Crystallographic and ^{1}H Nuclear Magnetic Resonance Studies of 5'-Deoxy-5'-adenosineacetic Acid; a Model Nucleotide of Adenosine-5'-monophosphate

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In order to elucidate the substrate specificity of adenosine-5'-monophosphate utilizing enzymes, the spatial conformation of 5'-deoxy-5'-adenosineacetic acid has been studied by *X*-ray crystallography and ^{1}H n.m.r. spectroscopy, and the results compared with those for adenosine-5'-monophosphate. In the crystal, the conformation about the glycosyl bond is *anti* with the torsion angle χ[O(1')–C(1')–N(9)–C(8)] equal to 50.7(9)°. The puckering of the ribose ring is C(3')-*exo*($_3E$). The significant conformational discrepancy between 5'-deoxy-5'-adenosineacetic acid and adenosine-5'-monophosphate was in the orientations of their exocyclic C(4')–C(5') bonds: *gauche-trans* for the former and *gauche-gauche* for the latter. These conformational characteristics were also observed in ^2H_2O solution by ^{1}H n.m.r. spectroscopy.

The intermolecular hydrogen bonding mode between the carboxyl group and the adenine ring found in the crystal would constitute a good model for the specific interaction between the acidic amino-acid and the nucleic acid base adenine.

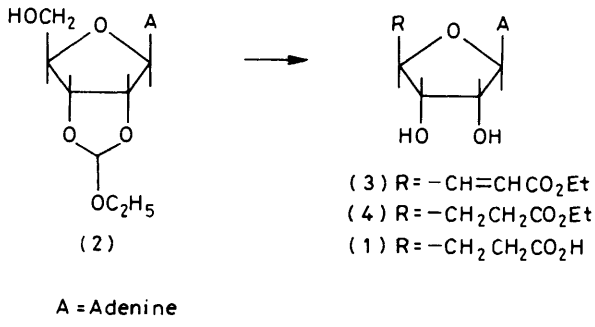
Adenosine-5'-monophosphate (AMP) is the important metabolic precursor of adenosine-5'-triphosphate, adenine nucleotide coenzymes, and other biologically important compounds, and thus it plays an essential role in the enzymic metabolism of living organisms.

In most of the cases where AMP acts as enzymic substrate or regulator, important interactions occur between the enzymes and the nucleotide.[1] Knowledge of these interactions is necessary for an understanding of the substrate specificity of AMP-utilizing enzymes.

One promising approach to a study of the nature of the interactions would be the use of AMP analogues capable of binding selectivity to the adenine nucleotide sites of the enzymes. Among them, adenine nucleotide analogues substituted at the exocyclic C(5') atom of the ribose group are effective candidates, and catalytic experiments have been performed for many enzymes such as AMP kinase, AMP aminohydrolase, and 5'-nucleotidase.[2]

5'-Deoxy-5'-adenosineacetic acid (AAA) could be a model nucleotide for AMP (a $CH_2CH_2CO_2H$ group replacing a $CH_2OPO_3H_2$ group), since it has been shown that AAA can replace AMP both as a building unit in oligonucleotides and as an enzyme substrate for AMP aminohydrolase.[3]

In order to deduce the substrate specificity of AMP aminohydrolase, it is necessary to determine the favoured conformation of AAA, and to compare it with that of AMP. We therefore studied its spatial conformation by *X*-ray crystallography † and ^{1}H n.m.r. spectroscopy.



Experimental

Synthesis of AAA (1).—Essentially the procedure of Walker, *et al.*,[5] was employed for the synthesis of AAA (1). When 2',3'-*O*-ethoxymethylene adenosine (2),[6] however, was used as the starting material instead of 2',3'-*O*-*p*-anisylideneadenosine, 9-(ethyl 5,6-dideoxy-β-D-*ribo*-hept-5-enofuranosyluronate)adenine (3) could be isolated in better yield (21.1%) than that obtained by Walker *et al.* (12.5%). Hydrogenation, followed by hydrolysis with Dowex 1-X2(OH^-) ion-exchange resin by the method of Walker *et al.*, yielded (1), the identity of which was established by elemental analysis and comparison with the data for authentic material.[5]

Crystal Data.—$C_{12}H_{15}N_5O_5$, M = 309.28, Orthorhombic, Space group $P2_12_12_1$, a = 5.197(1), b = 10.513(1), c = 24.258(5) Å, U = 1 325.4(4) Å^3, D_m = 1.549(2) (flotation in C_6H_6–CCl_4), D_c = 1.550 g cm^{-3}, Z = 4, μ(Cu-K_α) = 10.00 cm^{-1}.

Crystallographic Measurements.—Transparent platelets were obtained from an aqueous solution at room temperature. A single crystal (dimensions 0.5 × 0.3 × 0.1 mm^3) was used for the *X*-ray study. Cell parameters were obtained from a least-squares analysis of the setting of 45 reflections measured on a Rigaku four-circle diffractometer with graphite-monochromated Cu-K_α radiation. Out of 1 319 unique intensities (sin $\theta/\lambda \leq 0.583$ Å^{-1}) collected by the ω scan mode (scan width, 1.5°; scan speed, 2°/min), 1 164 having $I \geq 2\sigma(I)$ were considered observed. The intensities of four standard reflections measured every 100 reflections remained constant to within ±0.3% of their mean values. Lorentz and polarization corrections were applied. No absorption correction was made.

