Conformational Barriers in Nucleoside Analogs: The Crystal Structure of 3-Deazaadenosine

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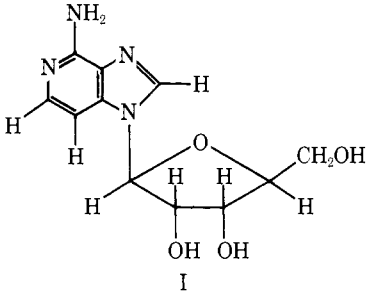
Abstract: The crystal and molecular structure of 3-deazaadenosine ($C_{11}H_{14}N_4O_4$) has been determined from three-dimensional x-ray data collected by counter methods. The nucleoside analog crystallizes in space group $P2_12_12_1$ of the orthorhombic system with four formula units in a cell of dimensions a = 4.637 (2), b = 12.549 (6), and c = 19.878 (10) Å. The observed and calculated densities are 1.54 (2) and 1.535 g cm^{-3}, respectively. The structure was refined by full-matrix least-squares methods to a conventional R factor of 0.039 using 976 independent intensities. The solid-state conformation around the glycosyl linkage is anti, with a χ value of +4.5°, and the conformation around the extracyclic bond C(4′)–C(5′) is gauche–gauche; while this latter conformation is the one most commonly observed in nucleoside structures, in the related molecule adenosine the conformation is gauche–trans. The sugar has the C(3′)-endo envelope (^{3}E) conformation. CNDO/2 molecular orbital calculations of the internal energy of the nucleoside as a function of glycosyl torsional angle, χ_{CN}, suggest that the observed solid-state χ_{CN} value of +4.5° may not be the lowest energy conformation for the isolated molecule, since the global energy minimum occurs in the syn range at χ_{CN} = 168°; the solid-state conformation, however, is calculated to lie less than 1 kcal/mol higher in energy than this global minimum. The analysis shows several energetically unfavorable torsional regions, and the sources of these rotational barriers have been shown to be intramolecular van der Waals contacts between sugar and base atoms.

Nucleoside analogs, in which the heterocyclic base has been modified, have shown considerable biological activity against microbial and tumor cells in vitro.[2a,b] One such class of nucleosides is the deaza derivatives of adenosine in which a nitrogen atom on the adenine base is formally replaced by a CH group. The best known examples of this class are the powerful antibiotic tubercidin (7-deazaadenosine; see Figure 1 for the numbering system) and its derivatives toyocamycin (7-cyanotubercidin) and sangivamycin (7-amidotubercidin). The crystal structure of tubercidin has been reported,[3,4] and it has been postulated[3] that the lack of hydrolysis of the glycosyl bond of this nucleoside may be directly related to the quite significant shortening of the glycosyl bond in tubercidin as compared with that in the corresponding natural nucleoside, adenosine.

Another potentially useful deaza derivative of adenosine is obtained by replacing the nitrogen at the 3 position of adenosine by a CH group, thus yielding 3-deazaadenosine, (I).[5,6] Since it has been proposed[7] that N(3) of adenosine at



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the -CCA end of tRNA may be playing an important role in the process of protein biosynthesis by acting as a hydrogen-bond acceptor, its absence in 3-deazaadenosine provides a fraudulent analog which, if incorporated in the -CCA terminus of tRNA, could produce a tRNA molecule incapable of accepting an amino acid. There are, however, no biochemical data available for 3-deazaadenosine to indicate that the nucleoside is actually incorporated in the tRNA. In order to provide a structural basis for the discussion of the possible role of 3-deazaadenosine in biochemical reactions, we have carried out an x-ray crystallographic investigation, a molecular orbital calculation of atomic charge densities, and a detailed conformational analysis of the rotational barrier around the glycosyl bond, using both the molecular orbital method and the van der Waals contact method.

Experimental Section

X-Ray Data Collection. Colorless crystals of 3-deazaadenosine were obtained by dissolving a small quantity in an ethyl acetate–methanol mixture and evaporating it at room temperature. The needle-shaped crystals were all of low quality and had a rather curious shape in that they were tapered with one end of the needle thinner than the other. One such crystal was cut in the middle and the thicker of the two fragments, which also showed the least amount of tapering, was used for data collection. The size of the fragment was approximately 0.40 × 0.15 × 0.15 mm. After preliminary Weissenberg and precession photography to determine the space group and approximate cell constants, the crystal was transferred to a Picker 4-Circle automatic diffractometer equipped with a copper tube, a scintillation counter and a pulse-height analyzer. The cell constants were determined by a least-squares refinement procedure[8] using 12 reflections which had been centered accurately with a narrow source at a tube take-off angle of 1.0°. The intensities were measured at a take-off angle of 3.0° by the θ–2θ scan technique at a scan rate of 1°/min with a stationary background count of 40 s on each side of a peak, which was scanned from 1.5° below the calculated α_1 peak position to 1.5° above the calculated α_2 peak position. The crystal centering and the stability of the instrument electronics were monitored by measuring three standard reflections after every 100 measurements. The data were collected out to a maximum 2θ of 124° and processed by the method of Corfield, Doedens, and Ibers[9] as described elsewhere.[10] In this procedure a standard deviation, σ, is assigned to each observation based on counting statistics with a term included to reduce the weights assigned to the strong reflections. The quality of the crystal fragment used was rather low but was judged to be marginally acceptable.

