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Comparison of Solid State and Solution Conformations of *R* and *S* Epimers of 8,5′-Cycloadenosine and Their Relevance to Some Enzymatic Reactions†

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ABSTRACT: The C(5′)-*R* epimer of 8,5′-cycloadenosine crystallizes in the monoclinic space group $P2_1$ ($Z = 2$) with unit cell dimensions $a = 5.755$ (1), $b = 16.895$ (1), and $c = 5.511$ (1) Å and $\beta = 104.16$ (1)°. X-ray intensity data were measured on a diffractometer, and the crystal structure was determined by direct methods. Least-squares refinement converged at $R = 0.037$ for 1108 reflections. The conformation about the glycosyl bond is anti, as imposed by the 8,5′ cyclization, with $\chi_{CN} = 29.8°$. The ribose ring adopts the unusual C(1′)endo–O(4′)exo (^1_OT) conformation with pseudorotation parameters $P = 289.0°$ and $\tau_m = 49.0°$. The six-membered ring formed by the 8,5′ linkage is approximately a half-chair with C(4′) and O(4′), respectively, above and below a plane defined by the other four atoms. These results are compared with those previously reported for the corresponding *S* epimer [Haromy, T. P., Raleigh, J., & Sundaralingam, M. (1980) *Biochemistry 19*, 1718–1722]. The conformations of the sugar rings and the exocyclic groups of both epimers in the solid state are compared to the conformations in solution, as determined by analysis of the systems of proton–proton vicinal coupling constants from the ^{1}H NMR spectra. The foregoing findings are employed to examine the role of the conformational parameters of adenosine and 5′-AMP in reactions catalyzed by the appropriate enzymes.

The *R* and *S* diastereoisomers of 8,5′-cycloadenosine and its 5′-phosphate, which are carbon-bridged analogues of adenosine and 5′-AMP, have been employed as model compounds to furnish useful information about the conformation of purine nucleosides in solution (Stolarski et al., 1980) and about the conformation of adenosine and its 5′-phosphate in enzyme–substrate complexes (Raleigh & Blackburn, 1978; Dudycz & Shugar, 1979). These structurally rigid analogues may be regarded as simulating adenosine and 5′-AMP with the aglycon in the anti conformation and with the exocyclic carbinol group in the conformation trans (the *R* epimer) or gauche⁻ (the *S* epimer). Notwithstanding that the conformation of the pentofuranose ring differs from that typically found for pentofuranosyl nucleosides in solution, the anticipated invariability of this conformation on formation of enzyme–substrate complexes points to their potential utility for determination of the conformational requirements dictated by enzymes with which they may interact.

In contrast to the reported findings of Hampton et al. (1972a,b) regarding the substrate properties of these analogues, we have found that neither the *R* or *S* epimers of 8,5′-cycloadenosine nor its 5′-phosphates are substrates of the aminohydrolases of adenosine and 5′-AMP, respectively. With snake venom 5′-nucleotidase, the *S* epimer of 8,5′-cycloadenosine 5′-phosphate was also found not to be a substrate (Dudycz & Shugar, 1979), in agreement with the findings of Raleigh & Blackburn (1978). On the other hand, the *R* epimer, although a substrate for this enzyme, was hydrolyzed at a much lower rate than that reported by Hampton et al. (1972b). Since the compounds synthesized by Hampton et al. (1972a,b) exhibited physicochemical properties at variance with those synthesized by us (Stolarski et al., 1980) and Matsuda et al. (1978) according to a procedure different from that employed by Harper & Hampton (1972) and Raleigh & Blackburn (1978), it was of obvious interest to establish unequivocally the structures of the foregoing model analogues. We have therefore extended previous results obtained by ^{1}H NMR spectroscopy (Stolarski et al., 1980) with X-ray diffraction data on single crystals.

We describe here the solid-state structure of the *R* epimer of 8,5′-cycloadenosine, which fully confirms and extends the assignment previously made in solution on the basis of ^{1}H NMR spectroscopy. Since this was prepared from the *S* diastereoisomer by a change in the configuration at C(5′) of the latter, the authenticity of the *R* epimer establishes that of the *S* epimer, as well as of the corresponding 5′-phosphates (Dudycz & Shugar, 1979). After the completion of this investigation a report appeared describing the single-crystal structure of the *S* epimer of 8,5′-cycloadenosine (Haromy et al., 1980), prepared according to the procedure of Raleigh & Blackburn (1978). Thus we can make a direct comparison between the two epimers, both in the solid state and in solution, and discuss their relevance to the substrate properties of both the nucleosides and nucleotides.

Experimental Procedures

The *R* and *S* epimers of 8,5′-cycloadenosine were synthesized essentially as described by Matsuda et al. (1978). The structures of the two compounds were established, apart from X-ray diffraction for the *R* epimer as described below, by means of ^{1}H NMR spectroscopy (Stolarski et al., 1980) with the aid of a Bruker-90 spectrometer operating in the Fourier

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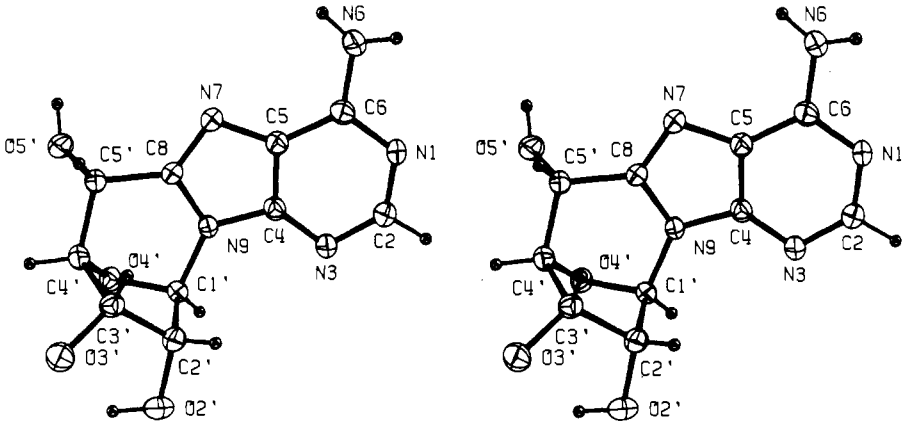