Structure Determination and Refinement.—The structure was solved by multisolution tangent refinement with the program MULTAN[7] using 188 reflections with $|E| \geq 1.42$.

† Preliminary results for *X*-ray analysis were reported in ref. 4.

Table 1. Fractional atomic co-ordinates and isotropic thermal parameters along with their standard deviations in parentheses

Atom	x	y	z	B_{iso}/Å^2 *
N(1)	0.007(1)	1.199 4(6)	0.443 7(2)	2.8(2)
C(2)	0.190(2)	1.121 7(7)	0.463 0(3)	2.8(2)
N(3)	0.225(1)	0.996 7(6)	0.452 2(2)	2.4(1)
C(4)	0.046(1)	0.956 8(6)	0.416 3(3)	2.1(2)
C(5)	−0.151(1)	1.026 0(7)	0.393 3(3)	2.4(2)
C(6)	−0.165(2)	1.156 7(7)	0.407 7(3)	2.6(2)
N(6)	−0.343(1)	1.236 1(6)	0.387 7(3)	3.3(2)
N(7)	−0.300(1)	0.950 2(5)	0.358 7(2)	2.6(2)
C(8)	−0.193(1)	0.837 4(6)	0.362 3(3)	3.6(2)
N(9)	0.017(1)	0.834 2(5)	0.396 5(2)	2.4(1)
C(1′)	0.200(1)	0.728 7(6)	0.405 0(3)	2.2(2)
C(2′)	0.070(1)	0.610 9(7)	0.432 7(3)	2.2(2)
O(2′)	0.125(1)	0.617 0(5)	0.490 4(2)	2.4(1)
C(3′)	0.211(2)	0.500 6(7)	0.403 7(3)	3.2(2)
O(3′)	0.462(1)	0.489 8(5)	0.427 4(2)	3.6(1)
C(4′)	0.243(2)	0.551 3(7)	0.345 4(3)	2.5(2)
O(1′)	0.286(1)	0.688 2(4)	0.352 4(2)	2.6(1)
C(5′)	0.016(2)	0.524 9(7)	0.307 8(3)	2.8(2)
C(6′)	0.025(2)	0.603 5(8)	0.256 4(4)	4.0(2)
C(7′)	−0.200(2)	0.592 4(9)	0.218 8(3)	3.6(2)
O(1)	−0.294(1)	0.476 1(6)	0.213 5(2)	4.1(2)
O(2)	−0.283(2)	0.681 4(6)	0.194 6(3)	5.6(2)
H(2)	0.35(1)	1.161(6)	0.490(2)	2(1)
H(6a)	−0.36(2)	1.298(10)	0.401(4)	6(2)
H(6b)	−0.47(2)	1.213(7)	0.358(3)	2(1)
H(8)	−0.27(2)	0.760(8)	0.341(3)	3(2)
H(1′)	0.35(1)	0.757(6)	0.431(3)	2(1)
H(2′)	−0.14(1)	0.606(7)	0.425(3)	2(1)
H(O2′)	−0.01(2)	0.578(9)	0.510(4)	5(2)
H(3′)	0.10(2)	0.421(8)	0.404(3)	2(1)
H(O3′)	0.49(3)	0.426(12)	0.439(5)	8(3)
H(4′)	0.42(2)	0.516(7)	0.327(3)	2(1)
H(5′a)	0.01(2)	0.426(9)	0.300(4)	4(2)
H(5′b)	−0.16(2)	0.546(7)	0.330(3)	2(1)
H(6′a)	0.06(2)	0.705(8)	0.263(3)	2(2)
H(6′b)	0.21(2)	0.568(9)	0.235(4)	5(2)
H(O1)	−0.45(2)	0.471(8)	0.190(3)	4(2)

* The equivalent isotropic temperature factors for non-hydrogen atoms have been calculated from anisotropic thermal parameters using the equation $B = 4/3(B_{11}a^2 + B_{22}b^2 + B_{33}c^2 + 2B_{12}ab\cos\gamma + 2B_{13}ac\cos\beta + 2B_{23}bc\cos\alpha)$, where B_{ij} are the principal components of mean square displacement matrix B.

When the set with the highest figure of merit was used to synthesize an *E*-map, geometrically acceptable positions for all non-hydrogen atoms were obtained. The structure was then refined by the full-matrix least-squares method with isotropic thermal parameters and then by the block-diagonal least-squares method with anisotropic ones. The positional parameters of all hydrogen atoms found on a difference Fourier map at this stage were included in the refinement. The final R index $[(\Sigma||F_o| - |F_c||)/(\Sigma|F_o|)]$ was 0.073. The quantity minimized was $\Sigma w(|F_o| - |F_c|)^2$, where the weight ($w$) was treated as unity for all reflections. Positional and isotropic thermal parameters are listed in Table 1. All numerical calculations were carried out at the Computation Center of Osaka University using the program UNICS.[8] The scattering factors cited in International Tables for *X*-ray Crystallography [9] were used for all atoms. Tables of observed and calculated structure factors, and anisotropic temperature factors are listed in Supplementary Publication No. SUP 23517 (8 pp.).