Solution and Refinement. The structure was solved by direct methods[11] with the aid of the multiple solution computer program, MULTAN,[12] using a total of 165 normalized structure factors, E, with magnitudes greater than 1.40. All the 19 nonhydrogen atoms were located from an E map of the set with the lowest R_{Karle} of 0.20 and the second highest figure of merit, FOM, of 1.1195. The refinement of the atomic parameters was carried out using NUCLS, which is Ibers' version of the full-matrix least-squares program of

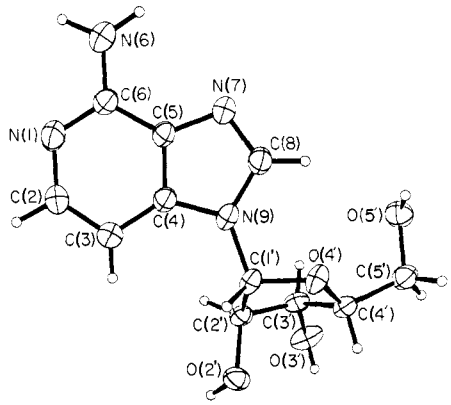

Figure 1. A computer drawing (C.K. Johnson, Oak Ridge National Laboratory, Oak Ridge, Tenn., Report No. ORNL-3794) of the 3-deazaadenosine molecule viewed along the normal to the planar base moiety. Hydrogen atoms are shown as small open circles and nonhydrogen atoms with 50% probability thermal ellipsoids.

Busing, Martin, and Levy;[13] the refinement was carried out on F, the function minimized being $\Sigma w(|F_o| - |F_c|)^2$. The weights, w, were taken as $w = 4F_o^2/\sigma^2(|F_o|)$.[2] The atomic scattering factors for C, N, and O were taken from the "International Tables for X-Ray Crystallography" [14] and those for H from Stewart, Davidson, and Simpson.[15] The hydrogen atoms were located from a difference Fourier synthesis performed after three cycles of anisotropic refinement of the nonhydrogen atoms. The hydrogen atoms were refined with isotropic temperature factors. Inspection of the observed and calculated structure amplitudes near the end of the refinement indicated that there was significant error in the data due to secondary extinction. A correction for secondary extinction was, therefore, applied in the manner described by Zachariasen.[16] The refined value of the extinction coefficient was $1.9(3) \times 10^{-7}$. No parameter shift in the final cycle of refinement was greater than 0.4 times its estimated standard deviation (esd) and, with the exception of those of O(3') and of H(O3') none was greater than 0.1 times its esd. A difference Fourier synthesis calculated at the end of the refinement by substracting out all 31 atoms in the asymmetric unit revealed no significant residual electron density.

The final agreement factors $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $R_w = (\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2)^{1/2}$ were 0.039 and 0.044, respectively.

Molecular Orbital Calculations. The MO calculations were performed by the CNDO/2 procedure of Pople and co-workers[17] with the Quantum Chemistry Program Exchange (QCPE) Program No. 141 (obtainable from the University of Indiana, Bloomington, Ind.). The geometry assumed for the molecule was that observed in the x-ray diffraction study with the exception that the C-H, N-H, and O-H bond lengths, which are normally observed to be too short in the x-ray studies, were changed to 1.08, 1.01, and 0.97 Å, respectively, keeping their bond directions unchanged. Calculations were performed at intervals of 30° in χ_{CN} as the ribose was rotated with respect to the base around the glycosyl bond N(9)-C(1'), except in the vicinity of the global energy minimum and the first local minimum where two extra calculations, 15° on each side of the minimum, were performed in order to define the position of the minimum more accurately.

Intramolecular Contact Method. Interatomic distances between selected pairs of atoms on the base and on the ribose were calculated as a function of the glycosyl torsion angle,[18] χ_{CN},[19] in a manner analogous to that of Haschemeyer and Rich.[20] The main base-sugar contacts for 3-deazaadenosine, a C(3')-endo sugar, involve[20] C(3) and HC(3) on the base with the atoms HC(2'), HC(3'), and O(4') on the sugar, and HC(8) on the base with HC(2') and HC(3') on the sugar. For other sugar puckers (e.g., the C(2')-endo-C(1')-exo found in 2-thio- and 2-chlorobenzimidazole nucleosides[21,22]), the intramolecular contacts would, necessarily, involve other atoms.[20]

Results

The cell constants and other pertinent crystallographic data are presented in Table I. The positional and thermal parameters for all atoms in the 3-deazaadenosine molecule, along with their esd, are presented in Tables IIA and IIB. The observed and calculated structure factors are given in Table III (see paragraph at the end of paper regarding supplementary material). A computer drawing of the molecule is shown in Figure 1. The bond lengths and bond angles, associated with the nonhydrogen atoms of the molecule, are depicted in Figure 2, and those associated with the hydrogen atoms are presented in Tables IV and V, respectively (see paragraph at end of paper regarding supplementary material). The deviations of atoms from their least-squares planes for the nine-atom 3-deazapurine moiety and the best four-atom plane of the ribose moiety are given in Table VI.