FIGURE 1: Stereoscopic view of the C(5′)-*R* epimer of 8,5′-cycloadenosine; the thermal ellipsoids correspond to 50% probability.

transform mode, on solutions at a concentration of 0.03 M. The structures were further confirmed by ^{13}C NMR spectroscopy of the 5′-phosphates of the *R* and *S* epimers (Dudycz & Shugar, 1979) with the aid of a Varian-80 spectrometer at a frequency of 20 MHz by using solutions at a concentration of 0.1 M. The values of the chemical shifts (in ppm vs. internal tetramethylsilane) were as follows for the heterocyclic carbons: C(2), 153.5; C(4), 147.5; C(5), 118.8; C(6), 155.8; C(8), 164.8 (identical for both the *R* and *S* epimers). The values for chemical shifts for the sugar carbons were as follows: C(1′), 85.08 (*S*) and 87.63 (*R*); C(2′) and C(3′), 69.92 and 75.63 (*S*), 71.00 and 74.49 (*R*); C(4′), 89.31 (*S*), 89.27 (*R*); C(5′), 67.39 (*S*), 68.06 (*R*). Signal assignments were based on a parallel ^{13}C NMR spectrum for the parent 5′-AMP and previously reported literature data for 5′-AMP (Uesugi & Ikehara, 1978). The assignments of the C(2′) and C(3′) signals are, however, not unequivocal.

Suitable crystals of the *R* epimer for X-ray diffraction were obtained by stepwise cooling of a hot aqueous solution of the compound. A colorless plate, measuring 0.25 × 0.20 × 0.05 mm, was mounted on an Enraf-Nonius CAD4 diffractometer. Unit cell dimensions were determined from a least-squares refinement of the angular settings of 18 high-order reflections ($2\theta > 110°$). The following crystal data were obtained: a = 5.755 (1), b = 16.895 (1), and c = 5.511 (1) Å and β = 104.16 (1)°; V = 519.6 Å^3; D_x = 1.70 g cm^{-3}; Z = 2; $F(000)$ = 276; μ(Cu Kα) = 11.0 cm^{-1}. Intensity data were measured with nickel-filtered copper radiation (Cu Kα, λ = 1.5418 Å). There were 1125 unique reflections with $2\theta \leq 155.8°$ of which 1100 had intensities higher than $1.5\sigma(I)$. The intensities were corrected for Lorentz and polarization factors, but absorption corrections were considered unnecessary.

The structure was determined by direct methods with the aid of the computer program MULTAN78 (Main et al., 1978). Of the 40 starting sets subjected to tangent refinement, the solution with the highest combined figure of merit yielded an *E* map on which all 19 nonhydrogen atoms were located. Atomic parameters were refined by block-diagonal least squares. All hydrogen atoms, except the one attached to O(3′), were found on difference Fourier maps and refined with isotropic temperature parameters. Scattering factors were taken from the *International Tables for X-Ray Crystallography* (1974). Throughout the refinement the function $\Sigma w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1 w_2$, where $w_1 = 1$ for $|F_o| \leq 8.0$, $w_1 = 8.0/|F_o|$ for $|F_o| > 8.0$, $w_2 = \sin^2\theta/0.4$ for $\sin^2\theta < 0.4$, and $w_2 = 1$ for $\sin^2\theta \geq 0.4$. This scheme made the average values of $w(\Delta F^2)$ independent of $|F_o|$ and $\sin^2\theta$. At the end of the refinement the average parameter shift was 0.15σ, and the largest one was 0.6σ. The final values of R ($\Sigma||\Delta F|/\Sigma|F_o|$) and R' ($\Sigma w\Delta F^2/\Sigma wF_o^2$) are 0.037 and 0.044, respectively, for 1108 reflections, including 11 unobserved ones for which $|F_o| < |F_c|$ but excluding three strong ones which were affected by extinction. All coordinates are listed in Table I; the thermal parameters as well as a list of observed and calculated structure factors are provided as supplementary material (see paragraph at end of paper regarding supplementary material).

Table I: Fractional Atomic Coordinates[a]

atom	*x/a*	*y/b*	*z/c*
N(1)	4599 (4)	9589 (2)	−239 (4)
C(2)	2672 (5)	9127 (2)	−458 (6)
N(3)	2373 (4)	8523 (1)	989 (4)
C(4)	4370 (5)	8387 (2)	2779 (5)
C(5)	6497 (5)	8811 (2)	3230 (5)
C(6)	6559 (5)	9452 (2)	1636 (5)
N(6)	8428 (5)	9955 (2)	1942 (5)
N(7)	8237 (4)	8471 (1)	5153 (4)
C(8)	7138 (4)	7860 (2)	5829 (5)
N(9)	4780 (4)	7789 (0)	4505 (4)
C(1′)	3257 (5)	7113 (2)	4738 (5)
C(2′)	3757 (5)	6435 (2)	3113 (5)
O(2′)	1953 (4)	5845 (1)	2874 (4)
C(3′)	6254 (4)	6142 (2)	4680 (5)
O(3′)	6367 (4)	5309 (1)	4947 (4)
C(4′)	6448 (5)	6526 (2)	7283 (5)
O(4′)	4048 (3)	6815 (1)	7191 (3)
C(5′)	8143 (5)	7235 (2)	7769 (5)
O(5′)	8317 (4)	7513 (1)	10229 (3)
H(2)	132 (6)	926 (2)	−192 (7)
H(61)	986 (7)	988 (2)	296 (7)
H(62)	845 (8)	1015 (3)	51 (9)
H(1′)	159 (6)	725 (2)	438 (7)
H(2′)	378 (8)	657 (3)	147 (8)
H(O2′)	212 (12)	555 (4)	438 (12)
H(3′)	753 (6)	633 (2)	406 (6)
H(4′)	696 (7)	614 (2)	859 (7)
H(5′)	977 (6)	707 (2)	759 (7)
H(O5′)	968 (9)	776 (3)	1059 (9)

[a] The coordinates of the nonhydrogen atoms were multiplied by 10^4 and those of the hydrogen atoms by 10^3.

