previous paragraph we obtain for the specific second-order rate constant for OH^- attack on 3-methylhistidine protonated only on the imidazole ring log $k_0 = 2.9$ (s^{-1} M^{-1}).

For the Pd(II) complex of 3-methylhistidine the observed first-order rate constant for H(2) exchange is given by

$$k_{obsd} = k_c[OH^-] = k_cK_w/[H^+]$$

From the average log k_{obsd} − pH = −15.67 in Table III at 61 °C we calculate for the specific second-order rate constant for OH^- attack on complexed 3-methylhistidine log $k_c = -1.9$ (s^{-1} M^{-1}).

The specific second-order rate constants for OH^- attack on severals forms of 3-methylhistidine may now be compared. Slowest is the chelated ligand in PdL_2, with log $k_c = -1.9$ (s^{-1} M^{-1}). The two rate constants for the protonated imidazole ligand are log $k_0 = 2.9$ for the minor tautomer with only the imidazole ring protonated and log $k_+ = 3.5$ for the major diprotonated species in weakly acidic solutions. Thus the rate constants for OH^- attack on the protonated imidazole ligand are $10^{4.8}$ and $10^{5.4}$ times greater than that for the Pd(II) complex. These ratios are similar to that of $10^{4.6}$ found for N(7)-protonated inosine and its complex with Pt(II) at N(7). Thus both imidazole and purine derivatives behave similarly.

The magnitude of $\sim 10^5$ found for the ratio of the specific second-order rate constants for OH^- attack on protonated and metalated ligand exceeds that inferred from consideration of pK_a values at the imidazole nitrogen. Because of the methyl group in 3-methylhistidine, we cannot measure the acidity of a proton at that position and turn our attention to histidine. In enPd(histidine) the imidazole nitrogen deprotonation across the ring from the chelated Pd(II) occurs with $pK_a = 10.8$.[28] When a proton replaces the Pd(II), the imidazole deprotonation in histidine occurs with $pK_a = 6.1$.[14] Applying the slope of −0.58 from the introduction to this difference of 4.7 log units in pK_a, we anticipate a specific second-order rate constant about $10^{2.7}$ times greater in the ligand than in the complex. That the calculated value is more than 10^2 times greater indicates that the Pd(II) complex falls more than 2 log units below the expected log k in a plot of log (second-order rate constant) vs. pK_a. That H(2) exchange was not observed in an imidazole complex of Co(III)[12] suggests that metal ions generally inhibit exchange more than anticipated on the basis of the pK_a of nitrogen-bound hydrogen in the complexed imidazole ring.

In both cases examined, inosine and 3-methylhistidine, the specific second-order rate constant for exchange occurs about 10^5 times faster in the protonated ligand than in the metal ion complexed ligand. (It is still less in the neutral ligand, which cannot form an ylide.) How then is the observation referred to in the introduction that metals promote H(8) exchange in nucleic bases and inhibit H(2) exchange in imidazoles consistent with the above conclusion? The resolution lies in the substantially different pK_a values at N(1) or N(3) in imidazoles and N(7) in nucleic bases. In our examples with $pK_a \simeq 6$ at N(1) in 3-methylhistidine, there is an appreciable amount of protonated species even in basic solutions so that the concentration product of N(1)-protonated species and $[OH^-]$ is relatively high. In contrast, for inosine with $pK_a = 1.0$ for N(7) protonation, the concentration product of N(7)-protonated species and $[OH^-]$ is much lower. Complexation of metal ions to inosine or another purine base at N(7) places a positive charge where a proton does not occur until quite low pH. In these instances the metal ion functions as a superacid catalyst in neutral solutions. In contrast, the much more basic imidazoles partially protonate even in neutral solutions, and the much greater polarizing power of the proton over a metal ion[29] reduces the apparent relative role of the metal ion.

Registry No. Ino, 58-63-9; dienPtIno^{2+}, 69215-32-3; enPtIno$_2^{2+}$, 80263-30-5; dienPtGuo^{2+}, 69667-81-8; enPtGuo$_2^{2+}$, 72950-44-8; 3-methylhistidine, 332-80-9; Pd(3-methylhistidine)$_2$, 80263-31-6.

(28) Pitner, T. P.; Wilson, E. W., Jr.; Martin, R. B. *Inorg. Chem.* **1972**, *11*, 738.

(29) Martin, R. B. *J. Am. Chem. Soc.* **1967**, *89*, 2501.

2-Amino-8-methyladenosine 5′-Monophosphate Dihydrate. A Nucleotide with Syn C4′-Exo Conformation and "Triple-Stranded" Packing

J. V. Silverton,*,† W. Limn,‡ and H. T. Miles‡

Contribution from the Laboratory of Chemistry, National Heart, Lung, and Blood Institute, and the Laboratory of Molecular Biology, National Institute of Arthritis, Metabolic, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received May 26, 1981*

Abstract: The preparation and crystal structure of 2-amino-8-methyladenosine 5′-monophosphate dihydrate, $C_{11}H_{14}N_6O_7P\cdot 2H_2O$, are described. The space group is $R3$, with $a = 20.192$ (1) Å and $c = 11.0849$ (7) Å. The final R factor was 2.3%, using counter data corrected for absorption and extinction, and the resulting esd's are the smallest yet reported for an X-ray structure of a nucleotide. The nucleotide molecules adopt the unusual syn C4′-exo conformation, and the extensively hydrogen-bonded components of the crystals are organized into crystallographic helices which, in turn, form triangular groupings reminiscent of the early Pauling and Corey model for the nucleic acids. The extensive involvement of water bridges in the packing is of interest. The observation of the syn C4′-exo conformation is relevant to modeling studies of polynucleotides and may indicate that the allowable ranges of sugar conformation are broader than previously suspected.

Mutual rotation of the base and sugar of nucleotides about the glycosidic bond has received extensive investigation in recent years.[1-14] X-ray crystallographic studies have shown a high preponderance of the anti conformation,[3] although, especially among purine nucleosides, a number of syn structures have been

†NHLBI.
‡NIAMDD.

(1) Donohue, J.; Trueblood, K. N. *J. Mol. Biol.* **1960**, *2*, 363.
(2) Haschemeyer, A. E. V.; Rich, A. J. *J. Mol. Biol.* **1967**, *27*, 369.
(3) Sundaralingam, M. *Biopolymers* **1969**, *7*, 821.

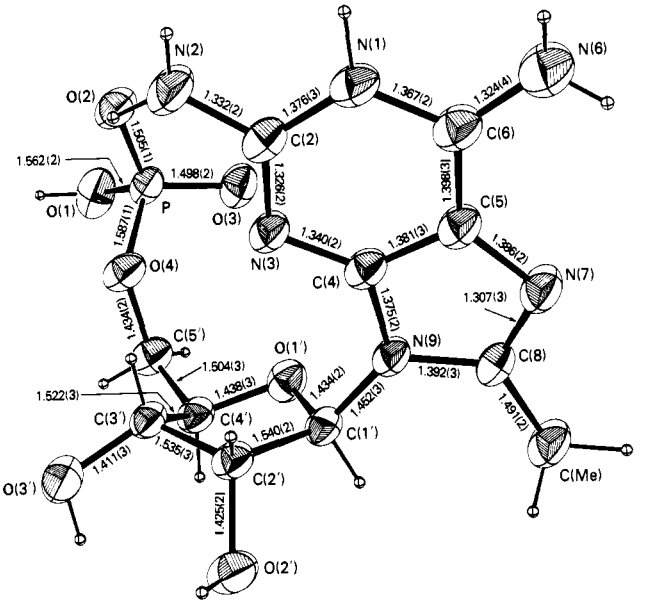

Figure 1. Bond lengths, esd's, and crystal conformation of the nucleotide. The thermal ellipsoids depict 40% probability and hydrogen atoms are represented as arbitrary spheres.