Table I. Crystallographic Data for 3-Deazaadenosine

Chemical formula: $C_{11}N_4O_4H_{14}$	Mol wt = 226.3
λ(Cu Kα) = 1.5405 Å	Space group: $P2_12_12_1$ (orthorhombic)
a = 4.637 (2), b = 12.549, c = 19.878 (10) Å	
U = 1156.7 Å^3 Z = 4	D_c = 1.535 g cm^{-3}
D_m = 1.54 (2) g cm^{-3}, by flotation in CCl_4–benzene solution	
Number of reflections >3σ = 976	Cu Kα(Ni-filtered)
R = 0.039	R_w = 0.044

The conformational energy map for rotation around the glycosyl bond by the CNDO/2 method is shown in Figure 3, and the variation of intramolecular distances for the same rotation is shown in Figure 4.

Discussion

Geometry of the Heterocyclic Base. The bond lengths in the heterocyclic ring of 3-deazaadenosine (Figure 2) are indicative of a highly conjugated system with considerable delocalization of the π-electrons. A comparison of the bond lengths with those in the parent nucleoside, adenosine,[23] indicates that the major change occurs, as expected, at position 3, which is the site of substitution, since it involves a change in bond type from C-N to C-C. Thus, the two C-C bonds, C(2)-C(3) and C(3)-C(4), in 3-deazaadenosine are longer than the two similarly situated C-N bonds, C(2)-N(3) and N(3)-C(4), in adenosine, by 0.041 and 0.051 Å, respectively. These changes presumably reflect the radius difference between N and C (0.04 Å). The only other significant difference in bond length, in this part of the molecule, between 3-deazaadenosine and adenosine is in the exocyclic bond C(6)-N(6), which at 1.352 Å is longer in the former by 0.020 Å.

The changes in the bond angles in the heterocyclic base occurring due to the 3-deaza substitution are depicted in Figure 5, where the two bases are superimposed with their N(9) atoms and the glycosyl bonds N(9)-C(1') (projected onto the plane of the base in the case of 3-deazaadenosine in which C(1') is out of the plane by 0.14 Å) in exact superposition. It is immediately obvious that the shape of the six-membered ring has changed considerably with only minor modifications in the five-membered imidazole ring. The actual differences (3-deazaadenosine-adenosine) in the internal ring angles in the six-membered ring at N(1), C(2), C(3), C(4), C(5), and C(6) are 0.0, −2.7, +3.4, −5.5, +3.2, and +1.6°, respectively. The net effect is to move the six-membered ring slightly away from the sugar moiety so that the expected interactions between the hydrogen atom attached to C(3) and the atoms of the sugar moiety are slightly relieved. An important consequence of the ring distortion is the displacement in the positions of atoms N(1) and N(6) in the 3-deaza analog (dotted portion in Figure 5) relative to their positions in adenosine. The amount of this displacement is approximately 0.2 Å for both N(1) and

Table IIA. Positional and Thermal Parameters ($\times 10^4$) for Nonhydrogen Atoms