Results and Discussion

Molecular Geometry. A stereoscopic representation of the *R* epimer of 8,5′-cycloadenosine is shown in Figure 1. The adenine moiety of the molecule is not completely planar, individual atoms deviating by up to 0.05 Å from the mean plane. There are significant deviations from planarity even in the pyrimidine and imidazole rings (up to 0.016 and 0.013 Å, respectively). The deviations are considerably larger than in adenosine (Lai & Marsh, 1972) and some of its analogues

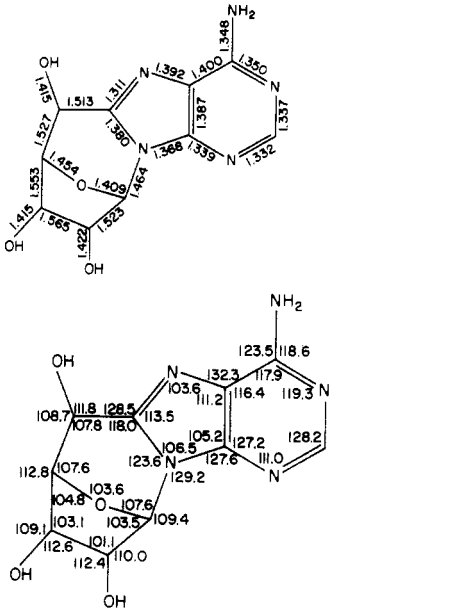


FIGURE 2: (Top) Bond distances (in ångströms); their estimated standard deviations (esd's) are 0.003–0.004 Å. (Bottom) Bond angles (in degrees); their esd's are 0.2–0.3°.

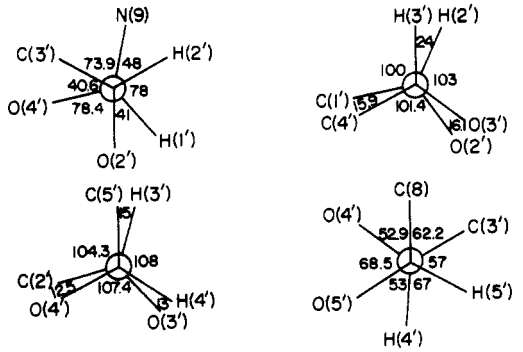


FIGURE 3: Newman projections along C(1′)–C(2′), C(2′)–C(3′), C(3′)–C(4′), and C(4′)–C(5′).

(Parthasarathy, 1977; Birnbaum & Shugar, 1978). From the data of Haromy et al. (1980) we have calculated that, although the pyrimidine ring is fully planar in the *S* epimer, there are deviations of individual atoms from the purine plane of up to 0.05 Å. These distortions from planarity are therefore ascribable to the C(8)–C(5′) cyclization. As usual, C(1′) is significantly displaced from the mean adenine plane (by 0.146 Å), and so is N(6) (by 0.134 Å).

As might have been anticipated, the C(8)–C(5′) linkage leads to distortion of the exocyclic angles at N(9), viz., C(8)–N(9)–C(1′) is 6.4° smaller than in adenosine, while C(4)–N(9)–C(1′) is 4.9° larger. All other bond angles as well as all bond lengths (Figure 2) are within the experimental error of normal values (Lai & Marsh, 1972; Birnbaum & Shugar, 1978).

The conformation of the glycosyl bond is anti, as imposed by the 8,5′ cyclization. The χ_{CN} torsion angle (29.8°) is 2.4° larger than that found in the *S* epimer (Haromy et al., 1980). The basic differences between the two epimers are illustrated in the Newman projection (Figure 3) along the C(4′)–C(5′) bond. In our *R* epimer, the torsion angle ψ [C(3′)–C(4′)–C(5′)–O(5′)] is −176.4° (trans); in the *S* epimer the orientations of O(5′) and H(5′) are interchanged, and ψ corresponds to the gauche$^-$ conformation.

The most interesting aspect of the ribose moiety is the conformation of the furanose ring (Figures 3 and 4). Its

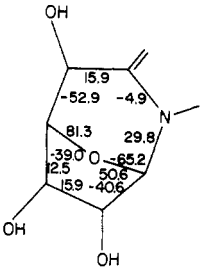


FIGURE 4: Endocyclic torsion angles in the two puckered rings.

conformational parameters (Altona & Sundaralingam, 1972) are P = 289.0° and τ_m = 49.0°. The phase angle of pseudorotation (P) corresponds to C(1′)endo–O(4′)exo puckering (^1_OT), a conformation which, displayed also by the *S* epimer, has not previously been encountered in nucleosides. Furthermore, the fact that the furanose ring is part of a 3.2.1 bicyclic system results in the maximum amplitude of puckering (τ_m) being 10° larger than normal. It is, consequently, of interest to examine what effect this unusual conformation has on the bond lengths and bond angles of the ribose moiety (Figure 2).