Conformational Analysis by ^{1}H *N.m.r. Spectroscopy.*—^{1}H N.m.r. spectra at 21 °C were measured on a Varian XL-200 (200-MHz, FT-mode) spectrometer. Chemical shifts were measured *vs.* internal Me_4Si, for $(C^2H_3)_2SO$ solution, and DSS (2,2-dimethyl-2-silapentane-5-sulphonate) for ^2H_2O solution. Samples were adjusted to 0.1M for $(C^2H_3)_2SO$, and 0.05M for ^2H_2O.

The AAA conformation was analysed by conventional methods, using the spin-lattice relaxation time of proton (T_1), the observed chemical shifts (δ), and the coupling constants (J).

(*a*) *Glycosyl bond.* In nucleosides and nucleotides, stable conformations about their glycosyl bonds are found in both the *anti* and *syn* ranges.* Measurement of the spin-lattice relaxation times (T_1) for H(8) and H(1′) by the pulse Fourier transform method, allowed the conformation about the glycosyl bond to be estimated, on the basis that the ratio of T_1's for these two protons depends strongly on the *anti-syn* conformation.[11]

Assuming that the ratio $(T_1)_8/(T_1)_{1'}$ is 0.53 for *anti*, and 1.52 for *syn* conformation,[11] the conformation could be roughly estimated by the following formula:

$$(T_1)_8/(T_1)_{1'obs} = 0.53 \times P_{anti} + 1.52 \times P_{syn},\ P_{anti} + P_{syn} = 1$$

where $(T_1)_8/(T_1)_{1'obs}$ represents the ratio of the observed spin-lattice relaxation times of H(8) and H(1′) protons, and P_{anti} and P_{syn} are the populations (%) of *anti* and *syn* conformers about the glycosyl bond.

(*b*) *Sugar puckering.* The puckering of ribose ring can be assessed by assuming a C(2′)-*endo* ⇌ C(3′)-*endo* equilibrium. The percentage of C(3′)-*endo* can be estimated by the following formula: [12] C(3′)-*endo*(%) = $100 \times [J_{3'4'}/(J_{1'2'} + J_{3'4'})]$.

(*c*) *Exocyclic* C(4′)–C(5′) *bond.* The conformation about C(4′)–C(5′) bond can be discussed in terms of a blend of the *gauche-gauche* (*gg*), *gauche-trans* (*gt*), and *trans-gauche* (*tg*) conformers. The following equation is among those which have been used to estimate the contribution from the *gg* conformer: [13] $P_{gg}(\%) = 10\ (13 - \Sigma)$, where $\Sigma = J_{4'5'} + J_{4'5''}$.

Results and Discussion

Molecular Dimensions and Planarities.—The bond lengths and angles between non-hydrogen atoms are shown in Figure 1. The average bond distances involving hydrogen atoms are C–H = 1.08, O–H = 0.83, and N–H = 0.87 Å. The average estimated standard deviations in bond lengths are (C, N, O–C) = 0.01 and (C, N, O–H) = 0.09 Å. The average standard deviations in bond angles between non-hydrogen atoms varied from 0.6 to 0.9°.

Average bond lengths and angles for neutral adenine base have been reported by Voet and Rich.[14] The geometry of the adenine ring of AAA molecule is comparable with those averages within their standard errors; only the length of C(2)–N(3), 1.353 Å, is considerably longer than the averaged one [1.315(8) Å].

It should be borne in mind that a prominent dimensional difference between AAA and AMP molecules [15,16] is in the bonding state of the N(1) atom: in contrast to the AAA molecule having a neutral adenine ring, the free AMP molecule exists as a zwitterion in the solid state with N(1)-protonation of the adenine base; [17] consequently, there is lengthening of the N(1)–C(2) bond (1.368 Å for monoclinic AMP [15]; 1.387 Å for orthorhombic AMP [16]) and widening of the angle at the N(1) atom [C(2)–N(1)–C(6) = 122.8° for the former, 121.9° for the latter]. Probably this structural dis-

* Definitions of specific terms, such as *anti* and *syn*, may be found in ref. 10.

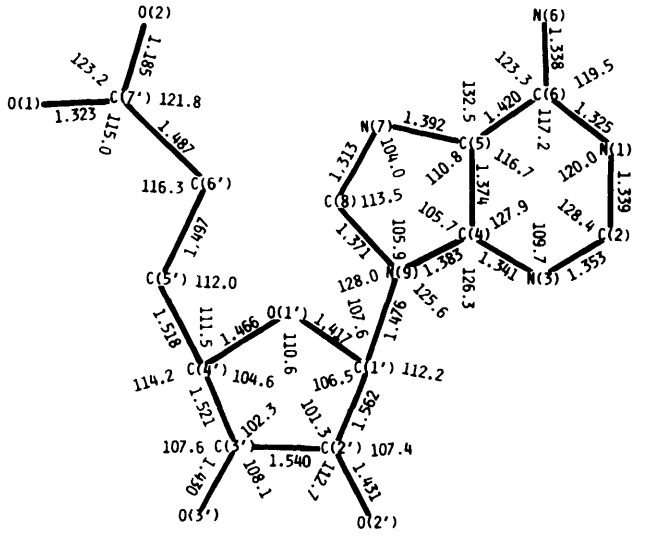


Figure 1. Intramolecular bond lengths (Å) and angles (°) between non-hydrogen atoms along with the atomic numbering used in this work. Estimated standard deviations are 0.009—0.011 Å for lengths and 0.6—0.9° for angles

crepancy between AAA and AMP molecules gives rise to a difference in the pK values of AMP phosphate (0.9) and AAA carboxy (4.8 *) [18] groups.