Atom	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	−2147 (7)	2328 (3)	2094 (1)	405 (17)	65 (2)	15 (1)	−20 (6)	7 (3)	5 (1)
C(2)	−4050 (10)	1511 (3)	2079 (2)	456 (21)	68 (3)	13 (1)	−36 (8)	−4 (4)	1 (1)
C(3)	−5295 (9)	1031 (3)	2626 (2)	362 (19)	56 (3)	16 (1)	−35 (7)	−6 (4)	2 (1)
C(4)	−4427 (7)	1466 (3)	3243 (2)	274 (17)	38 (2)	14 (1)	−2 (6)	2 (3)	3 (1)
C(5)	−2447 (8)	2282 (3)	3283 (2)	271 (17)	36 (2)	13 (1)	3 (6)	0 (4)	5 (1)
C(6)	−1305 (8)	2723 (3)	2688 (2)	283 (16)	40 (2)	15 (1)	−2 (6)	1 (4)	3 (1)
N(6)	537 (8)	3558 (3)	2694 (2)	538 (22)	68 (3)	17 (1)	−55 (7)	16 (4)	4 (1)
N(7)	−1941 (7)	2560 (2)	3950 (1)	365 (15)	50 (2)	14 (1)	−18 (5)	6 (3)	2 (1)
C(8)	−3641 (8)	1936 (3)	4293 (2)	357 (18)	41 (2)	14 (1)	−1 (6)	4 (4)	2 (1)
N(9)	−5181 (7)	1239 (2)	3902 (1)	314 (14)	43 (2)	12 (1)	−2 (5)	7 (3)	5 (1)
C(1')	−7417 (8)	481 (3)	4106 (2)	221 (15)	40 (2)	12 (1)	8 (5)	12 (3)	4 (1)
C(2')	−6561 (7)	−673 (3)	4005 (2)	188 (16)	43 (2)	15 (1)	−15 (6)	13 (3)	−2 (1)
O(2')	−9125 (5)	−1260 (2)	3897 (1)	243 (12)	52 (2)	19 (1)	−17 (4)	10 (3)	−9 (1)
C(3')	−5265 (8)	−932 (3)	4687 (2)	187 (15)	33 (2)	20 (1)	−3 (5)	4 (4)	4 (1)
O(3')	−4958 (7)	−2030 (2)	4825 (2)	239 (13)	38 (2)	33 (1)	17 (4)	13 (3)	10 (1)
C(4')	−7302 (8)	−353 (3)	5154 (2)	207 (16)	39 (2)	15 (1)	−10 (6)	2 (3)	7 (1)
O(4')	−7983 (5)	620 (2)	4801 (1)	293 (12)	38 (1)	14 (1)	26 (4)	16 (2)	4 (1)
C(5')	−6226 (9)	−96 (3)	5852 (2)	306 (18)	50 (2)	16 (1)	−20 (7)	−1 (4)	6 (1)
O(5')	−3763 (6)	569 (2)	5861 (1)	284 (12)	63 (2)	21 (1)	−16 (5)	−4 (3)	−3 (1)

Table IIB. Positional and Thermal Parameters for Hydrogen Atoms

	x/a	y/b	z/c	B
HC(2)	−0.463 (11)	0.126 (3)	0.162 (2)	5 (1)
HC(3)	−0.640 (9)	0.042 (3)	0.262 (2)	5 (1)
H1N(6)	0.131 (9)	0.377 (3)	0.227 (2)	4 (1)
H2N(6)	0.115 (9)	0.385 (3)	0.310 (2)	3 (1)
HC(8)	−0.376 (12)	0.190 (3)	0.478 (2)	5 (1)
HC(1')	−0.923 (8)	0.061 (2)	0.382 (2)	2 (1)
HC(2')	−0.515 (8)	−0.076 (3)	0.361 (2)	2 (1)
HO(2')	−0.870 (10)	−0.185 (3)	0.361 (2)	5 (1)
HC(3')	−0.355 (9)	−0.060 (3)	0.476 (2)	3 (1)
HO(3')	−0.650 (13)	−0.228 (5)	0.488 (3)	7 (2)
HC(4')	−0.893 (10)	−0.077 (3)	0.524 (2)	3 (1)
H1C(5')	−0.584 (8)	−0.073 (3)	0.611 (2)	3 (1)
H2C(5')	−0.764 (9)	0.027 (3)	0.617 (2)	2 (1)
HO(5')	−0.459 (11)	0.126 (4)	0.585 (2)	6 (1)

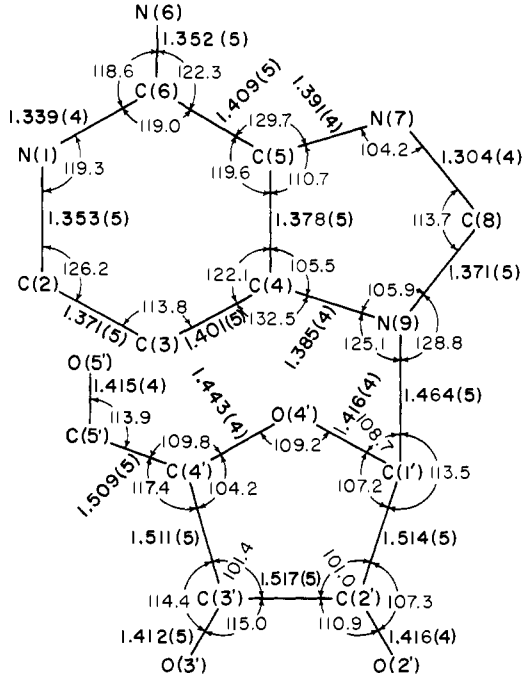

Figure 2. Line drawing of the 3-deazaadenosine molecule showing the bond lengths and bond angles associated with the nonhydrogen atoms.