Table I. Bond Angles for the Heavier Atoms (deg)[a]

O(1)-P-O(2)	112.3 (1)	O(1)-P-O(3)	107.6 (1)
O(1)-P-O(4)	105.9 (1)	O(2)-P-O(3)	116.3 (1)
O(2)-P-O(4)	104.1 (1)	O(3)-P-O(4)	110.0 (1)
P-O(4)-C(5')	120.8 (1)	C(2)-N(1)-C(6)	123.3 (2)
N(1)-C(2)-N(2)	115.6 (2)	N(1)-C(2)-N(3)	124.0 (2)
N(2)-C(2)-N(3)	120.5 (2)	C(2)-N(3)-C(4)	112.1 (2)
N(3)-C(4)-C(5)	128.6 (2)	N(3)-C(4)-N(9)	126.2 (2)
C(5)-C(4)-N(9)	105.2 (1)	C(4)-C(5)-C(6)	117.5 (2)
C(4)-C(5)-N(7)	111.1 (2)	C(6)-C(5)-N(7)	131.4 (2)
N(1)-C(6)-C(5)	114.5 (2)	N(1)-C(6)-N(6)	119.2 (2)
C(5)-C(6)-N(6)	126.4 (2)	C(5)-N(7)-C(8)	104.9 (2)
N(7)-C(8)-C(Me)	124.4 (2)	N(7)-C(8)-N(9)	112.3 (1)
C(Me)-C(8)-N(9)	123.3 (2)	C(4)-N(9)-C(8)	106.6 (2)
C(4)-N(9)-C(1')	126.0 (2)	C(8)-N(9)-C(1')	127.2 (1)
N(9)-C(1')-O(1')	108.6 (1)	N(9)-C(1')-C(2')	114.8 (1)
O(1')-C(1')-C(2')	107.2 (1)	C(1')-O(1')-C(4')	107.0 (1)
C(1')-C(2')-O(2')	107.8 (1)	C(1')-C(2')-C(3')	103.4 (1)
O(2')-C(2')-C(3')	111.2 (2)	C(2')-C(3')-O(3')	114.6 (1)
C(2')-C(3')-C(4')	102.2 (1)	O(3')-C(3')-C(4')	113.0 (2)
O(1')-C(4')-C(3')	103.4 (2)	O(1')-C(4')-C(5')	110.3 (2)
C(3')-C(4')-C(5')	115.6 (1)	O(4)-C(5')-C(4')	110.1 (2)

[a] Esd's are given parenthetically.

reported.[3] Solution studies of dinucleoside monophosphates containing 8-methyladenosine suggest the presence of both syn and anti forms, with a relatively low rotational barrier.[15,16]

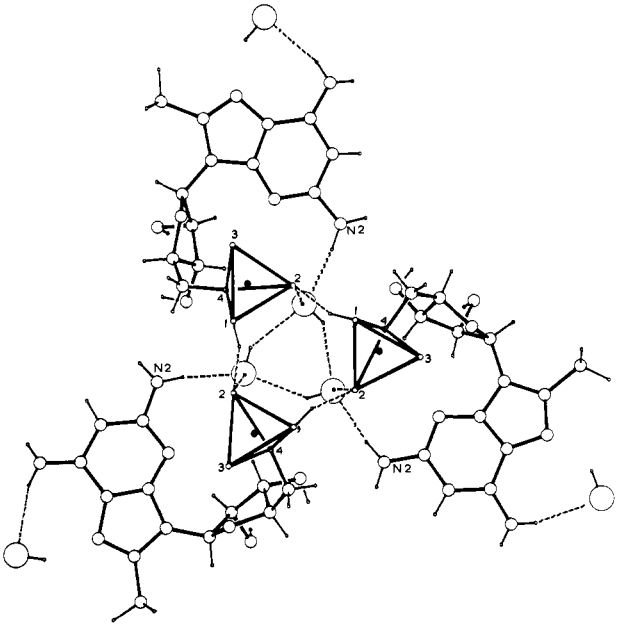

Figure 2. The triad of molecules around a crystallographic 3-fold axis. Water molecules are depicted as large circles with O(S1) atoms in the center of the picture. The phosphate groups are shown as tetrahedra with numbers corresponding to Figure 1. Hydrogen bonds are depicted by broken lines.

Virtually all of the naturally occurring syn purine nucleosides investigated crystallographically have had a 5'-O–H···N(3) hydrogen bond and C2'-endo ring puckering of the sugar.[3] Introduction of bulky substituents at C(8) increases steric hindrance in the anti range, thus favoring the syn conformation, and other substitutions can also alter the energetic balance, providing information on factors controlling nucleoside and nucleotide conformation. In the present study we examine the effects of introducing both an 8-methyl substituent, important because of its potential for steric interference, and a 2-amino group, of interest for its hydrogen-bonding potential. The structure is novel both in the conformation of the nucleotide and in the striking organization of the molecules into triads with phosphate groups in the center and bases on the periphery. Extensive hydrogen bonding and water bridges control molecular association.

Molecular Structure and Bonding

The final bond lengths, atomic nomenclature adopted, and the crystal conformation of the nucleotide are shown in Figure 1 (ORTEP[17] drawing), and the bond angles are given in Table I. Bond lengths, not involving the phosphorus atom, have esd's (derived from inversion of the normal equations matrix) comparable with better X-ray studies of compounds without heavy atoms, and the P–O bond lengths have esd's less than 0.002 Å. In the present structure, the hydrogen atom positions lead to appropriate molecular dimensions which do not differ significantly from expected values. Probably as a consequence of the extensive hydrogen bonding, the thermal parameters for the heavier atoms of the crystal are very small except for those of the water molecules.