In the ribose ring including the C(5') atom, the atomic bond distances and angles are roughly in the range of average ones within the margin of standard error,[10,19] although a bond length of C(1')–C(2'), 1.562 Å, is considerably longer than the average, 1.52 Å.[10]

Bond lengths and angles within the carboxy-group are similar to the averaged values for neutral carboxylic acids.[20,21] Characteristically, there are differences in the bond lengths and angles involving the carboxy oxygen O(2) and the oxygen atom O(1) with a proton attached to it. The C(7')–O(2) bond is *ca.* 0.14 Å shorter than the C(7')–O(1)H bond, and the C(6')–C(7')–O(2) bond angle is *ca.* 7° larger than the corresponding angle C(6')–C(7')–O(1).

The planarities of the two parts of the AAA molecule (the adenine ring and the carboxy group), were examined by the equation of the least-squares plane. Equations using the nine atoms for adenine ring and four atoms [C(6'), C(7'), O(1) and O(2)] for carboxy-group described in orthogonal axes along a, b, and c are: $0.596\,78X + 0.233\,79Y - 0.767\,59Z = -5.274\,76$ for the adenine ring, $-0.622\,91X + 0.177\,34Y + 0.761\,92Z = 5.786\,77$ for the carboxy-group.

The nine-membered ring of the adenine base is essentially planar with a maximum deviation of 0.019(8) Å for N(1), and N(6) lying approximately on the plane (−0.03(1) Å), while C(1') deviates as much as 0.15(1) Å from the best plane.

Atoms C(6'), C(7'), O(1), and O(2) are strictly coplanar with fluctuations of −0.003 to 0.009 Å. The dihedral angle between the adenine ring and the carboxy-group is 23.8(4)°.

Molecular Conformation in the Crystal Structure.—A stereoscopic view of the AAA molecule is shown in Figure 2. The conformational parameters of AAA and AMP (free and metal-complexed [22,23]) molecules, for the purposes of comparison, are given in Table 2, in which the torsion angle notation used for the nucleoside and nucleotide is according to Sundaralingam.[24]

The puckering of the ribose moiety is best described by the pseudorotation concept.[25] The pseudorotational phase angle P is 191.3°, and the maximum amplitude of puckering τ_m is 37.6°. Thus, the ribose ring displays a C(3')-*exo* ($_3E$) envelope conformation, which deviates slightly from the range commonly found in type S β-purine nucleosides and nucleotides.[25] The same ribose puckering has been found in 2'-deoxy-

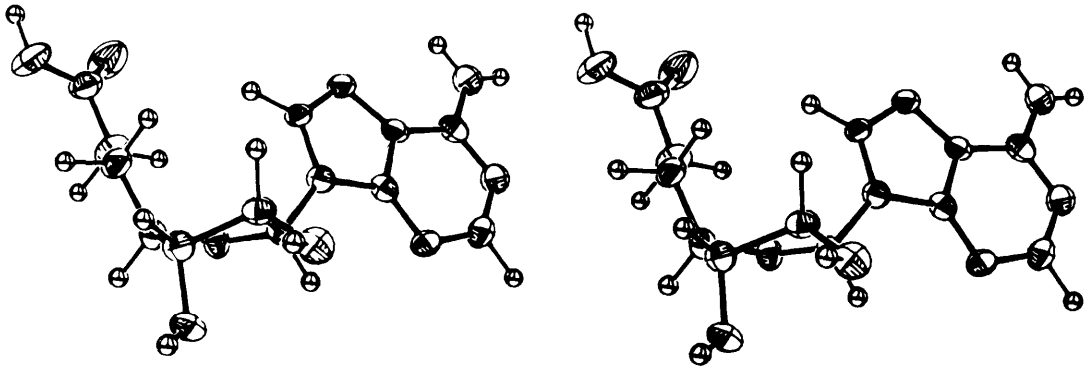


Figure 2. Stereoscopic drawing of AAA molecule. Thermal ellipsoids for non-hydrogen atoms are drawn at the 50% probability level

adenosine (P = 194.3°, τ_m = 36.3°) [26] and 5'-methylammonium-5'-deoxyadenosine (P = 180.8°, τ_m = 37.9°).[27]

The relative orientation of the base with respect to the ribose is described by the glycosyl torsion angle χ[O(1')–C(1')–N(9)–C(8)]. The AAA molecule is *anti* with angle χ = 50.7°. This orientation seems reasonable because in β-purine nucleosides and nucleotides, the correlation between the glycosyl torsion angle χ and ribose puckering shows that type S ribose puckering favours $38° < \chi < 73°$.[28,29]

The orientation about the C(4')–C(5') bond is: O(1')–C(4')–C(5')–C(6') = 47.1°, C(3')–C(4')–C(5')–C(6') = 165.4°, *i.e.* the AAA molecule has a *gauche-trans* (*gt*) conformation. This orientation, although less favoured than *gauche-gauche* (*gg*) when all nucleosides and nucleotides are considered, is also observed in 2'-deoxy-adenosine,[26] adenosine,[30] 5'-methylammonium-5'-deoxyadenosine,[27] and adenosine-3'-monophosphate.[31]

In a survey of the molecular conformation of AMP in crystal structures, a striking correlation in the conformation (*anti*-gg) about the glycosyl and exocyclic C(4')–C(5') bonds was noted. In the five AMP conformations listed in Table 2, only one takes the *tg* orientation probably as a result the effects of the intercalating chloroterpyridineplatinum(II).[23] Indeed an empirical energy calculation [32] suggested that the

* The value is the one of n-butyric acid.

