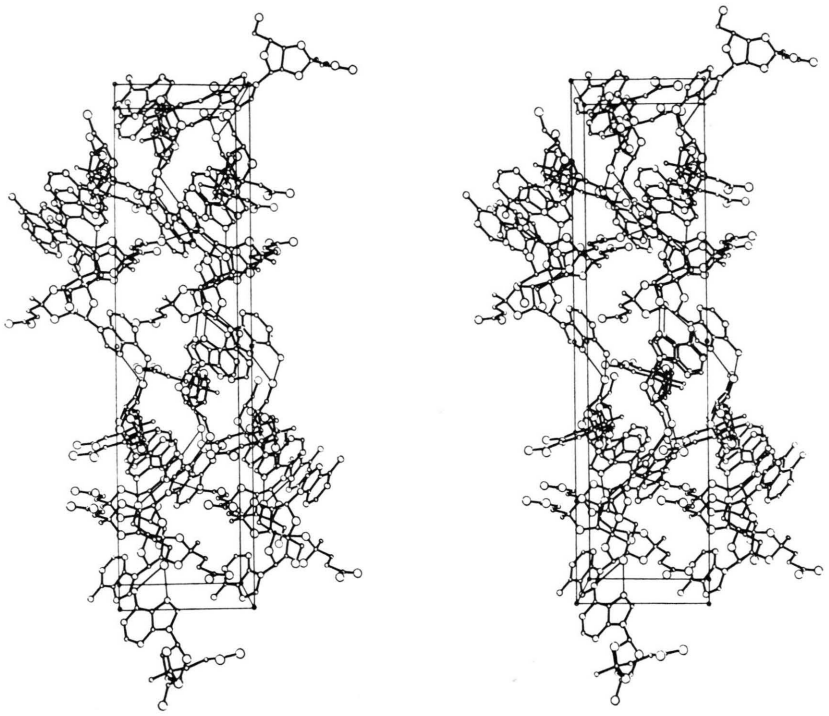

Fig. 3. ORTEP-stereogram of the unit cell, projection along b, view distance 30 Å.

parallel to the adenine plane with C(1″) deviating by 1.94 Å from it (Table IV). The spacer really expands the molecule, placing the carboxyl group at 11 Å from the adenine amino group.

The packing of the molecules in the unit cell is determined by base stacking and by hydrogen bonds between N(1) and N(6) of adenine and carboxyl groups of adjacent molecules. Further, O(5′) is attached simultaneously to N(6) and N(7) of the same base (Table II). The arrangement of the molecules in the unit cell is displaced in the stereo diagram Fig. 3.

b) NMR data

Although the acetal carbon of **1** is a chiral center, it had been shown that only one diastereoisomer was formed by treatment of adenosine with ethyl levulinate [6]: Compound **1** shows *one* signal for the acetal methyl group at 1.35 ppm in the proton NMR and at 23.51 ppm in the carbon NMR respectively. In contrast 2′,3′-O-isopropylideneadenosine gives two signals for the acetal methyl groups at 1.38 and 1.65 ppm in the ^{1}H and at 27.1 and 25.1 ppm in the ^{13}C NMR.

Since the X-ray analysis of **1** shows the methyl group in *exo*-position, the ^{1}H-signal at 1.38 ppm can be assigned to that group. The X-ray structure of **1** also allows the conclusion, that in 2′,3′-O-cyclic acetal derivatives like isopropylideneadenosine, the deshielded methyl signal (1.65 ppm) belongs to the endo group and the unshielded methyl signal (1.35 ppm) indicates the exo methyl group. The latter assignment agrees with the data of Imbach [15], imposing that the unshielding is due to the 2′,3′ protons at the ribose.

Table V. ^{13}C NMR shifts (δ values in ppm) relative to TMS (solvent: DMSO-d_6); mode: Fourier-transform; Bruker WP-270 spectrometer.

	Adenosine	Compound **1**	2′,3′-O-Isopropylidene-adenosine
C-2	152.7	152.6	152.7
C-4	149.3	148.9	148.9
C-5	119.5	119.2	119.2
C-6	156.3	156.1	156.2
C-8	140.3	139.6	139.6
C-1′	88.4	89.6	89.8
C-2′	73.9	81.3	81.4
C-3′	74.7	83.3	83.3
C-4′	87.0	86.6	86.4
C-5′	62.7	61.6	61.7
C-1″		174.2	
C-2″		33.8	
C-3″ or CH_3-*endo*		28.4	27.1
C-4″ or C-acetal		113.9	113.1
C-5″ or CH_3-*exo*		23.5	25.1

Acetal formation at the 2′,3′-hydroxyls in **1** can easily be detected by the strong downfield shift of the ^{13}C signals of the 2′,3′ carbons, which is similar to that of isopropylideneadenosine (Table V). Furthermore, the ^{13}C signal of the acetal substituents indicate that the exo methyl group absorbs in the high field region and the endo methyl group in the low field region.

c) Biospecificity of spacer extended 2′,3′-O-cyclic adenosine acetals

In this crystal structure, the side chain of **1** occurs in an all trans conformation as one would hope for a spacer. The *endo* conformation of the carboxyethyl chain together with the *syn* conformation of the nucleoside leads to an extended shape of the molecule. If the nucleoside derivative **1** is used as a ligand for affinity resins and shows similar stereochemistry as in the crystalline state the interaction with the active site of adenosine deaminase should not be reduced. In fact highly specific affinity resins for this enzyme have been synthesized recently [5].

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