It is usually difficult to assign P–O bond types solely on a basis of bond lengths,[18] especially since the differences are of the order of the esd's. In the present case, the two lengths are P–O(2), 1.505

(4) Lakschminarayanan, A. V.; Sasisekharan, V. *Biopolymers* **1969**, *8*, 475.
(5) Sundaralingam, M. In "Conformation of Biological Molecules and Polymers"; Bergmann, E. D., Pullman, B., Eds.; Israel Academy of Sciences and Humanities: Jerusalem, 1973; pp 417–455.
(6) Sasisekharan, V. In "Conformation of Biological Molecules and Plymers"; Bergmann, E. D., Pullman, B., Eds.; Israel Academy of Sciences and Humanities: Jerusalem, 1973; pp 247–258.
(7) Teng, N. H.; Itzkowitz, M. S.; Tinoco, I., Jr. *J. Am. Chem. Soc.* **1971**, *93*, 6257.
(8) Miles, D. W.; Inskeep, W. H.; Robins, M. J.; Winkley, M. W.; Robins, R. K.; Eyring, H. J. *J. Am. Chem. Soc.* **1970**, *92*, 3872.
(9) Schweizer, M. P.; Broom, A. D.; Ts'o, P. O. P.; Hollis, D. P. *J. Am. Chem. Soc.* **1968**, *90*, 1042.
(10) Ikehara, M.; Tazawa, I.; Fukui, T. *Biochemistry* **1969**, *8*, 736.
(11) Michelson, A. M.; Monny, C.; Kapuler, A. M. *Biochim. Biophys. Acta* **1970**, *217*, 7.
(12) Pullman, B.; Berthod, H. In "Conformation of Biological Molecules and Polymers"; Bergmann, E. D., Pullman, B., Eds., Israel Academy of Sciences and Humanities: Jerusalem, 1973; pp 209–244.
(13) Howard, F. B.; Frazier, J.; Miles, H. T. *J. Biol. Chem.* **1974**, *249*, 2987.
(14) Govil, G.; Fisk, C.; Howard, F. B.; Miles, H. T. *Nucleic Acids Res.* **1977**, *4*, 2573.
(15) Uesugi, S.; Limn, W.; Ikehara, M. *Chem. Pharm. Bull.* **1978**, *26*, 3732.
(16) Ikehara, M.; Limn, W.; Uesugi, S. J. *J. Carbohyd. Nucleosides, Nucleotides* **1978**, *5*, 1963.
(17) Johnson, C. K. Oak Ridge, TN, 1965, Oak Ridge National Laboratory Report ORNL-3794. All figures, except Figure 3, were made by using ORTEP.
(18) Watson, D. G.; Kennard, O. *Acta Crystallogr. Sect. B* **1973**, *B29*, 2358.

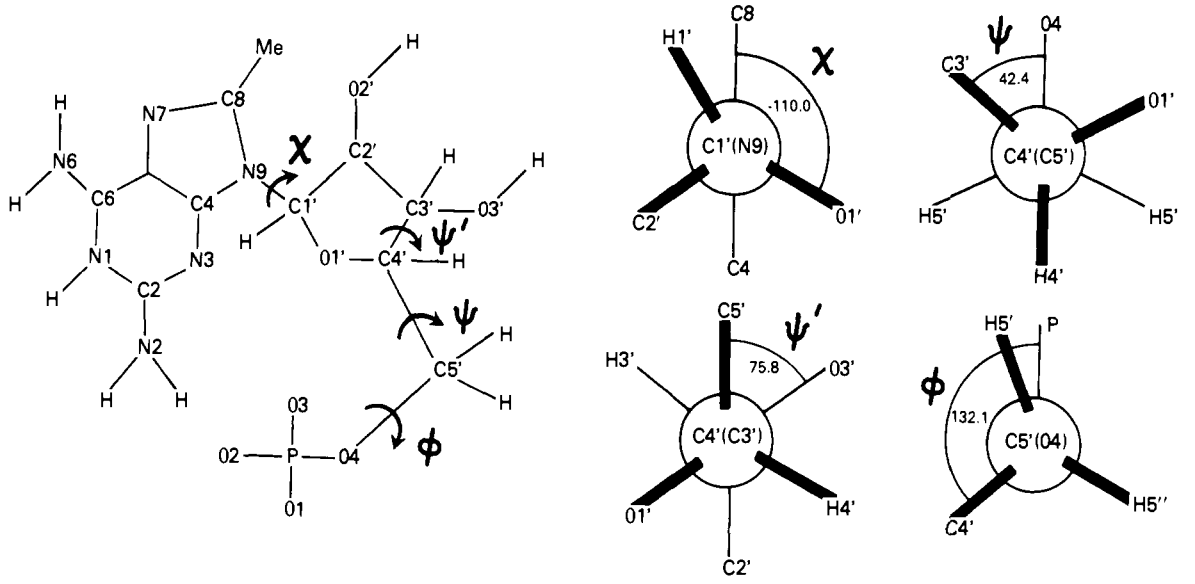

Figure 3. Torsion angles delineating molecular conformation.

(1) Å, and P–O(3), 1.498 (2) Å, and the difference, although small, is significant at the 3σ level. One might be tempted to suggest that the shorter bond, P–O(3), is the formal double bond, but the observed packing may indicate an opposite assignment. There is a triad of phosphate groups around a 3-fold axis with the O(3) atoms (Figure 2), as far away from symmetry-related atoms as possible, and it is logical for O(3) to carry most of the charge of the ionized phosphate group, since the other possible oxygen atom, O(2), is much closer to its symmetry-related atoms. O(3) also forms the shortest N–H···O hydrogen bond, that to N(1), along a 3-fold screw axis. Since N(1) is protonated and thus carries a significant proportion of the positive charge on the base, it is possible that the short distance observed is caused by both the hydrogen bond and the attraction of unlike charges. In accord with Srikrishnan, Fridey, and Parthasarathy,[19] who gave extensive tables of P–O bonds in nucleosides and nucleotides, the conclusion of Watson and Kennard,[18] that it is not possible to make a distinction among P–O bond types solely on bond lengths, still seems to be valid. The third phosphate oxygen atom, O(1), is protonated and hydrogen bonded to O(2) in the adjacent nucleotide of the triad. This proton and hydrogen bond can be present only in a terminal phosphate and not in an ionized nucleic acid.

The base moieties of the molecules in the crystal are not rigorously planar, but deviations are less than 0.020 Å. The substituents, N(2), N(6), and C(Me), are −0.042, 0.067, and 0.019 Å, respectively, from the mean plane. The two component rings individually deviate even less from planarity; the maximum deviation from the least-squares plane is 0.018 Å for the six-membered ring and 0.007 Å for the five-membered ring. N(2) and N(6) are −0.039 and −0.058 Å, respectively, from the plane of the six-membered ring and C(Me) is 0.037 Å from the plane of the five-membered ring. The two rings make an angle of 0.8° with each other. The amino hydrogen atoms are not exactly coplanar with the rings. The distances from the mean plane of the base are −0.02 and −0.25 Å for the atoms attached to N(2) and −0.22 and −0.25 Å for those attached to N(6). The deviations result in the planes of the N(2) and N(6) amino groups making angles of 14 and 23°, respectively, with the plane of the six-membered ring.

The crystallographic analysis of the analogous nucleotide 8-methyladenosine 3'-monophosphate has been reported.[20] This nucleotide also has the syn conformation and has corresponding bond lengths and angles which are very similar to those reported here; however, the sugar conformation is C2'-endo and the crystal packing is entirely different.