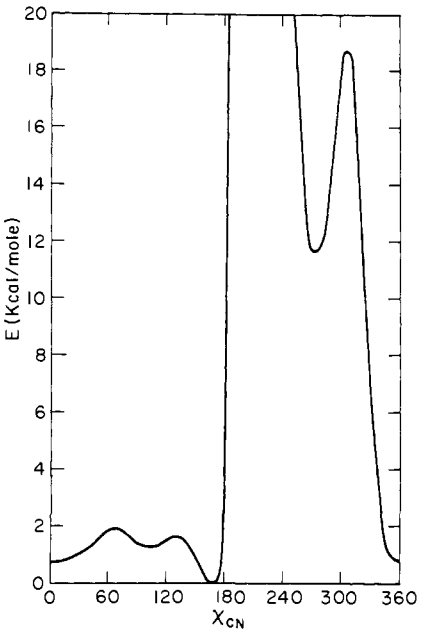

Figure 3. Conformational energy map calculated by the CNDO/2 MO method for rotation around the glycosyl bond N(9)-C(1') for 3-deazaadenosine. The angle χ_{CN} is that defined by Sundaralingam.[19] The conformation around the C(4')-C(5') bond was fixed at the crystallographically observed value (gauche-gauche). The energy of the global minimum is taken as zero. The crystallographically observed χ_{CN} angle is +4.5°. The height of the large barrier near χ_{CN} = 230° is calculated to be >65 kcal/mol.

N(6). In view of the involvement of both of these atoms in the Watson-Crick base-pairing scheme in nucleic acids, it would appear that the incorporation of 3-deazaadenine in these macromolecules could affect the strength of the pairing interaction. It is noteworthy, however, that the replacement of N(3) by CH results in an enhanced basicity of N(1)[5] and also the loss of the potential binding site N(3). In a recent study, Ohtsuka et al.[24] observed a slightly decreased stimulation by poly(3-deazaadenosine) of the binding of [^{14}C]Lys-tRNA to ribosomes, which could be attributed to a cumulative effect of all of the above factors.

The nine-atom heterocyclic base is planar within experimental error, as seen from Table VI, with no atom deviating from the least-squares plane by more than 0.02 Å. It should be noted, however, that the C(1') of the ribose is displaced from this plane by 0.14 Å; this result is different from that observed in adenosine,[23] where it is lying precisely in the plane of the heterocycle. A displacement of C(1') from the heterocyclic plane is sometimes observed for nucleosides and nucleotides. It may also be of interest to note that the

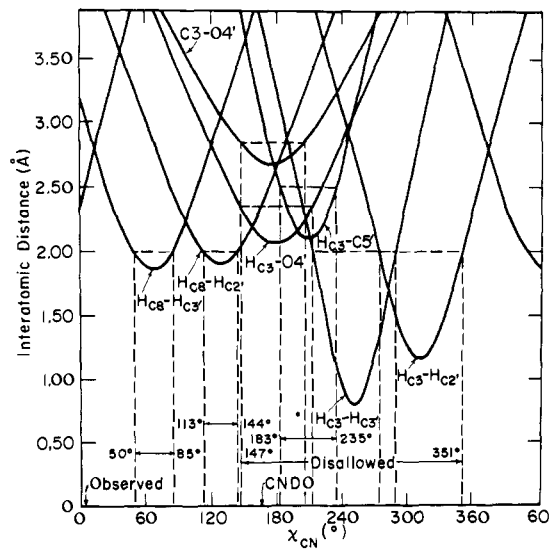

Figure 4. Intramolecular van der Waals interactions between selected sugar and base atoms as a function of χ_{CN}.[19] The horizontal dashed lines on each curve indicate the intramolecular van der Waals contact limit[20] (see text). Regions of severe contact and the disallowed regions are indicated by double-headed arrows between vertical dashed lines. Contacts between C(3) and the sugar atoms are omitted for clarity since they are generally similar to those between HC(3) and the sugar atoms.

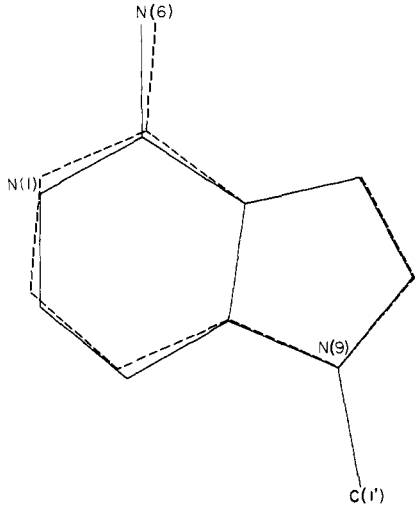

Figure 5. Comparison of the geometry of the base in adenosine (solid line) and 3-deazaadenosine (dashed line).

hydrogen atom HC(3) is displaced from the heterocyclic plane by the same amount as C(1'), i.e., 0.14 Å, but in the opposite direction.