The C(2′)–C(3′) bond (1.565 Å) is ~0.04 Å longer than in other furanose rings with cis hydroxyl groups (Lai & Marsh, 1972; Parthasarathy, 1977; Birnbaum & Shugar, 1978; Ekiel et al., 1979). This may be attributable to a repulsion between O(2′) and O(3′), which are separated by only 2.675 Å. This relatively short nonbonded distance is correlated with the low value of the torsion angle τ_2 [C(1′)–C(2′)–C(3′)–C(4′)] in the ^1_OT conformation. The C(2′)–C(3′) bond length in the *S* epimer, 1.548 (5) Å, appears at first sight to be shorter than in our structure, but the difference is not statistically significant. The substituents on the C(3′)–C(4′) bond are also more eclipsed than in most other nucleosides, and it is tempting to ascribe the lengthening of the C(3′)–C(4′) bond to this fact. Haromy et al. did indeed suggest such an interpretation in the case of the *S* epimer, even though the bond length they found, 1.535 (4) Å, does not differ significantly from those in other adenosine nucleosides (Lai & Marsh, 1972; Parthasarathy, 1977; Birnbaum & Shugar, 1978). There are, however, reasons for questioning such an interpretation. In our recent crystal structure analyses of 5-hydroxymethyl-2′-deoxyuridine (Birnbaum et al., 1980a) and pseudoisocytidine hydrochloride (Birnbaum et al., 1980c), the furanose rings were found in the C(1′)exo conformation, with the values of the torsion angle τ_3 [C(2′)–C(3′)–C(4′)–O(4′)] only 4.2° and 4.3°, respectively. Nonetheless, the C(3′)–C(4′) bond lengths in these structures, 1.532 (5) and 1.529 (5) Å, respectively, are normal and definitely shorter than in the present structure. These results cast some doubt on the presumed correlation between the endocyclic bond lengths and the torsion angles about them (Westhof & Sundaralingam, 1980), at least in those cases in which one of the eclipsed substituents is a hydrogen atom.

There is, on the other hand, no doubt about the existence of a correlation between bond angles and the pseudorotational parameters of the furanose ring in nucleosides, recently examined by Murray-Rust & Motherwell (1978) and Westhof & Sundaralingam (1980). The latter study was restricted to endocyclic bond angles, but it is the more valuable in that it takes into account not only P but also τ_m. In view of the unusual ring conformation in our structure, it is pertinent to compare its bond angles with those derived by Westhof & Sundaralingam (1980). The P vs. θ graphs in that paper are periodic in nature, with a period of 180°, so that we may use P = 109° in place of P = 289°. The values of the coefficients

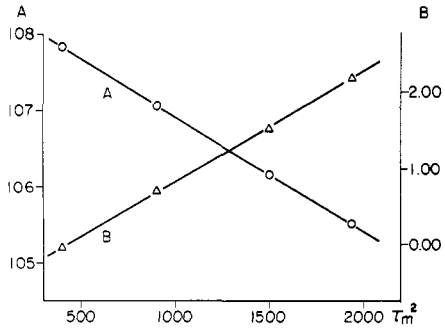


FIGURE 5: Relationship between coefficients A and B at C(1′) and the maximum amplitude of pseudorotation, τ_m.

Table II: Coefficients for Equation 3, Correlating the Conformation of a Furanose Ring with Its Endocyclic Bond Angles[a]

atom	θ^0 (deg)	θ'	α	β
O(4′)	112.96	−0.6779	3078	−1471
C(1′)	108.42	−0.6007	1502	−1429
C(2′)	105.74	0.4349	2404	−594
C(3′)	106.05	−0.0560	2130	−633
C(4′)	107.23	0.3926	1523	−779

[a] The values of α and β were multiplied by 10^6.

A and B used by these authors in their eq 5 are, however, listed for τ_m in the range 20–44°, while τ_m values for the S and R epimers are 47.7° and 49.0°, respectively. For extrapolation of values of A and B, they were plotted vs. τ_m^2. For each endocyclic angle, the points lie on a straight line [Figure 5 shows the dependence for the angle at C(1′)] with the exception of the value of A for $\tau_m = 30°$ for the angle at O(4′). This value appears to be in error, and, in place of 116.27°, it should probably be 110.27°, which fits well to the line.

The dependence of the coefficients A and B on τ_m may then be expressed analytically by eq 1 and 2

$$A_i = \theta_i^0 - \alpha_i \tau_m^2 \quad (1)$$

$$B_i = \theta_i' - \beta_i \tau_m^2 \quad (2)$$

where $i = 0, 1, 2, 3, 4$ for the endocyclic angles at O(4′), C(1′), C(2′), C(3′), and C(4′). When combined with eq 5 of Westhof & Sundaralingam (1980), this leads to

$$\theta_i = (\theta_i^0 - \alpha_i \tau_m^2) + (\theta_i' - \beta_i \tau_m^2) \cos(2P - 72i) = \theta_i^0 - [\alpha_i + \beta_i \cos(2P - 72i)]\tau_m^2 + \theta_i' \cos(2P - 72i) \quad (3)$$

This equation differs from that of Dunitz (1972), derived for infinitesimal changes of an ideal five-membered ring, by the last term. It means that the procedure adopted by Westhof & Sundaralingam (1980) was based on the more general eq 3 rather than on the equation of Dunitz (1972).

Values of the coefficients θ_i^0, θ_i', α_i, and β_i, calculated from values of A_i and B_i given by Westhof & Sundaralingam (1980), are listed in Table II and may now be used in eq 3 to calculate endocyclic angles for various values of P and τ_m. Table III gives the values of the endocyclic angles thus calculated, and those observed, for the R and S epimers. The largest deviations from the experimentally obtained results will be noted at C(1′) in the R and S epimers (1.4° and 1.0°) and at C(3′) in the S epimer (1.2°); they are no larger than some others found by Westhof & Sundaralingam (1980). Consequently, we may conclude that their equation is valid for τ_m values as high as 49°.

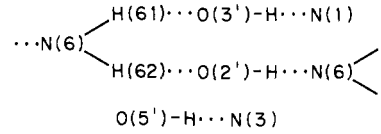
Murray-Rust & Motherwell (1978) also analyzed the correlation between P and the exocyclic angles of the furanose ring. In most instances the values of the bond angles were located within a range of several degrees for a given value of P. It can be seen, however, that the angle N(9)–C(1′)–C(2′) is smaller in our structure than in any other. In particular, it is also significantly smaller (by 2.2°) than in the S epimer (a difference of 1.2° is statistically significant), this being compensated for by a 2.2° increase in the angle N(9)–C(1′)–O(4′). It is not clear why epimerization at C(5′) should affect these angles. In addition, the angles C(1′)–C(2′)–O(2′) and C(2′)–C(3′)–O(3′) are 3.7° and 2.1°, respectively, larger than in the S epimer, while the angle O(3′)–C(3′)–C(4′) is 2.5° smaller. It appears most likely that the different geometries of the two epimers are a consequence of the different hydrogen-bonding schemes in which O(2′) and O(3′) participate.