Molecular Conformation and Packing

The present work represents the first nucleotide crystal structure in which the less common syn conformation is combined with a C4'-exo puckering of the sugar. Diagrams showing the torsion angles which delineate nucleotide conformation are given as Figure 3. Since χ is −110.0°, the conformation is syn. In the ribose ring, the torsion angle about the C(1')–C(2') bond is close to zero (−2.7°), and accordingly, by far the best four-atom plane is through O1',C1',C2',C3'. The corresponding ribose conformation is C4' exo, betokened by the Altona and Sundaralingam[21] pseudorotation angle (P) of 51.4°. The corresponding maximum torsion angle (θ_m) is 41.7°. This conformation has not been previously observed in a nucleotide but was recently found in the nucleoside 5-acetyl-2'-deoxyuridine[22] (P = 62.1°, θ_m = 36.7°), although, in this case, P is somewhat further from the ideal value of 54° for the conformation. In Sundaralingam's[5] notation, the present conformation is ${}_4E$, and the other is almost exactly intermediate between ${}_4E$ and ${}^0_4T$. The observed C4'-exo conformation is about 15° further along the pseudorotation pathway from the usual values for sugar conformation in nucleotides, although the adjacent C3'-endo conformation would also be very unusual for a syn nucleotide. We suggest, on the basis of this study and ref 26, that the usually syn C2'-endo correlation is valid only in the presence of a 5'-O–H···N(3) hydrogen bond, which has been present in virtually all of the previously reported syn purine nucleoside or nucleotide structures and which is also present in the syn C2'-endo nucleotide 8-methyladenosine monophosphate.[20] In the absence of a 5'-O–H···N(3) hydrogen bond, the choice between the C3'-endo or C2'-endo range of conformations may depend on external factors, including molecular packing, and the allowable ranges, as the present observations and ref 22 indicate, may be broader than heretofore suspected. In another structure without the 5'-O–H···N(3) hydrogen bond, the C2'-endo conformation has been observed.[23] The syn conformation is presumably favored in the present structure by greater steric interference of the sugar with the 8-methyl group in the anti range (cf. ref 15, 16, and 20), although recent studies suggest the hindrance is not sufficient to preclude an anti conformation in solution,[15,16] where solvation might stabilize this form. The presence of C2'-endo puckering

(19) Srikrishan, T.; Fridey, S. M.; Parthasarathy, R. J. *J. Am. Chem. Soc.* **1979**, *101*, 3739.
(20) Yasuniwa, M.; Tokuoka, R.; Ogawa, K.; Yamagata, J.; Fujii, S.; Tomita, K-H.; Limn, W.; Ikehara, M. *Biochim. Biophys. Acta* **1979**, *561*, 240.
(21) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
(22) Barr, P. J.; Chananont, P.; Hamor, T. A.; Jones, A. S. O'Leary, M. K.; Walker, R. T. *Tetrahedron* **1980**, *36*, 1269.
(23) Ginell, S. L.; Parthasarathy, R. *Biochem. Biophys. Res. Commun.* **1978**, *84*, 886–894.

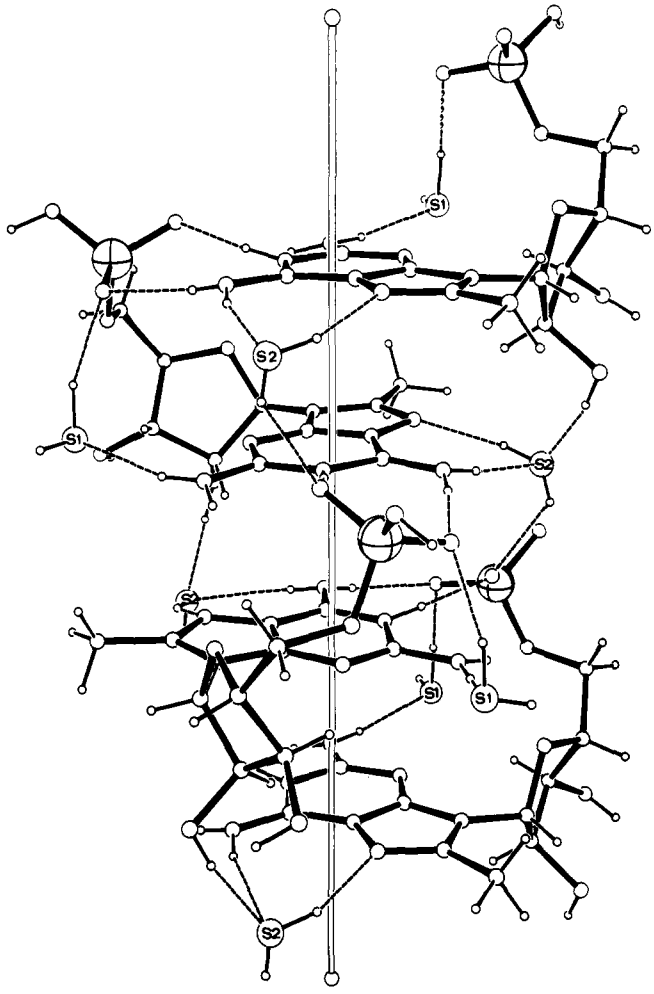

Figure 4. Arrangement of molecules around a crystallographic 3_1 axis, showing hydrogen bonds holding together the helix.

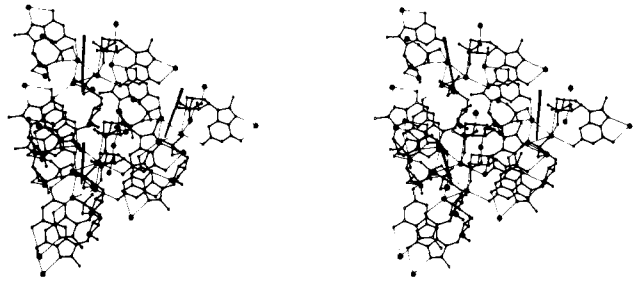

Figure 5. Stereo diagram showing molecular packing and hydrogen bonds. The solvent molecules are shown as larger black spheres and crystallographic 3-fold axes as vertical bars. The dense packing of molecules, interleaving of water molecules and phosphate groups, and the helices of parallel bases can be seen.

in previously reported syn nucleosides and nucleotides has evidently led to the assumption that the syn C2′-endo correlation was a principal of general validity for model building.[3,24,25] That this correlation is not necessarily valid was also shown in the case of the syn polynucleotide, poly(8BrA), for which NMR spin-coupling data indicated a C3′-endo conformation.[26]

In the crystal structure, a fairly large energetic contribution will come from the large number of hydrogen bonds forming the extensive hydrogen-bonding network shown in Figures 2 and 4. A stereo picture showing the packing of the molecules in the crystal is given as Figure 5. Each asymmetric unit has 12 hydrogen atoms, including those attached to the ribose oxygen atoms, which appear likely to be involved in hydrogen bonds. In fact, 11 such bonds are formed without postulating O–C hydrogen bonds (detailed in Table II). It will be noted that there are no intramolecular hydrogen bonds, although such linkages occur through water bridges.