Geometry and Conformation of the Ribose. The conformation of the ribose is C(3')-endo envelope (^{3}E)[25] since, as is evident from Table VI, C(3') is displaced in the same direction as C(5') from the plane formed by the remaining four atoms C(1'), C(2'), C(4'), and O(4'). The bond lengths and bond angles are in good agreement with the values observed for a C(3')-endo puckered ribose.[26b,27] As usual, the C(4')-O(4') bond is slightly longer than the C(1')-O(4') bond.

The conformation of the C(5')-O(5') bond around the C(4')-C(5') bond is gauche-gauche, the dihedral angles O(5')-C(5')-C(4')-O(4') and O(5')-C(5')-C(4')-C(3') being 58.2 and 60.5°, respectively. This conformation, which makes the O(5') atom lie on "top" of the sugar ring, is a commonly observed conformation for nucleosides in the crystalline state. The parent nucleoside, adenosine,[23] however, has the gauche-trans conformation.

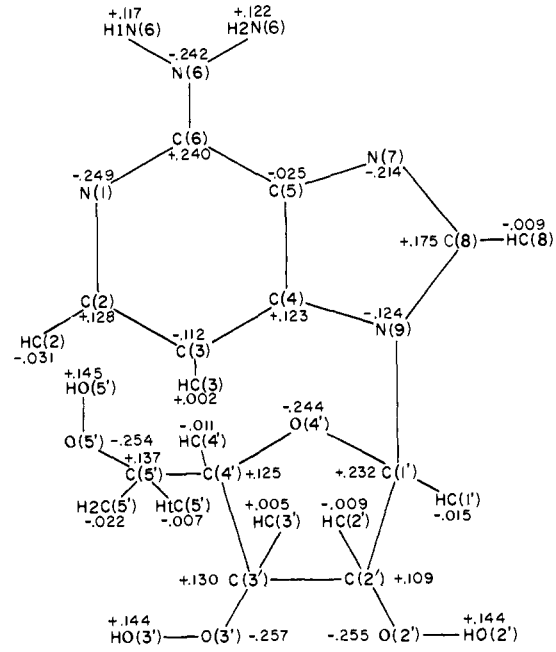

Figure 6. Net atomic charges, (total atomic electron density minus nuclear charge), in e, for the crystallographically observed conformation of 3-deazaadenosine.

Net Charge Density. The net electronic charge on each atom, as calculated by the CNDO/2 procedure (vide supra), is shown in Figure 6. Of the two basic nitrogen atoms, N(1) and N(7), on the heterocyclic ring, the former is seen to have a slightly more negative charge than the latter and is, therefore, presumably more basic.[28] The carbon atom C(3), which has been substituted for N(3) of adenosine, has a rather substantial negative charge, almost as much (−0.11 e) as N(9) (−0.12 e). This may partly explain the calculated χ_{CN}- torsional conformation of the molecule (vide infra). Another noteworthy feature of the charge densities is the slight excess of electron density (−0.07 e) on the six-atom imidazole moiety and an equal amount of positive charge on the 11-atom α-aminopyridine moiety. The 15-atom heterocyclic base moiety as a whole has a slight excess (−0.10 e) of electron density associated with it, which it gets from the ribose which, in turn, has an equal amount of positive charge. The OH protons, as expected, are slightly more positive (by approximately +0.02 e)[29] than the NH_2 protons. The CH and CH_2 protons are calculated to have a slight negative charge (approximately −0.02 e), except for HC(3) and HC(3'), which is probably an artifact of the CNDO/2 method since they should, more correctly, have a slightly positive charge in conformity with the electronegativity difference between a carbon and a hydrogen atom. This error is, however, small and should not affect, to any great extent, the qualitative nature of the conformational energy map shown in Figure 3.

The Glycosyl Torsion Angle and the Analysis of the Rotational Barrier around the Glycosyl Bond by the CNDO/2 Molecular Orbital and the Intramolecular Contact Methods. The value of the torsion angle, χ_{CN} for rotation around the glycosyl bond, N(9)-C(1'), is observed to be +4.5° in the crystal of 3-deazaadenosine. This χ_{CN} value is one of the smallest ever observed for a β-nucleoside and is consistent with the observation of Sundaralingam[26b] that, for β-nucleosides, the C(3')-endo sugar rings, in general, have smaller χ_{CN} values than the C(2')-endo rings. In order to assess the stability of the observed solid state conformation for this torsion angle two types of calculations were performed. In the first type of calculation, the internal energy of the mole-