Table 2. Comparison of the molecular conformations of AAA and AMP (free and metal-complexed) molecules. Torsion angle notations for the molecule are after Sundaralingam [24] and for pseudorotation parameters (P and τ_m) after Altona and Sundaralingam [25]

	AAA	AMP [15] (monoclinic)	AMP [16] (orthorhombic)	AMP·Ba [22]	AMP·Pt [23] Mol. I	AMP·Pt [23] Mol. II
Glycosyl (°)	50.7(9) (*anti*)	25.6 (*anti*)	72.5 (*anti*)	68.7 (*anti*)	40.3 (*anti*)	70.3 (*anti*)
Ribose pucker	C(3′)*exo* ($_3E$)	C(3′)*endo* (^3E)	C(2′)*endo* (^2E)	C(4′)*exo* ($_4E$)	C(2′)*endo* (^2E)	C(4′)*exo* ($_4E$)
Ribose conformation						
τ_0	−5.0(8)	4.8	−14.2	−16.3	−15.7	−24.9
τ_1	26.5(7)	−29.8	27.8	−11.1	30.3	−1.3
τ_2	−36.9(7)	42.3	−30.2	32.3	−31.7	26.3
τ_3	35.1(7)	−40.1	23.3	−42.4	23.1	−41.7
τ_4	−19.1(8)	22.9	−5.8	37.3	−4.9	42.3
P	191.3	12.3	172.1	40.5	169.5	53.0
τ_m	37.6	43.3	30.5	42.1	32.2	43.7
Backbone torsion angle						
ψ	165.4(7) (*tg*)	40.0 (*gg*)	62.3 (*gg*)	58.5 (*gg*)	39.4 (*gg*)	−174.8 (*tg*)
φ	184.6(8)	177.2	137.2	−143.3	156.1	174.2
ω	142.3(9)	177.5	179.3	176.6	−174.8	−179.5

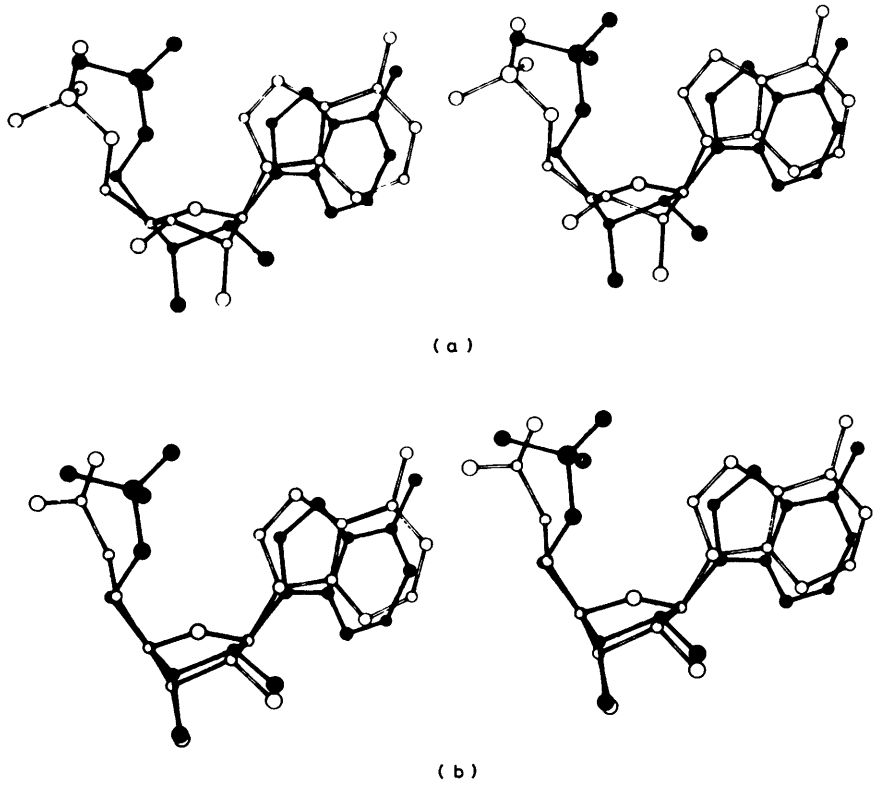


Figure 3. Comparison of the molecular conformation between monoclinic [C(3′)-*endo*] (O) and orthorhombic [C(2′)-*endo*] (●) AMP molecules. (a), and between AAA (O) and orthorhombic AMP (●) molecules. These molecules were drawn from crystallographic co-ordinates in such a way that the C(1′)–O(1′)–C(4′) bond sequence are common to each other

anti-gg conformation of AMP is energetically the most favoured one irrespective of whether the puckering of the ribose ring is C(2′)-*endo* or C(3′)-*endo*.