Table II. Details of Hydrogen Bonds[a]

X-H	Y	sym op	distances, Å X···Y	X-H	H···Y	angles, deg
O(1)-H	O(2)	2	2.614 (2)	0.91 (4)	1.75 (4)	157 (3)
O(S1)-H	O(S1)	3	2.969 (2)	0.87 (4)	2.23 (6)	144 (6)
O(S1)-H	O(2)	1	2.946 (2)	1.09 (5)	1.90 (5)	160 (4)
O(S2)-H	N(7)	1	2.809 (4)	0.96 (7)	1.93 (6)	151 (3)
O(S2)-H	O(3)	4	2.798 (2)	0.81 (6)	2.19 (7)	132 (7)
N(1)-H	O(3)	5	2.658 (2)	0.91 (3)	1.75 (3)	176 (3)
N(2)-H	O(S1)	1	2.882 (3)	0.87 (5)	2.03 (5)	165 (3)
N(6)-H	O(2)	5	2.876 (2)	0.75 (4)	2.13 (4)	172 (4)
N(6)-H	O(S2)	1	3.117 (3)	0.82 (4)	2.33 (3)	163 (5)
O(2′)-H	O(S2)	5	2.693 (2)	0.84 (3)	1.86 (3)	169 (3)
O(3′)-H	O(1′)	6	2.816 (2)	0.86 (3)	2.08 (4)	144 (4)

[a] Esd's are indicated parenthetically. Each hydrogen bond is designated X-H···Y, with Y being the acceptor atom. The symmetry operation generating Y from the listed coordinates is given in the column designated "sym op". The symmetry operations used are as follows: $1, x, y, z$; $2, -y, x-y, z$; $3, -x+y, -x, z$; $4, {}^2/_3 - x + y, {}^1/_3 - x, z - {}^2/_3$; $5, {}^1/_3 - y, x - y - {}^1/_3, z - {}^1/_3$; $6, {}^1/_3 - x + y, {}^2/_3 - x, z - {}^1/_3$.

There can be considered to be two basic motifs in the crystal packing: triads triads (the word "triad" is used in its primary dictionary sense of "a group of three") of nucleotides around crystallographic 3-fold axes, held together by interior water bridges involving O(S1) (Figure 2), and infinite crystallographic 3_1 helical "strands", held together by external water bridges involving O(S2) (Figure 4). The triads of strands, although they are not discrete, have phosphates in the center and bases on the periphery. The structure, as seen in Figure 2, bears an intriguing resemblance to the three-stranded nucleic acid helix proposed by Pauling and Corey[27] in 1952, although in the present case the crystallographic 3_1 helices do not interlace. The Pauling–Corey model also had three strands with phosphates in the center, hydrogen bonded to each other, and bases on the outside. It was subsequently realized that one major defect of that model was that the nucleic acid phosphate groups are ionized and thus hydrogen atoms for bonding are absent.[28] It was also thought that mutual electrostatic repulsion of the negative charges on the phosphates would cause strand separation. While this destabilizing effect is undoubtedly important, especially in completely ionized phosphates, it is interesting to consider how, in the present structure, local overall attraction of charges and hydrogen bonding involving water bridges can effectively stabilize such an arrangement.

The triad of nucleotides around the 3-fold axis is held together by three water molecules, hydrogen bonded to form a triangle, with the phosphate groups and N(2) of the nucleotide strongly bonded to the triangle. There is also a O(1)–H···O(2) bond, between molecules related by the 3-fold axis, which reinforces the triangular group. It will be noted that the O(S1) atom achieves roughly tetrahedral coordination. As previously mentioned, consistent with their carrying the charge on the phosphate group, the O(3) atoms are quite far apart. The triad has crystallographic 3-fold symmetry, and there are a total of 12 rather strong hydrogen bonds holding it together. One could anticipate an enthalpy contribution of ca. 12 kcal from hydrogen bonds formed per mol of nucleotide.[29] To accurately evaluate the effect of electrostatic repulsions and attractions, we would have to know the local dielectric constants and exact charge distributions. If an overall

(24) Olson, W. K. *Biopolymers* **1973**, *12*, 1787.
(25) Sarma, R. H.; Lee, C-H.; Evans, F. E.; Yathindra, N.; Sundaralingam, M. *J. Am. Chem. Soc.* **1974**, *96*, 7337.
(26) Howard, F. B.; Frazier, J.; Miles, H. T.; *J. Biol. Chem.* **1975**, *249*, 2987.
(27) Pauling, L.; Corey, R. B. *Proc. Natl. Acad. Sci.* **1953**, *39*, 84.
(28) Olby, R. "The Path to the Double Helix"; University of Washington Press: Seattle, 1974; pp 381–383.
(29) Pimentel, G. C.; McClellan, A. L. In "The Hydrogen Bond"; W. H. Freeman: San Francisco, 1960; p 213.

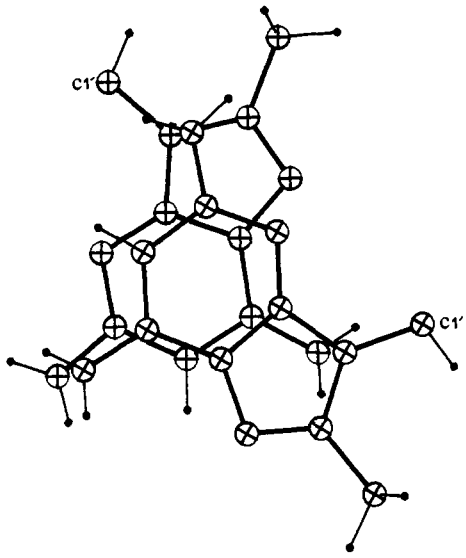

Figure 6. Overlap of two base moieties in the helix.

dielectric constant were appropriate, its value would probably lie between that of water or ice[30a] (80–100) and that appropriate to the alkyl phosphoric esters[30b] (10–20). Other analogues might be KH_2PO_4 (46)[30c] and $NH_4H_2PO_4$ (87 or 21, depending on direction).[30d] For 1 mol of material, two point charges, q_1 and q_2, would have an electrostatic energy of $331.6q_1q_2/(Dr)$ kcal, where D is the dielectric constant and r the distance in Å between the charges. In the triad, assuming opposite charges on N(1) and O(2), there would be attractive forces between each O(3) atom and the three symmetrically related N(1) atoms and repulsive forces between each pair of symmetrically related N(1) and O(3) atoms. If O(3) and N(1) were to have opposite unit point charges and if we were to insert the appropriate distances, we would obtain a total electrostatic energy for an Avogadro number of triads of $3(331.6/D)(-1/11.425 - 1/8.331 - 1/6.068 + 1/7.243 + 1/11.536)$ kcal. For D ranging from 10 to 80, the corresponding energy would range from −14.8 to −1.8 kcal. The significance of the calculation does not lie in the numerical values but in the fact that there is a lowering of energy for any overall dielectric constant, and considering the contribution of the hydrogen bonds, the triad of nucleotides and water molecules could be stable in isolation. The above point-charge electrostatic model is not intended to account for all atomic interactions and it is not necessarily the only model, but it does illustrate that electrostatic energy does not have to disrupt the triad. A similar calculation can also be performed with the negative charge distributed between O(2) and O(3), and here again, although numerical values are smaller, the energy contribution is still negative. We do not maintain that such a hypothetical isolated triad would have exactly the same conformation as in the crystal since a better balance of electrostatic forces might be possible, but an analysis of the forces indicates that the syn conformation could be preserved. The added amino group may be an important factor in stabilizing the syn conformation, since N(2) is intimately involved in the triad, and the anti conformation would require an entirely different packing of molecules. The phosphate hydrogen atom is responsible only for one-fourth of the hydrogen bonding, and thus it is at least possible to speculate that such a water-bridged structure could be stable in a polymer of this nucleotide where the hydrogen atom would not be available.