cule was calculated by the CNDO/2 molecular orbital method as a function of the torsion angle χ_{CN}. The results, depicted in Figure 3, indicate that the global minimum in energy occurs at $\chi_{CN} \sim 168°$ which is in the classical syn range.[18] The crystallographic conformation at χ_{CN} of 4.5° is calculated to lie approximately 0.75 kcal/mol higher in energy than the global minimum; such a small calculated energy difference is probably not significant in the CNDO/2 approximation, but it is noteworthy that the barrier between these two minima is also very small and only about 1.3 kcal/mol of excitation energy is required to go from one conformer to the other. It is, therefore, quite likely that, under suitable conditions, the syn conformation can be obtained. It might be pointed out that a global energy minimum in the syn range has also been calculated for C(3′)-endo adenosine by Pullman and Berthod,[30] whereas the crystallographic conformation of this molecule is observed to be anti.[23] In order to further investigate the factors responsible for the existence, in the MO calculations, of the global energy minimum in the syn rather than the crystallographically observed anti range for 3-deazaadenosine, nonbonded interatomic distances between selected atoms on the base and on the sugar were calculated as the glycosyl torsion angle χ_{CN} was changed at intervals of 15°. The van der Waals radii were taken to be of the intramolecular type[20] (H, 1.0; O, 1.35; and C, 1.50 Å). The results shown in Figure 4 are, with one exception, in remarkable agreement with those obtained by the more sophisticated molecular orbital calculations shown in Figure 3. The lone exception is the global energy minimum at $\chi_{CN} \sim 168°$ in the molecular orbital calculations, (Figure 3), which lies in the disallowed range, $147° \leq \chi_{CN} \leq 351°$, in the van der Waals contact diagram (Figure 4). With this one exception (vide infra), it can be seen from the molecular orbital conformation map (Figure 3) that the two low energy local minima, one at $\chi_{CN} \sim 5°$ (experimentally observed) and the other at $\chi_{CN} \sim 100°$, correlate very well with the lack of any van der Waals interaction (Figure 4) in the ranges $\chi_{CN} \sim -9$ to +50° and 85 to 113°. Even the mild van der Waals interactions of the base hydrogen atom, HC(8), with the two sugar hydrogen atoms, HC(3′) and HC(2′) (Figure 4), surprisingly, show up as small humps in energy around χ_{CN} of 70 and 130°, respectively, in the molecular orbital map (Figure 3). The primary rotational barriers are due to the short contacts made by C(3) and its attached hydrogen atom HC(3) with four of the sugar ring atoms, C(2′), C(3′), C(4′), and O(4′) and with HC(2′), HC(3′), O(5′), and HO(5′).

The occurrence of the global energy minimum in the molecular orbital conformational map (Figure 3) is probably a result of Coulombic attractions which would necessarily be absent from the "hard sphere" type van der Waals interactions (Figure 4). That this might be the case can be seen by considering the atomic charges (Figure 6) and distances for the interacting atoms. The main attractive interaction is probably between C(3) on the base, which has a substantial negative charge of −0.11 e, and HO(5′) on the sugar which, being attached to an electronegative atom, has a positive charge of 0.16 e, the distance between the two atoms being 2.23 Å (sum of the respective van der Waals radii = 2.50 Å). CNDO/2 calculations suggest that, in going from the conformation observed in the crystalline state ($\chi_{CN} = 4.5°$) to that calculated as the global minimum ($\chi_{CN} \simeq 168°$), the O(5′)-HO(5′) bond has acquired more polarity, the oxygen atom becoming more negative (by 0.012 e) and the hydrogen atom more positive (by 0.014 e). The C(3)-O(5′) and C(3)-O(4′) distances of 2.90 and 2.69 Å, respectively, at the global minimum are short, but the C(3)-H-O(5′) angle of 93° and C(3)-H-O(4′) angle of 111° preclude any significant C--O type hydrogen bonding interaction. The absence of any significant HC(3)-O(5′) and HC(3)-O(4′) bond order as determined from the density and overlap matrices in the MO calculations is consistent with the above observation.

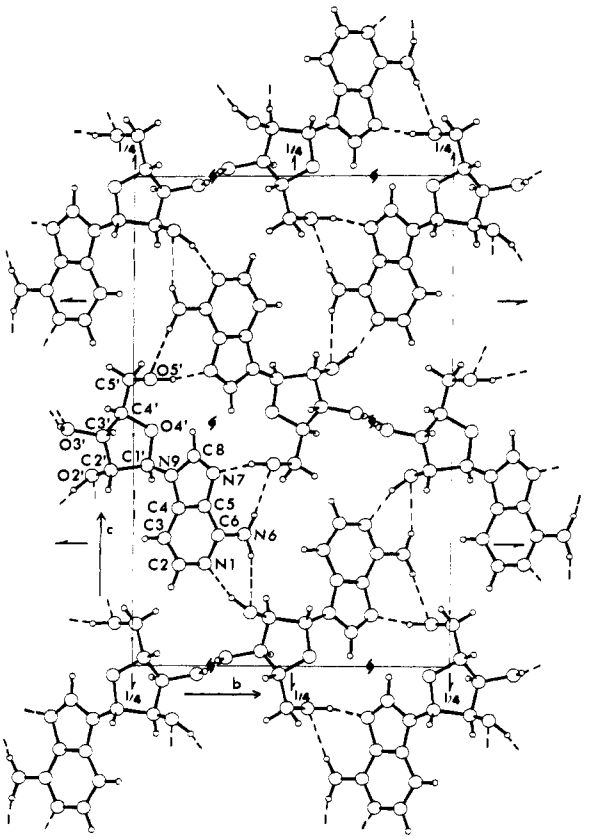

Figure 7. Hydrogen bonding and packing viewed normal to the *bc* plane. One unit cell is outlined and the symmetry elements are shown. Dashed lines indicate possible hydrogen bonds.