Table III: Calculated and Observed Furanose Endocyclic Bond Angles in R and S Epimers of 8,5′-Cycloadenosine

	R epimer		S epimer	
atom	calcd	obsd	calcd	obsd
O(4′)	103.3	103.6	103.8	103.3
C(1′)	102.5	103.5	102.8	104.2
C(2′)	100.5	101.1	100.8	101.4
C(3′)	102.4	103.1	102.6	103.8
C(4′)	104.4	104.8	104.5	105.1

The six-membered ring formed by the 8,5′ linkage is approximately a half-chair with C(4′) 0.317 Å above and O(4′) 0.587 Å below a plane defined by the other four atoms. Calculation of the Cremer & Pople (1975) puckering parameters yielded the following values: $Q = 0.612$ Å; $\theta = 47.8°$; $\phi = 200.0°$; $q_2 = 0.454$; $q_3 = 0.411$. The angle θ is closest to 50.8°, the value for the ideal half-chair. Such a conformation is not surprising in view of the presence of two sp^2 hybridized atoms, C(8) and N(9), in the ring, it being well-known that the preferred conformation of cyclohexene is a half-chair (Anet & Yavari, 1978). However, because it is part of a bicyclic system, the ring is much more puckered than cyclohexene, as reflected in larger torsion angles and smaller bond angles. In the S epimer the ring is even more puckered ($Q = 0.644$ Å), and its conformation is slightly different; its angle θ (53.8°) is closer to 54.7°, the value for the ideal envelope (half-boat, sofa), than to 50.8°, the value for the ideal half-chair (in the original publication the ring was denoted as a half-chair but described as an envelope). Consequently, some of the torsion angles, C(4′)–C(5′)–C(8)–N(9) and C(5′)–C(8)–N(9)–C(1′), differ by ~9°. It is not clear why the S epimer should prefer this conformation, particularly since it results in a relatively short intramolecular contact (2.43 Å) between O(5′) and H(3′), while a shift toward a half-chair would have increased this distance.

Hydrogen Bonding. There are five protons capable of participating in hydrogen bonding, and, in fact, all of them do so:



The hydrogen bonds can be seen in the packing diagram (Figure 6). All distances and angles, except those involving H(O3′), which could not be located, are listed in Table IV. As commonly observed in X-ray analyses, some O–H and N–H bonds appear shorter than their real values of 0.97 and 1.04 Å, respectively. By extending the covalent bond lengths in the direction of the bond to their real values, one obtains corrected H···A distances which more accurately reflect the strength of the hydrogen bonds.

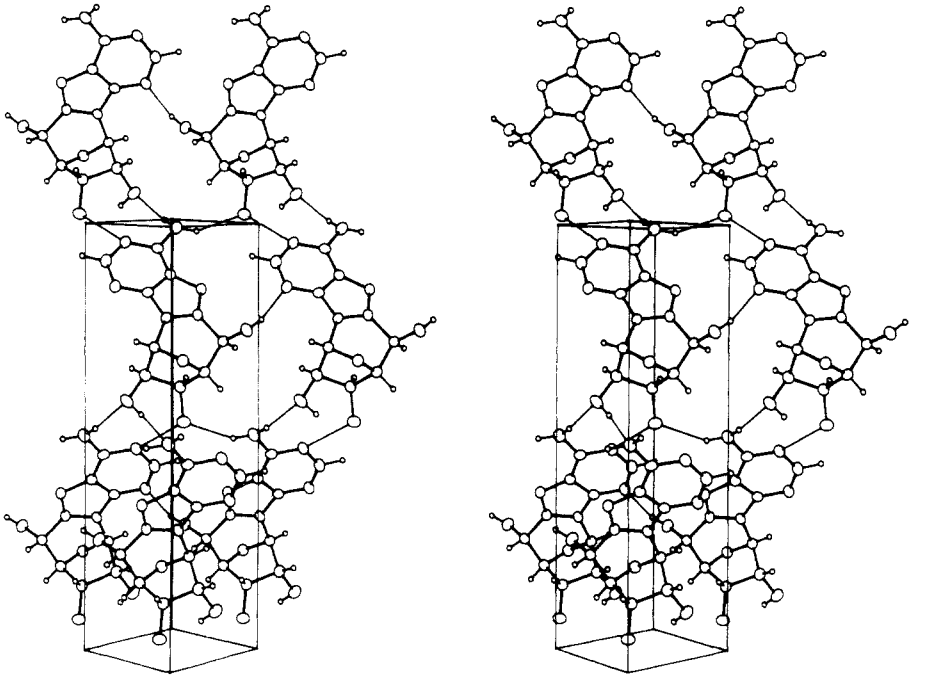


FIGURE 6: Stereoscopic view of the molecular packing in the crystal.