The second water molecule, S2, is attached by two hydrogen bonds to the atoms of the triad nucleotides, and the remaining five hydrogen bonds are involved in holding the helical structures together. Since the helix has exact 3_1 space-group symmetry, the nearly parallel orientation of the bases to the *ab* plane requires

Table III. Crystal and Experimental Data

molecular formula:	$C_{11}H_{14}N_6O_7P$
habit:	trigonal prismatic, elongation along c
radiation:	Cu Kα (graphite monochromator)
wavelength:	1.5418 Å
space group:	$R3$ (No. 146)
temperature:	23 ± 1 °C
cell dimensions (from least-squares refinement of $\pm\theta$ data)	
$a = b$ =	20.192 (1) Å
c =	11.0849 (7) Å
V =	3914.16 Å^3
Z =	9 (asymmetric unit includes $2H_2O$)
asymmetric unit weight:	409.27 daltons
d_{obsd} =	1.55 (1) g cm^{-3}
d_{calcd} =	1.562 g cm^{-3}
crystal size:	sphere, radius 0.20 mm
absorption factor:	19.84 cm^{-1}
μR:	0.40
reflections:	1694 (21 unobserved 1σ)
maximum $(\sin\theta)/\lambda$:	0.61 Å^{-1}
theoretical resolution of data:	0.54 Å
diffractometer:	Nonius CAD-4
least-squares weighting:	after Peterson and Levy[33]
function minimized:	$\Sigma w\Delta^2$
anisotropic temperature factor:	$\exp[-2\pi^2(\Sigma_i\Sigma_j U_{ij}a_i^*a_j^*h_ih_j)]$

that they should also be nearly parallel to each other. (The angle between the two mean planes is 2.7°.) Figure 6 shows that the overlap of the atoms is considerably greater than that of any of the nucleotides described by Bugg, Thomas, Sundaralingam, and Rao.[31] However, the interplanar spacing is 3.701 Å, in contrast to distances of 3.3–3.4 Å usually observed in base stacking,[31] suggesting a smaller stacking stabilization of the overall structure than the extensive overlap would otherwise produce. Even with the bases as far apart as they are, there are several distances between nonbonded atoms in the helix which correspond to van der Waals contacts, all of which may be considerably shortened if the interplanar spacing were as small as 3.4 Å.

The second water molecule, S2, is an important factor in stabilizing the helices. Like S1, its oxygen atom also achieves tetrahedral coordination. It is involved both as a donor and as an acceptor to N(7) and N(6) in the adjacent nucleotide molecule and forms an acceptor bond to O(2') and a donor bond to O(3) in molecules related by the 3-fold screw. To a major extent the present structure appears to be organized and stabilized by extensive use of water bridges, which bond each molecule to those around it. Intermolecular hydrogen bonds evidently play a lesser role. It is possible that water bridges also play an important role in ordered polynucleotide structures in aqueous solution. NMR observations of sugar hydroxyl groups in glucose,[32] poly(U),[33] and several polynucleotides[34] have been made in water solution and interpreted as evidence of water bridges.[34]

The helix is stabilized further by the N(1)–H···O(3) hydrogen bond and also by the probable electrostatic attraction of N(1) and O(3). The unique O(3')–H···O(1') bond provides a link to molecules related by the other crystallographic screw axis and adds further stabilization. Only one previous observation of a hydrogen bond involving O(1') in a nucleotide has been reported, and in that case, the other atom involved was O(2').[19] In polynucleotide helices, hydrogen bonding of O(2') to O(1') of the adjacent ribose has been proposed as a stabilizing interaction.[33-35]

Finally, we note some interesting differences in bond lengths from those given by Sundaralingam[3] for his fundamental nucleotide unit. The phosphate ester bond is shorter, 1.587 Å as compared to Sundaralingam's value of 1.605 Å, and the C(1')–N(9) bond is also shorter, 1.452 vs. 1.469 Å. Obviously,

(30) "Landolt-Bornstein Tables", 6th Ed.; Hellwege, K. H., Hellwege, A. M., Eds. Springer Verlag: Berlin, 1959; Vol. II, Part 6, pp 453(a); 656(b); 459(c), 463(d).

(31) Bugg, C. E.; Thomas, J. M.; Sundaralingam, M.; Rao, S. T. *Biopolymers* **1971**, *10*, 175.
(32) Harvey, J. M.; Simons, M. C. R.; Naftalin, R. J. *Nature* **1970**, *261*, 435.
(33) Young, P. R.; Kallenbach, N. R. *J. Mol. Biol.* **1978**, *126*, 467.
(34) Bolton, P. H.; Kearns, D. R. *Biochim. Biophys. Acta* **1978**, *517*, 329.
(35) Rabczenko, A.; Sugar, D. *Acta Biochim. Pol.* **1972**, *18*, 387.

adoption of the observed conformation has not introduced any particular bond strain. Bugg, Thomas, Sundaralingam, and Rao[31] comment that the C(1′)–O(1′) bond is usually shorter than the C(4′)–O(1′) bond, which might indicate some double-bond character. In the present case the two bonds are identical within the esd's.

Experimental Section

Crystallographic Work. X-ray experimental details are given in Table III. The basic crystal habit is indicated in the table, although there was also some development of other faces at the end of the needles. As obtained, the crystals were about 4 mm long and had cross-sectional dimensions of about 0.6 mm. The preliminary X-ray examination showed that the crystals possessed a rhombohedral primitive cell with no systematic absences and X-ray symmetry 3. Two space groups are thus possible: $R\bar{3}$ and $R3$, but since the density corresponds to nine molecules in the hexagonal unit cell (which is used throughout this paper), the space group must be $R3$. The crystals were quite stable mechanically, and it was possible to grind a fairly large sphere for data collection allowing measurement of X-ray intensities such that only 21 of the 1694 independent reflections had I less than 1σ. Three standard reflection intensities, measured after each period of 3-h X-ray exposure, gave no evidence of significant radiation damage.

Solution of the phase problem was carried out using the MULTAN78[37] set of programs. The presence of the phosphorus atom made the various tests less selective than usual, but with the tests ABSFOM, PSIZERO, and RKARLE weighted 0.5:1.5:1.0, the solution with the best overall figure of merit proved to be correct. In the nucleotide itself, only the carbon atom of the methyl group was missing from the E map. Although when the X-ray work commenced the degree of hydration of the crystals was unknown, the crystal density was compatible with the presence of two molecules of water for each nucleotide molecule, and the two oxygen atoms of the water molecules and the methyl carbon atom were readily found by standard sequences of least-squares refinements and difference maps (all calculations, except for those otherwise indicated, used the XRAY72[38] series of programs). All hydrogen atoms expected were found. Final least-squares refinement utilized anisotropic thermal parameters for the heavier atoms and isotropic parameters for the hydrogen atoms. Dispersion corrections[39] were applied to the scattering factors of the nonhydrogen atoms. It was apparent that extinction was affecting the intense low-angle reflections, and when the refinement had converged at an R factor of 3%, an extinction correction of the Zachariasen[40] type was evaluted by using MLAB.[41] The data were corrected for extinction and a spherical absorption correction was also applied by using local programs. The resulting data set refined to the very low R factor of 2.3%, although no bond lengths changed significantly from the results before the application of the corrections. The precision of this structure, in terms of R factor and esd's, is the highest yet attained for an X-ray structure of a nucleotide, and given the low temperature factors and the high-quality corrected data, the structue should also be of high accuracy. Final atomic parameters are given in Table IV. Anisotropic temperature factors and a table of observed and calculated structure factors are available. (See paragraph at end of paper regarding supplementary material.)