On the other hand, some variations are observed in the sugar puckering of AMP: C(2′)-*endo*, C(3′)-*endo*, and C(4′)-*exo*. Both C(2′)-*endo* and C(3′)-*endo* can be considered as the primary modes of sugar puckering for the AMP molecule, since they are also encountered, equally commonly, in numerous nucleosides and nucleotides; [25] a C(4′)-*exo* ⇌ C(3′)-*endo* transition also occurs readily,[33] in a similar fashion to C(2′)-*endo* ⇌ C(3′)-*exo* and C(3′)-*endo* ⇌ C(2′)-*exo* transitions.[34]

Thus it seems reasonable to assume that the two kinds of conformations observed in free AMP crystals (monoclinic

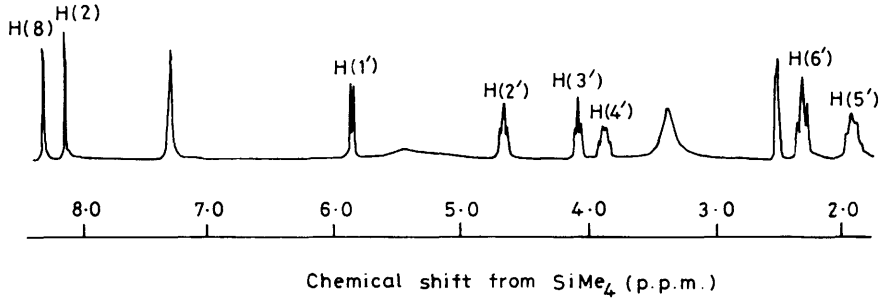


Figure 4. 200-MHz ^{1}H n.m.r. spectrum of AAA in $(C^2H_3)_2SO$ solution

and orthorhombic forms) represent the most energetically stable ones.

Figure 3 shows the conformational comparisons between monoclinic and orthorhombic AMP molecules (a) and between AAA and orthorhombic AMP molecules (b) observed in their crystal structures.

If AMP aminohydrolase recognizes these two AMP conformers as equivalently suitable substrates, the substrate specificity of the enzyme would be less severe, considerable spatial variation, especially in the ribose puckering, being found between the two AMP conformers. Thus when the high specificity of this enzyme for the chemical nature of substituents, is considered as well as the steric requirements,[35] it is clear that it distinguishes selectively between the two AMP conformers. It is interesting that the observed conformation in AAA with respect to the glycosyl bond and ribose puckering is very similar to that of orthorhombic AMP [Figure 3(*b*)].

There is a significant spatial difference between AAA and AMP conformers in their orientation about the exocyclic C(4′)–C(5′) bond: this is *gauche-trans* for the AAA molecule and *gauche-gauche* for the AMP molecule. This conformational discrepancy, along with the dimensional one between carboxyethyl and phosphate groups may, at least partly, be responsible for the difference between the model AAA and the natural AMP substrates for the catalytic reaction by the enzyme; for AMP aminohydrolase, the Michaelis constants (K_m) for AAA is 2.7 times greater than that for AMP, and the reaction velocity ($V_{max.}$) is 0.28 times less than that for AMP.[3]

On the other hand, the conformational similarity observed in AAA and AMP crystals lies in the glycosyl bond, an *anti* conformation of the adenine base with respect to sugar ring being adopted. As has already pointed by Follman,[3] this *anti* conformation may primarily be necessary for the binding of substrates to AMP aminohydrolase.

Molecular Conformation in ^2H_2O *and* $(C^2H_3)_2SO$ *Solutions.*—Substrate and enzyme interaction occurs in a dynamic not a static state. Thus, although the insights achieved by *X*-ray crystallographic studies provide valuable information concerning the interaction, solution studies would provide even more valuable information. Thus, we have studied the conformation of the AAA molecule in ^2H_2O and $(C_2H_3)_2SO$ solutions by ^{1}H n.m.r. spectroscopy.

Assignment of all the proton resonances was made by homonuclear decoupling, spin multiplicities, and comparison with previously published data.[12,35,36] The ^{1}H n.m.r. spectrum of the AAA molecule in $(C^2H_3)_2SO$ solution is shown in Figure 4; the chemical shifts, coupling constants, and the spin-lattice relaxation times (T_1) of H(8) and H(1′) are summarized in Table 3. The conformational populations (%) about the glycosyl and exocyclic C(4′)–C(5′) bond and the ribose puckering for AAA and AMP are listed in Table 4; the population for the latter molecule was calculated by using published data.[11,12]

As has already been suggested by Follman and Gremels,[35] the high preference for the *anti* orientation about the glycosyl bond was observed in both the molecules in ^2H_2O solution, although the populations of the *anti* and *syn* conformers

Table 3. Proton chemical shifts (p.p.m.), coupling constants (Hz) and spin-lattice relaxation times (s) in ^2H_2O and $(C^2H_3)_2SO$ solutions at 21 °C