Table IV: Distances and Angles for the Hydrogen Bonds

		distances (A)			angles (deg)	
D-H···*A*	*A* at	*D*···*A*	H···*A*	H···A_{cor}	*D*-H···*A*	H-*D*···*A*
N(6)-H(61)···O(3′)	$(2-x, {}^{1}/_{2}+y, -1-z)$	3.124	2.31	2.18	152	20
N(6)-H(62)···O(2′)	$(1-x, {}^{1}/_{2}+y, -2-z)$	3.011	2.17	2.00	168	9
O(3′)-H···N(1)	$(1-x, -{}^{1}/_{2}+y, -2-z)$	2.796				
O(2′)-H···N(6)	$(1-x, -{}^{1}/_{2}+y, -2-z)$	3.283	2.35	2.33	166	10
O(5′)-H···N(3)	$(1+x, y, 1+z)$	2.839	1.98	1.89	167	9

From this it is apparent that some of the hydrogen bonds are rather weak, in particular O(2′)–H···N(6). Nonetheless, the H···N(6) distance and the O(2′)–H···N(6) angle point to the reality of this hydrogen bond. An additional indication is the fact that N(6) is slightly tetrahedral, being significantly displaced toward the proton-donating O(2′). To our knowledge, this is the first example of a crystal structure in which an exocyclic $-NH_2$ of purine or pyrimidine nucleoside functions as a hydrogen-bond acceptor. This may be taken to imply that, when bound to an enzyme, the nucleoside (or nucleotide) could accept a proton from a direction approximately perpendicular to the base moiety. It is noteworthy that H(O2′) approaches N(6) from the same side on which O(5′) is located in the active *R* epimer (in the 5′-nucleotidase system).

Comparison of Solid-State and Solution Conformational Data. The values of the measured vicinal proton–proton coupling constants for the *R* and *S* epimers of 8,5′-cycloadenosine are listed in Table V. The three coupling constants from which the sugar ring conformation can be derived, viz., *J*(1′,2′), *J*(2′,3′), and *J*(3′,4′), establish the solution conformation of 8,5′(*R*)-cycloadenosine as O(4′)exo–C(1′)endo; hence, it is identical with that in the crystal. The solution conformation of the *S* epimer is also closely similar to that in the crystal, but in this instance the low accuracy of the value for *J*(3′,4′) makes it difficult to establish whether the agreement is as good as for the *R* epimer.

The crystallographic data were used to determine the dihedral angles between the planes containing the coupled protons H(*i*)–C(*i*)–C(*j*) and C(*i*)–C(*j*)–H(*j*), with *i*, *j* = 1′, 2′, 3′, 4′. The values of the dihedral angles were used, in turn, to calculate the corresponding vicinal coupling constants for the sugar ring with the aid of the Karplus (1959) relation by using the parameters normally employed for nucleosides and nucleotides (Davies & Danyluk, 1974), viz., $J(i,j) = 9.8\cos^2\phi_{i,j} - 0.9\cos\phi_{i,j}$ (see Table V). The resulting calculated values of the coupling constants are only in qualitative agreement with the measured values. There is a lack of strict quantitative agreement for the cisoidal *J*(2′,3′), where the discrepancy for the *R* epimer is 1.4 Hz and for the *S* epimer 2.2 Hz.

The foregoing discrepancies cannot be accounted for by differences between conformations in the crystal and in solution, since the results for the system of coupling constant values exclude sugar conformations other than O(4′)exo–C(1′)endo.

Table V: Comparison of Measured and Calculated[a] Vicinal Coupling Constants $^3J(i,j)$ for the *R* and *S* Epimers of 8,5′-Cycloadenosine

vicinal protons H(*i*),H(*j*)	1′,2′	2′,3′	3′,4′	4′,5′
R epimer				
dihedral angle $\phi_{i,j}$ (deg)	78	24	108	67
calcd *J*(*i*,*j*) (Hz)	0.2	7.3	1.2	1.2
measured *J*(*i*,*j*) (Hz)	0.0[b]	5.9	0.9	1.1
S epimer				
dihedral angle $\phi_{i,j}$[c] (deg)	94	12	109	49
calcd *J*(*i*,*j*) (Hz)	0.2	8.5	1.3	3.7
measured *J*(*i*,*j*) (Hz)	0.0[b]	6.3	~0[d]	6.1

[a] Calculated from crystallographic results with the aid of the Karplus relations: $J(i,j) = 9.8\cos^2\phi_{i,j} - 0.9\cos\phi_{i,j}$ for $\phi_{1',2'}$, $\phi_{2',3'}$, and $\phi_{3',4'}$; and $J(4',5') = 5.05 - 1.0\cos\phi_{4',5'} - 5.5\cos^2\phi_{4',5'}$ (Remin & Shugar, 1973). [b] Value below resolution of instrument, but < 0.4 Hz. [c] Calculated from the data of Haromy et al. (1980). [d] Difficult to evaluate precisely because of signal overlapping.

Assumptions of other sugar conformations or dynamic equilibria between two conformations did not yield a system of coupling constants with a transoidal constant close to zero and a cisoidal one of ~6 Hz, as observed experimentally (Table V). Such a system is characteristic for conformations close to O(4′)exo on the pseudorotational cycle. Furthermore the low, but measurable, values of $J(3',4')$ clearly different from zero, and the zero values for $J(1',2')$ within the limits of resolution of the measurements, point to solution conformations of O(4′)exo–C(1′)endo.

Nor can the discrepancies be attributed to poor parametrization of the Karplus relation, which furnishes credible results for analyses of ribonucleosides and ribonucleotides exhibiting the conformational equilibrium N ⇌ S. Analysis of the mutual interdependence of the coupling constants for nucleosides with different values of the equilibrium constant $K = N/S$ provides approximate values of the parameters for a given class of nucleosides and nucleotides with different sugar moieties (Ekiel et al., 1979).

The lower precision associated with the location of hydrogen atoms by X-ray diffraction methods led to standard deviations for dihedral angles not exceeding 3°; hence, this cannot be the source of the differences between the expected and measured values of the cisoidal coupling constants. The dihedral angle corresponding to $J(2',3')$ = 5.9 Hz is 35°, significantly different from the 24° angle observed in the solid state. The only remaining interpretation is that the difference in the values of the dihedral angles between protons in sugar rings with the same conformation is due to packing forces in the crystal and the possible necessity of using somewhat different parametrizations of the Karplus relation for cyclonucleosides and nucleosides in the conformation C(2′)endo and C(3′)endo.