Synthesis of Nucleotide. 8-Methyl-2-amino-5′-adenylic acid was synthesized by the following series of reactions: 8-methylguanosine (I) → tri-O-acetyl-8-methylguanosine (II) → 2-amino-6-chloro-8-methyl-(tri-O-acetyl-β-D-ribofuranosyl)purine (III) → 2-amino-8-methyladenosine (IV) → 2-amino-8-methyladenosine monophosphate (V). The following reaction conditions and purification methods were used, and the following properties of the compounds were observed.

I → II. Acetic anhydride in pyridine, 65°, 1 h. II recrystallized from EtOH: mp 212–215 °C; UV in 50% EtOH, max 253.8, 275 nm (sh). Anal.: C, 48.04, H, 5.03, N, 16.54.

Table IV. Atomic Parameters[a]

atom	x/a	y/b	z/c	U
P	15944 (2)	8879 (2)	89237	302
O(1)	1123 (1)	1145 (1)	9716 (1)	471
O(2)	1155 (1)	46 (1)	8641 (1)	392
O(3)	2358 (1)	1178 (1)	9512 (1)	413
O(4)	1695 (1)	1288 (1)	7652 (1)	338
O(S1)	747 (1)	−175 (1)	6062 (2)	487
O(S2)	5779 (1)	662 (1)	5182 (2)	652
N(1)	2918 (1)	−660 (1)	5733 (2)	334
C(2)	2458 (1)	−337 (1)	5698 (2)	315
N(2)	1709 (1)	−829 (1)	5634 (2)	388
N(3)	2720 (1)	409 (1)	5720 (1)	297
C(4)	3486 (1)	814 (1)	5766 (2)	282
C(5)	3998 (1)	550 (1)	5779 (2)	319
N(6)	4096 (1)	−599 (1)	5709 (2)	463
C(6)	3699 (1)	−240 (1)	5737 (2)	342
N(7)	4746 (1)	1152 (1)	5812 (2)	346
C(8)	4686 (1)	1768 (1)	5804 (2)	291
C(Me)	5348 (1)	2563 (1)	5856 (2)	377
N(9)	3929 (1)	1598 (1)	5766 (1)	270
C(1′)	3653 (1)	2135 (1)	5649 (1)	270
O(1′)	3303 (1)	2152 (1)	6766 (1)	331
O(2′)	3332 (1)	2587 (1)	3867 (1)	360
C(2′)	3055 (1)	1942 (1)	4648 (1)	272
O(3′)	1873 (1)	2029 (1)	4702 (1)	401
C(3′)	2344 (1)	1819 (1)	5341 (1)	268
C(4′)	2692 (1)	2295 (1)	6473 (2)	288
C(5′)	2158 (1)	2102 (1)	7529 (2)	353
		Hydrogen Atoms		
O(1)	65 (2)	101 (1)	943 (2)	55 (7)
O(S1)	27 (2)	−53 (2)	603 (3)	89 (12)
O(S1)′	76 (2)	−14 (2)	704 (4)	99 (14)
O(S2)	587 (3)	65 (3)	447 (5)	115 (17)
O(S2)′	558 (2)	99 (2)	537 (4)	94 (13)
N(1)	267 (1)	−117 (1)	586 (2)	48 (7)
N(2)′	157 (1)	−129 (1)	544 (2)	45 (6)
N(2)	137 (2)	−67 (1)	564 (2)	53 (8)
N(6)	391 (1)	−101 (2)	555 (2)	51 (8)
N(6)′	455 (2)	−34 (2)	556 (2)	56 (8)
C(Me)	536 (2)	283 (2)	657 (4)	95 (13)
C(Me)′	588 (2)	261 (2)	572 (3)	90 (12)
C(Me)″	535 (2)	284 (2)	512 (4)	90 (13)
C(1′)	407 (1)	261 (1)	547 (2)	32 (5)
O(2′)	314 (1)	239 (1)	319 (3)	51 (7)
C(1′)	296 (1)	151 (1)	415 (2)	26 (5)
O(3′)	216 (2)	247 (2)	441 (2)	55 (7)
C(3′)	205 (1)	132 (1)	555 (1)	24 (4)
C(4)	290 (1)	282 (1)	625 (2)	31 (5)
C(5)	184 (1)	228 (1)	738 (2)	36 (6)
C(5)′	242 (1)	237 (1)	822 (2)	40 (6)

[a] The positional parameters for nonhydrogen atoms are multiplied by 10 000, except for those of P where an extra factor of 10 is applied, and the thermal parameters by 10 000. The geometric mean of the diagonal terms of the vibration tensor is given as U for the heavier atoms. The actual anisotropic parameters are available as supplemental material. All parameters for hydrogen atoms are multiplied by 1 000. Hydrogen atoms are designated by the heavier atom to which they are attached.

II → III. II (5.5 g) added to 37.5 mL of $POCl_3$ and 0.9 mL of diethylaniline at 25 °C. Reflux 3 min. Evaporate $POCl_3$, add ice water, and extract with $CHCl_3$. III was a glass, single spot on TLC ($CHCl_3$, EtOH, 30:1), R_f (0.85; recrystallized from EtOH: mp 147 °C, UV in 50% EtOH, max 251.3, (10 200), 311.3 nm (8400). Anal.: C, 46.01, N, 15.60, H, 4.49, Cl, 8.05.

III → IV. Add III to 100 mL of EtOH saturated with NH_3 (0 °C) and cool with dry ice/acetone. Place in steel bomb; heat 7 h at 100 °C. Evaporate solvent. Recrystallize from hot water. IV: mp 275–276 °C; TLC (cellulose) single spot R_f 0.58 (BuOH, AcOH, H_2O 5:2:3); UV in 0.05 M phosphate buffer, pH 6.8, max 257 (11 000), 281.3 nm (10 200); in 0.01 M HCl, max 253.7 (12 400), 293.5 nm (9980).

IV → V. IV (1.1 g) stirred in 11 mL of $(MeO)_3PO$ and 0.55 mL of $POCl_3$ at 0 °C for 5 h. Store 16 h at 5 °C. Add 0.15 mL of $POCl_3$. After 2 h at 0 °C, pour into 30 mL of ice water, neutralize with 1 N NH_4OH, and apply to a AG 1-X2 (formate) column. Wash with 1 L of 0.05 M HCO_2H and elute with linear gradient of 0.05 M HCO_2H and

(36) Peterson, S. W.; Levy, H. A. *Acta Crystallogr.* **1957**, *10*, 70.
(37) Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J-P.; Woolfson, M. M. MULTAN78; Universities of York and Louvain, 1978. A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data.
(38) Stewart, J. M.; Kruger, G. J.; Ammon, H. L.; Dickinson, C.; Hall, S. R. Computer Center, University of Maryland, 1972, XRAY72 Technical report TR-192.
(39) "International Tables for X-Ray Crystallography", Kynoch Press: Birmingham, England, 1974, Vol. IV, p 149
(40) Zachariasen, W. H. *Acta Crystallogr.* **1963**, *16*, 1139.
(41) Knott, G. D.; Shrager, R. I. "On-line Modeling by Curve-fitting; Computer Graphics: Proceedings of the SIGGRAPH Computers in Medicine Symposium, 1972", ACM, SIGGRAPH Notices, Winter; Association for Computing Machinery: Chicago, 1972; Vol. 6, No. 4, pp 138–151.