	^2H_2O	$(C^2H_3)_2SO$
Chemical shifts [e]		
H(8)	8.34	8.32
H(2)	8.25	8.15
H(1′)	6.05	5.85
H(2′)	— [b]	4.66
H(3′)	4.25	4.08
H(4′)	4.13	3.88
H(5′) [c]	2.00	1.92
H(6′)	2.31	2.31
Coupling constants [d]		
$J_{1'2'}$	5.7	5.1
$J_{2'3'}$	5.0	5.3
$J_{3'4'}$	4.7	4.9
$J_{4'5'}$ [e]	6.0	5.3
$J_{5'5''}$ [f]	—	—
$J_{5'6'}$	7.0	7.5
Spin-lattice relaxation time (T_1) [g]		
$(T_1)_8$	1.71(5)	0.86(1)
$(T_1)_1$	2.57(9)	0.80(4)

[a] Estimated error: ±0.02 p.p.m. [b] The signal of H(2′) proton overlaps with that of H_2O proton. [c] Splitting between H(5′) and H(5″) could not be clear-cut at 200 MHz. [d] Estimated error: ±0.2 Hz. [e] $J = (J_{4'5'} + J_{4'5''})/2$. [f] We could not determine the $J_{5'5''}$ unequivocally. [g] Measured with the pulse Fourier transform mode by applying a 180°-τ-90° pulse sequence.

Table 4. Calculated population (%) [a] of certain conformers for AAA molecule. For comparison, that of AMP molecule is also listed.

		AAA		AMP
		^2H_2O	$(C^2H_3)_2SO$	H_2O
Glycosyl bond	*anti*	86	45	83
Ribose ring	C(3′)-*endo*	45	49	38
C(4′)–C(5′) bond	*g* . *g*	10	24	66
	g . *t* (or *t* . *g*)	90	76	34

[a] Error is ±0.5—10%.

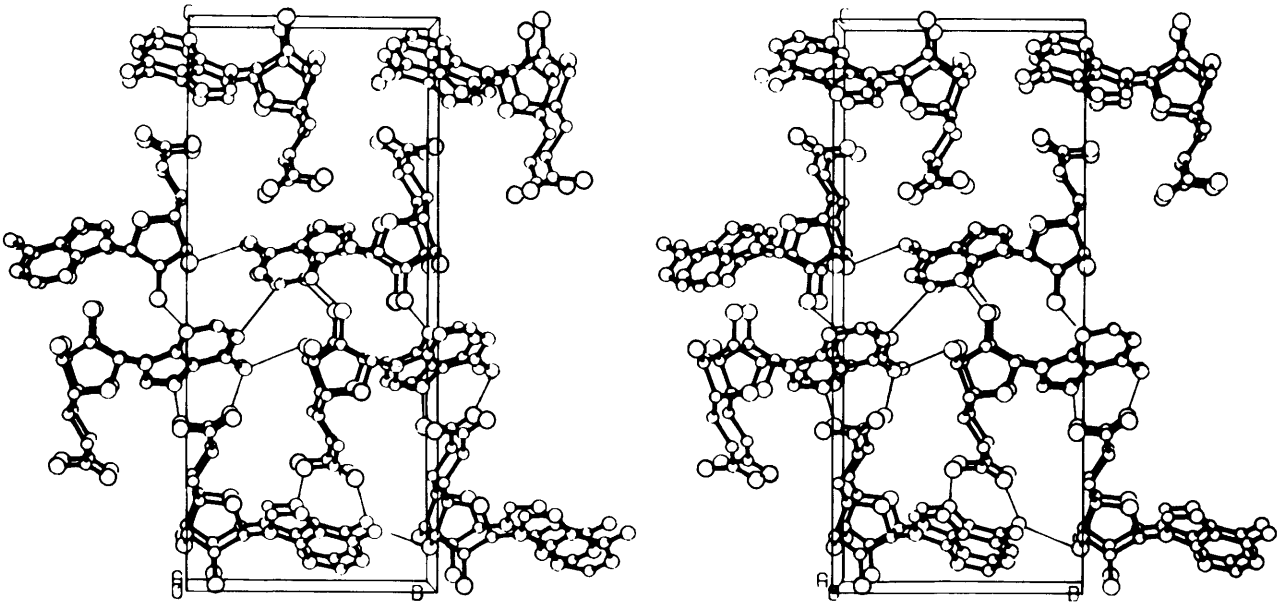


Figure 5. Stereoscopic drawing of AAA molecules viewed along the *a* axis. The thin lines represent the possible hydrogen bonds

Table 5. The hydrogen bond distances (Å) and angles (°) and the short contacts less than 3.5 Å observed in AAA crystal

Superscript numbers represent the symmetry equivalent number

Hydrogen bonds

Donor (D)	Acceptor (A)	Distance D···A	Distance H···A	Angle of D–H···A
C(2)[1]	N(1)[2]	3.371(11)	2.33(7)	153(5)
N(6)[1]	O(2)[3]	2.846(11)	1.86(8)	175(7)
N(6)[1]	O(3′)[4]	3.011(9)	2.29(11)	164(12)
O(2′)[1]	N(3)[5]	2.774(8)	2.32(11)	179(9)
O(1)[1]	N(7)[6]	2.756(9)	1.77(10)	173(9)