The above results, therefore, suggest the existence of two favored conformations for rotation around the glycosyl bond, one in the syn range at $\chi_{CN} \sim 168°$ from the MO calculation, and the other in the anti range at $\chi_{CN} \sim 5°$, observed crystallographically.

Hydrogen Bonding, Packing, and Stacking Interactions. The hydrogen bonding pattern in the crystal is depicted in Figure 7 and the distances and angles associated with it are given in Table VII. All hydrogen atoms attached to oxygen and nitrogen atoms are probably involved in the formation of hydrogen bonds. One possible hydrogen bond, N(6)···H1N(6)···O(2′), however, formed by the $-NH_2$ group, is rather long and considerably bent, the N···O distance being 3.24 Å and the angle at hydrogen being 131°. The H1N(6)···O(2′) distance (2.53 Å), on the other hand, is slightly smaller than the sum of the van der Waals radii (2.60 Å) of the respective atoms, a criterion suggested by Hamilton and Ibers[32] to be indicative of a hydrogen-bonding interaction. It may be noted that a long N-H···O (3.19 Å) hydrogen bond has also been suggested in the intermolecular complex phenobarbital-2-(8-bromo-9-ethyl)adenine.[33]

There are no interbase hydrogen bonds in the crystal of 3-deazaadenosine, and the packing is dictated mainly by the requirements of the electronegative, hydrogen bond forming atoms N(1), N(6), and N(7) all lying on one side of the molecule, and atoms O(2′), O(3′), and O(5′) with similar properties lying on the opposite side.

There is very little base stacking interaction observed in

the crystal of 3-deazaadenosine. The molecules which do interact, to some extent, are those related by the *a*-axis translation. The planar base moieties of these molecules are separated by approximately 3.47 Å with an almost complete overlap of atoms C(6), H1N(6), and H2N(6) of one molecule with atoms C(3), N(1), and C(5), respectively, of the other molecule. The interatomic separations, C(6)-C(3) = 3.51 Å, H1N(6)—N(1) = 3.55 Å and H2N(6)-C(5) = 3.55 Å, when compared with the interplanar separation of 3.47 Å indicate quite clearly an almost perfect overlap of the respective atoms but, as stated above, very little stacking interaction because of the large distances involved.

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Supplementary Material Available. Tables III (Observed and Calculated Structure Amplitudes for 3-Deazaadenosine), IV (Bond Lengths Involving the Hydrogen Atoms), V (Bond Angles Involving the Hydrogen Atoms), VI (Deviations of Atoms from the Least-Squares Planes), and VII (Distances and Angles Associated with the Hydrogen Bonds) (11 pages). Ordering information is given on any current masthead page.

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Syntheses of Isoalloxazines and Isoalloxazine 5-Oxides. A New Synthesis of Riboflavin[1]

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Abstract: The nitrosative and nitrative cyclizations of 6-(*N*-alkylanilino)uracils gave the corresponding isoalloxazine 5-oxides including riboflavin 5-oxide. The reduction of the 5-oxides with sodium dithionite in water gave the corresponding isoalloxazines. The condensation of 6-alkylaminouracils with nitrosobenzenes in acetic anhydride also gave isoalloxazines. The *m*-chloroperbenzoic acid oxidation of riboflavin in acetic acid gave 7,8-dimethyl-10-acetoxymethylisoalloxazine 5-oxide, which was unstable toward hydrolysis, producing lumichrome.

The traditional synthetic routes to isoalloxazines involve the condensations of (a) *o*-phenylenediamines with alloxan, alloxantin, dialuric acid, or halobarbituric acid; (b) 2-arylazoanilines with barbituric acid; (c) anilines with violuric acid; (d) *o*-benzoquinones with 5,6-diaminopyrimidines; (e) dimeric biacetyl with diaminouracils, or diacetyl with preformed lumazines: and (f) quinoxalines with guanidine. These procedures are well documented in the literature.[11] We now wish to report two new and convenient syntheses of isoalloxazines including riboflavin, which consist of (1) the nitrosative and nitrative cyclizations of 6-(*N*-alkylanilino)uracil precursors followed by deoxygenation of the isoalloxazine 5-oxides thus obtained, and (2) the condensation reaction of 6-alkylaminouracils with nitrosobenzenes.[1] Ad-