Comparison of the data for the *R* and *S* epimers of 8,5′-cycloadenosine demonstrates that, with virtually identical pseudorotational parameters *P* and τ_m, and differences in endocyclic torsion angles in the sugar ring not exceeding 2°, there are differences of up to 16° between the dihedral angles involving protons (Table V). This most likely results from differences in packing in the crystal, in part by formation of intermolecular hydrogen bonds involving sugar hydroxyls. Supporting this view are some relatively short intermolecular distances in the crystal structure of the *R* epimer: H(1′)···H(5′), 2.28, H(1′)···H(O5′), 2.28, and H(2′)···O(4′), 2.44 Å. A change in dihedral angles of 16° may alter the value of $J(2',3')$ by 1 Hz or more. Furthermore, the parameters of the Karplus relation are strongly dependent on the values of the bond angles (Barfield & Grant, 1965) as well as on the orientation of the sugar hydroxyls (Jaworski et al., 1978) and of the sugar ring oxygen relative to coupled protons.

An analogous situation prevails in a comparison of the calculated and measured values of $J(4',5')$ in the *S* epimer, which describes the conformation of the exocyclic 5′-CH_2OH, identical in solution and in the crystal for the cyclonucleosides. An additional perturbing factor here is obviously the C(8)–C(5′) linkage, not present in the normal nucleosides.

The foregoing underlines the difficulties associated with tests of the reliability of the Karplus relation, even with model compounds exhibiting identical conformations in solution and in the solid state. With other systems hitherto investigated (Plochocka et al., 1977; Manor et al., 1974; Kung et al., 1977; Birnbaum et al., 1980b), there have also been differences between the conformations in solution and in the solid state or, where similar conformations have been assumed, differences between calculated and measured coupling constants of up to 3–4 Hz. The most satisfactory agreement has been noted for nucleoside cyclic 3′,5′-phosphates (Watenpaugh et al., 1968; Lee & Sarma, 1976), but even in these instances there is no certainty as to the identity of these conformations. Good agreement with crystal data was also achieved by assuming a three-component conformational equilibrium in solution for the sugar ring (Birnbaum et al., 1979, 1980a).

Relatively good results emerged from a test of the Karplus relation for $^{13}C-^{1}H$ vicinal coupling constants, using dihedral angles determined by X-ray diffraction, for pyrimidine nucleosides with rigid structures (Delbaere et al., 1973). The foregoing authors also observed deviations of some coupling constants (up to 2.8 Hz) from the values predicted by the Karplus relation. They interpreted them as due to anomalies in the residual electron density distribution which modified the observed coupling constants.

On the other hand, comparisons of conformations in solutions and the solid state are essential for evaluation of packing forces of molecules in the crystal. The conformation assumed in solution is determined primarily by intramolecular interactions, whereas in the solid state the magnitude of the intermolecular energy is comparable to the internal energy of the individual molecules (Dauber & Hagler, 1980). Interpretation of the nature of the interactions in the crystal, in particular, the form of the potential of interaction and the types of interaction which make up this potential, requires analysis of a large variety of molecules in the solid state and in solution. It is to be anticipated that studies of molecules with rigid structures, which undergo only minor changes in conformation on transition from the solid state to solution, would be most profitable.

Enzymological Aspects. The conformation of the structures of the individual *R* and *S* epimers of 8,5′-cycloadenosine and, by analogy, their 5′-phosphates unequivocally establishes their previously observed lack of substrate properties toward adenosine deaminase and 5′-AMP deaminase, respectively (Dudycz & Shugar, 1979), in contrast to the substrate properties previously reported for a nonseparable mixture of the presumed epimers (Hampton et al., 1972). It follows that these structures do not reflect those of the natural substrates. Nonetheless, these findings, taken in conjunction with the substrate properties toward adenosine deaminase of other cycloadenosine analogues, such as 8,2′-anhydro-8-oxoadenosine (Ikehara & Fukui, 1974), and of nucleosides with bulky substituents at C(8) (Simon et al., 1970; Ikehara & Fukui, 1974; Stolarski et al., 1980), lead to the conclusion that the enzyme–substrate complex of adenosine with adenosine deaminase is such that the nucleoside is in the high anti conformation with exclusion of the typical syn and anti conformations.

With the adenosine deaminase system, the *R* epimer is also not an inhibitor. The *S* epimer is a weak inhibitor, $K_i \simeq 3 \times 10^{-4}$ M, but this value is perhaps too high to unequivocally establish a requirement for the gauche$^-$ conformation of the exocyclic 5′-CH_2OH group.

The foregoing is in apparent contradiction with the reported results on the susceptibility to enzymatic deamination of the 2,5′- and 4,5′-anhydroformycins (Zemlicka, 1975; Makabe et al., 1975), which are formally anti and syn analogues of formycin A, an adenosine analogue. The former of these, reported as susceptible to adenosine deaminase under conditions where the latter is unaffected, is in the anti conformation with a glycosidic torsion angle, χ_{CN}, similar to that found in the *R* and *S* epimers of 8,5′-cycloadenosine and in 8,5′-cyclo-5′-deoxyadenosine and 8,5′-anhydro-8-oxoadenosine, all of which are resistant to adenosine deaminase (Ikehara &

Fukui, 1974; Dudycz & Shugar, 1979). Closer examination of the published results for the anhydroformycins, however, reveals that susceptibility to deamination was examined with the use of inordinately high concentrations of the enzyme (10–100 units/mL) and a large excess of enzyme to substrate (1 unit enzyme to 0.02–1 μmol of substrate), and hence, they are not comparable with the above results.[1]

Furthermore, the difference in substrate properties of the two anhydroformycins was interpreted in terms of the difference in conformation of the aglycon in the two analogues, without taking into account the fundamental differences in structure between them. It has been noted that the substrate properties of *N*-methyl derivatives of formycin toward human adenosine deaminase are markedly dependent on the site of methylation, e.g., *N*(2)-methylformycin is a feebler substrate than the parent formycin, whereas *N*(4)-methylformycin is not a substrate (Giziewicz et al., 1975; Crabtree et al., 1979), notwithstanding that the latter is constrained to the anti conformation by the *N*(4)-methyl substituent.