Short contacts

Atom	Atom	Distance	Atom	Atom	Distance
C(4)[1]	O(1′)[1]	3.454(9)	C(8)[1]	C(2′)[1]	3.234(11)
C(8)[1]	O(1′)[1]	2.949(9)	C(2)[1]	O(3′)[5]	3.135(10)
N(3)[1]	O(3′)[5]	3.228(8)	C(4)[1]	O(2′)[5]	3.240(9)
C(5)[1]	O(2′)[5]	3.401(9)	N(9)[1]	O(2′)[5]	3.453(9)
N(6)[1]	C(2)[7]	3.270(11)	N(7)[1]	N(3)[7]	3.387(9)
N(7)[1]	O(1′)[7]	3.499(8)	C(8)[1]	O(1′)[7]	3.140(9)
C(2′)[1]	O(3′)[7]	3.409(9)	C(4)[1]	O(1)[8]	3.409(10)
C(5)[1]	C(7′)[8]	3.347(11)	C(6)[1]	O(2)[8]	3.413(11)
C(8)[1]	O(1)[8]	3.451(10)	N(9)[8]	O(1)[8]	3.380(9)
O(1′)[1]	O(1)[8]	3.422(8)	N(1)[1]	C(3′)[9]	3.479(11)

Symmetry equivalent no.

(1)	x, y, z	(2)	$0.5 + x, 2.5 - y, 1 - z$
(3)	$-1 - x, 0.5 + y, 0.5 - z$	(4)	$-1 + x, 1 + y, z$
(5)	$-1 + x, y, z$	(6)	$-0.5 + x, 1.5 - y, 1 - z$
(7)	$-1 - x, -0.5 + y, 0.5 - z$	(8)	$-x, 0.5 + y, 0.5 - z$
(9)	$x, 1 + y, -z$		

were almost equal in $(C^2H_3)_2SO$ solution. On the basis of the calculated population using the formula of Davies and Danyluk,[12] there was for AAA no marked preference for the C(3′)-*endo* or the C(2′)-*endo* conformation in either solution; AMP however shows a preference for C(2′)-*endo* rather than C(3′)-*endo*. A significant difference between the two molecules lies in the population of the exocyclic C(4′)–C(5′) orientation: the AAA molecule exhibits a marked preference for a *gauche-trans* (or *trans-gauche*) conformation in both solutions, while the *gauche-gauche* orientation is highly favoured in the AMP molecule. The C(6′) protons of the AAA molecule appear as a triplet in both solutions, the 5′6′ coupling being fully described by just one coupling constant; this suggests that the C(5′)–C(6′) bond rotates freely.

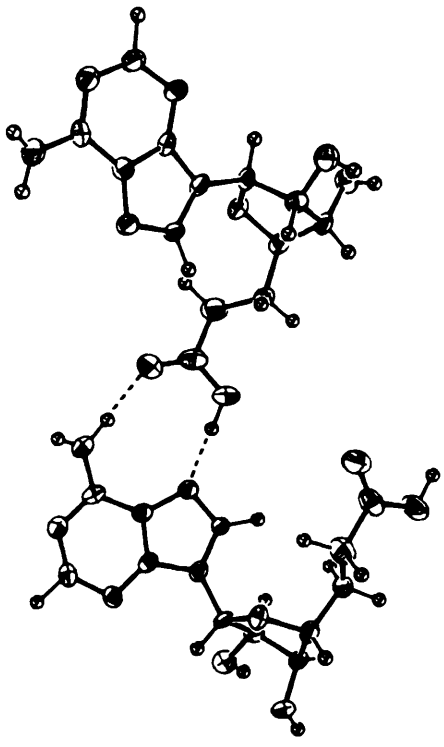


Figure 6. Hydrogen bonding mode between the adenine ring and the carboxy-group observed in AAA

As a result, these ^1H n.m.r. results show clearly that AAA and AMP show similar conformational differences both in their solid and solution (^2H_2O) states.

Hydrogen Bonding and Molecular Packing Modes.—Figure 5 shows the packing of AAA molecules in the unit cell looking along the *a* axis. Possible hydrogen bonds and the short contacts less than 3.5 Å are summarized in Table 5.

The polarizable atoms of the adenine base participated in the many hydrogen bonds and short contacts. The adenine base is linked by three hydrogen bonds [N(1) · · · C(2), 3.371 Å; N(6) · · · O(2), 2.846 Å; N(3) · · · O(2'), 2.774 Å] and many short contacts with the neighbouring adenine or ribose ring related by a diad screw axis or *b*-axis translation, so that AAA molecules form double layers parallel to the *b* axis. The layers are mutually linked by hydrogen bonds between the adenine base and the carboxy-group: the carboxy-group is linked by two hydrogen bonds to N(6) and N(7) of the neighbouring adenine ring [O(2) · · · N(6), 2.846 Å; O(1) · · · N(7), 2.756 Å] (see Figure 6). This interaction would be important in specific binding of the acidic amino acid with the nucleic acid base adenine, because it is a model for the way in which an adenine, base-paired *via* N(6) and N(1) atoms to uracil (or thymine) in double-stranded RNA (or DNA), might form hydrogen bonds with the carboxy-group of glutamic or aspartic acid. This kind of hydrogen bond is also observed in the crystal structure of *N*-(9-β-D-ribofuranosylpurin-6-yl)glycyl-L-alanine.[37]

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