0.2 M HCO_2H. Lyophilize the nucleotide fraction and recrystallize from hot water to give 886 mg of colorless crystals: cellulose TLC (BuOH, AcOH, H_2O 5:2:3) R_f 0.23; UV in 0.05 M phosphate buffer, pH 6.8, 257.5 (10100), 281.2 nm (9930); in 0.01 M HCl, max 253.8 (11300), 292.5 nm (9200).

Registry No. I, 36799-17-4; II, 80326-48-3; III, 80326-49-4; IV, 80326-50-7; $V{\cdot}2H_2O$, 80326-51-8.

Supplementary Material Available: A table of observed and calculated structure factors and full refinement parameters for the heavier atoms (13 pages). Ordering information is given on any current masthead page.

Kinetics of Model Threonine Aldolase Reactions‡

Joseph A. Marcello† and Arthur E. Martell*

Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received February 27, 1981*

Abstract: Kinetics of dealdolation of Schiff bases of pyridoxal and β-hydroxy amino acids and of their Zn(II), Al(III), and Ga(III) chelates are reported. Disappearance of reactants and appearance of products of the metal-free model systems, and their complexes consisting of 1:1:1 and 2:2:1 molar ratios of pyridoxal:amino acid:metal, were followed by proton NMR. The rates of dealdolation were determined for threonine, β-hydroxyvaline, and β-hydroxyleucine and the corresponding first-order rate constants are reported. Under comparable conditions the rates of Al(III) and Ga(III) chelate catalysis were found to be 2–6 times higher than those of the metal-free systems and of the corresponding Zn(II) Schiff base chelates. The rates of dealdolation of the Zn(II) chelates were approximately equivalent to the rates observed for the proton-catalyzed (metal free) Schiff bases. Catalysis in the 1:1 chelates was found to be stronger than the catalysis observed in the Schiff base chelates having a 2:1 ligand–metal ratio. The electron-donating effect of the methyl groups of β-hydroxyvaline is evident in that dealdolation is observed for the metal-free systems containing β-hydroxyvaline, but not for the analogous L-threonine systems. A reaction mechanism for the dealdolation reaction in these model systems is proposed. The rate constants obtained for the metal-free β-hydroxyvaline–pyridoxal Schiff base were resolved into the specific rate constants for the individual solution components, and the variations obtained for the specific rate constants are interpreted in terms of the proposed reaction mechanism.

Threonine is converted to glycine and acetaldehyde, and serine is converted to glycine and formaldehyde, respectively, in pyridoxal-activated enzyme systems.[1-3] The reaction has been shown to take place with pyridoxal and metal ions albeit at slower rates.[4-8] This reaction also occurs with β-hydroxy amino acids that do not have an α-hydrogen atom.[9]

Two mechanisms have been proposed[9,10] for these model system reactions. The Snell mechanism[9] suggests that the reaction proceeds through direct α–β carbon–carbon cleavage of the reactive Schiff base chelate. Although it was shown that α-methylserine reacts to give α-alanine and formaldehyde under conditions similar to those under which serine and threonine react to give formaldehyde and acetaldehyde, respectively, it has not been demonstrated that the reaction does not proceed at least in part through an α-deprotonated intermediate (in the absence of an α substituent), as has been suggested by Braunstein.[10] In the absence of quantitative kinetic studies, it has not been possible to propose with confidence a reaction mechanism for these model reactions.

This paper reports the first detailed kinetic measurements of the pyridoxal and pyridoxal–metal-ion-catalyzed dealdolation of threonine, β-hydroxyleucine, and β-hydroxyvaline. The rate constants of these model system reactions have now been determined and a probable reaction mechanism is proposed.

Experimental Section

Materials. Pyridoxal hydrochloride was obtained from Mann Laboratories as Mann Analyzed grade and was used without further purification. The following amino acids were obtained from the sources indicated: DL-threonine and DL-serine from Sigma Chemical Co., α-methylserine from Calibochem; L-threonine from Mann Laboratories; β-hydroxyleucine from United States Biochemical; and β-hydroxyvaline from Calbiochem and Dr. T. Wilkinson, Department of Chemistry, Texas A&M University. All of these amino acids were of sufficient quality to use without further purification.

The deuterium oxide used as a solvent for this study was obtained from Aldrich Chemical Co. and was specified as 99.8% deuterium. The aluminum(III) solutions were prepared by dissolving hydrated $Al_2(SO_4)_3$ in D_2O and evaporating to dryness. This procedure was repeated several times to remove residual H_2O. The standard Al(III) solutions were prepared from the deuterated material by dilution to the appropriate concentration with D_2O. The standard Zn(II) solutions were prepared from hydrated $Zn(NO_3)_2$ by a procedure similar to that employed for the Al(III) solutions. The Al(III) and Zn(II) solutions were standardized by conventional chelatometric titrations.[11] The standard Ga(III) solution was prepared by dissolving gallium metal in DCl (20%) and diluting to the appropriate volume. The ionic strengths of the samples used in the measurements were maintained at unity with reagent grade KCl. All of the solutions were initially 0.1000 M in both pyridoxal hydrochloride and amino acid. In the 1:1:1 systems (pyridoxal–amino acid–metal ion) the concentration of the metal ion was 0.1000 M and in the 2:2:1 systems it was 0.0500 M.

Procedures. The NMR spectra were obtained with a Varian HA-100 nuclear magnetic resonance spectrophotometer and were measured at the ambient probe temperature (30 ± 2 °C). The chemical shifts are given

‡This work was supported by research Grant No. AM-11694 from the National Institute of Arthritis, Metabolism and Digestive Diseases, U.S. Public Health Service.

†Abstracted in part from a dissertation submitted to the Faculty of Texas A&M University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December 1979.

(1) Meister, A. "Biochemistry of the Amino Acids"; Academic Press: New York, 1957.
(2) Gilbert, J. B. *J. Am. Chem. Soc.* **1957**, *79*, 2242.
(3) Karasek, M. A.; Greenberg, G. M. *J. Biol. Chem.* **1957**, *191*, 227.
(4) Metzler, D. E.; Ikawa, M.; Snell, E. E. *J. Am. Chem. Soc.* **1954**, *76*, 639.
(5) Metzler, D. E.; Snell, E. E. *J. Am. Chem. Soc.* **1953**, *75*, 2786.
(6) Longenecker, J. B.; Snell, E. E. *J. Biol. Chem.* **1957**, *225*, 409.
(7) Abbott, E. H.; Martell, A. E. *J. Chem. Soc., Chem Commun.* **1968**, 1501.
(8) Marcello, J. A.; Martell, A. E.; Abbott, E. H. *J. Chem. Soc., Chem. Commun.* **1975**, 16.
(9) Longenecker, J. B.; Ikawa, M.; Snell, E. E. *J. Biol. Chem.* **1957**, *227*, 663.
(10) Braunstein, A. E.; Shemyakin, M. M. *Biokhimiya* **1953**, *18*, 393.
(11) Schwarzenbach, G. "Complexometric Titrations"; Interscience: New York, 1957.

0002-7863/82/1504-1087$01.25/0