As regards the observed susceptibility toward 5′-nucleotidase of the *R* epimer of 8,5′-cycloadenosine 5′-phosphate, coupled with the inertness toward this enzyme of the corresponding *S* epimer, this may be regarded as evidence that the *R* epimer does reflect the conformation adopted by the parent 5′-AMP in its interaction with this enzyme. The relatively low rate of hydrolysis of the *R* epimer, relative to 5′-AMP, may be accounted for, on the one hand, by the fact that the phosphate group in this model compound is linked to a secondary hydroxyl and not to a primary hydroxyl as in 5′-AMP and, on the other hand, by the fact that the sugar conformation in the *R* epimer differs somewhat from that in the parent 5′-AMP. It follows that the conformation of the latter, when complexed to 5′-nucleotidase, is anti and gauche$^-$. This is consistent with the observation that AMP analogues containing a non-carbon-bridged secondary 5′-hydroxyl esterified with phosphoric acid, viz., *talo*-5′-AMP and *allo*-5′-AMP, are similar feeble substrates, dephosphorylated at a rate 200–400 times slower than 5′-AMP (Hampton et al., 1973), and that model purine nucleoside 5′-phosphates constrained to the syn conformation by bulky C(8) substituents are very weak, or totally inactive as, substrates for 5′-nucleotidase (Pless et al., 1978). Furthermore, preliminary observations (unpublished) have shown that the *S* epimer is not an inhibitor of 5′-nucleotidase, whereas the *R* epimer does inhibit, with a K_i value not much inferior to the K_m for the substrate, and hence are in agreement with the above observations and conclusions.

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Supplementary Material Available

Anisotropic thermal parameters for nonhydrogen atoms, isotropic thermal parameters for hydrogen atoms, deviations of atoms from least-squares planes, and a list of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

[1] It is true that adenosine deaminase from Takadiastase hydrolyzes 2,5′-anhydroformycin at a rate higher than for formycin itself (Makabe et al., 1975), but this enzyme is highly nonspecific (Sharples & Wolfenden, 1967) and hence not pertinent to the present discussion.

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Amino Acid Sequence of Residues 1–98 of the H-2K^d Murine Major Histocompatibility Alloantigen: Comparison with H-2K^b and H-2D^b Reveals Extensive Localized Differences†

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ABSTRACT: The complete primary structure of the amino-terminal 98 residues of the murine histocompatibility antigen H-2K^d has been determined by using radiosequencing methodology. Cyanogen bromide cleavage of the detergent-solubilized molecule yielded several peptides, two of which span residues 1–52 and 53–98. The latter peptide bears one of the carbohydrate moieties of the H-2K^d molecule. The complete sequence of the amino-terminal fragment (residues 1–52) was accomplished by analyses of peptides derived from cleavage by staphylococcal V8 protease. Analyses of peptides obtained from tryptic digests and NH_2-terminal sequence data were used to confirm the alignment of the V8 peptides. The complete sequence of the glycopeptide spanning residues 53–98 was achieved by NH_2-terminal sequence analyses and from fragments derived by thrombic cleavage. Alignment of the two CNBr fragments was deduced from a tryptic overlap peptide derived from the whole molecule. Positive identification was possible for all amino acids except Asp and the C-terminal Met (position 98). The sequence obtained in the study is Gly-Pro-His-Ser-Leu-Arg-Tyr-Phe-Val-Thr-Ala-Val-Ser-Arg-Pro-Gly-Leu-Gly-Glu-Pro-Arg-Phe-Ile-Ala-Val-Gly-Tyr-Val-*Asp*-*Asp*-Thr-Gln-Phe-Val-Arg-Phe-*Asp*-Ser-*Asp*-Ala-*Asp*-Asn-Ala-Arg-Phe-Glu-Pro-Arg-Ala-Pro-Trp-Met-Glu-Gln-Glu-Gly-Pro-Glu-Tyr-Trp-Glu-Glu-Gln-Thr-Gln-Arg-Val-Lys-Ser-*Asp*-Glu-Gln-Trp-Phe-Arg-Val-Ser-Thr-Arg-Thr-Ala-Gln-Arg-Tyr-Tyr-Asn-Gln-Ser-Lys-Gly-Gly-Ser-His-Thr-Phe-Gln-Arg-*Met*. These data allow the first extensive comparison of the primary structure of two allelic histocompatibility gene products (H-2K^d and H-2K^b). This comparison revealed one 22-residue-long region of amino acid sequence which contained 12 of the 23 amino acid differences noted between these molecules. Eleven of the remaining differences were not distributed randomly but localized to four discrete regions. No amino acid sequence distinctive of H-2K gene products was revealed when the NH_2-terminal 98 residues of the H-2K^d, H-2K^b, and H-2D^b amino acid sequences were compared.

One of the most striking aspects of the classical transplantation antigens of the murine histocompatibility (H-2) system is their high level of genetic polymorphism, as demonstrated by serological and cellular assays (reviewed by Klein, 1975; Snell et al., 1976). A systematic study of those H-2 antigens (H-2K, H-2D, and H-2L) has been undertaken in order to discern on a molecular level the basis for antigenic polymorphism and to ascertain the extent of evolutionary relationships that might prevail among H-2 antigens.

These H-2 antigens have been shown to be integral membrane glycoproteins (Shimada & Nathenson, 1969; Schwartz et al., 1973) that have approximate molecular weights of 45 000 and exist in noncovalent association with β_2-microglobulin (β_2m),[1] a nonintegrated protein (Rask et al., 1974; Nakamuro et al., 1973; Cresswell et al., 1973). The recent

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[1] Abbreviations used: β_2m, β_2-microglobulin; HPLC, high-performance liquid chromatography; Gdn·HCl, guanidine hydrochloride; F_3Ac-OH, trifluoroacetic acid.

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