a convenient and reliable basis for the establishment of the absolute configuration at phosphorus in *all* of the known chiral metabolites of **1** (see Scheme I) which may be synthesized from **1** using reactions that do not involve stereochemical changes at the asymmetric phosphorus center. Our results in this connection will be reported in the future.

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Supplementary Material Available: Tables of observed and calculated structure factors (3 pages). Ordering information is given on any current masthead page.

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8-Azaadenosine. Crystal Structure of Its Monohydrate and Conformational Analysis for Rotation around the Glycosyl Bond

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Abstract: The crystal and molecular structure of the synthetic nucleoside analogue 8-azaadenosine has been determined using three-dimensional x-ray counter data. The nucleoside crystallizes as a monohydrate ($C_9H_{12}N_6O_4 \cdot H_2O$) in the space group $P2_12_12_1$ of the orthorhombic system with four formula units in a cell of dimensions a = 17.125 (2), b = 9.830 (1), and c = 7.428 (1) Å. The crystal structure is isomorphous with that of formycin monohydrate. The structure was refined using 1136 nonzero intensity data to an R factor of 0.033. The conformation of the nucleoside around the glycosyl bond is high-anti with a χ value of 103.7°. The conformation of the C(5′)-O(5′) bond around the C(4′)-C(5′) bond is gauche-trans, and the puckering of the ribose is C(2′)-endo-C(1′)-exo. A CNDO/2 molecular orbital calculation of the energy of the molecule as a function of the torsional angle χ shows that the most stable conformation for the isolated molecule is syn with a χ value of approximately 265°; the solid-state conformation with χ observed in the high-anti range, however, is the next most stable conformation and is only 0.5 kcal mol^{-1} higher in energy than the global minimum. The two energy barriers between the syn and the high-anti conformations are estimated to be 1.25 and 1.5 kcal mol^{-1}, which are considerably lower than the value of approximately 6 kcal mol^{-1} reported for the naturally occurring nucleoside, adenosine. A nonbonded contact search calculation and calculations of atomic charge density by the MO method show that a low-anti conformation with $\chi \lesssim 44°$ is precluded owing to a severe contact between negatively charged atoms N(8) on the base and ring oxygen atom O(4′) on the sugar. The high-anti conformation may be stabilized by an electrostatic attraction between N(8) on the base and C(2′) on the sugar. It is shown that the 8-azapurine nucleosides occurring in the high-anti conformation have their exocyclic bond angle C(4)-N(9)-C(1′) larger than N(8)-N(9)-C(1′) while the naturally occurring purine nucleosides occurring in the high-anti conformation have the reverse relationship.

Ever since the discovery of the antibacterial[1] and the antitumor[2] properties of 8-azaguanine there has been considerable interest in the biochemical[3] and biophysical[4,5] properties of 8-azapurines and their nucleosides (I, II). Our interest in these compounds is primarily structural and is motivated by the belief that the precise structural information provided by x-ray diffraction experiments and theoretical calculations based on the observed geometry would be of value in eventually unra-

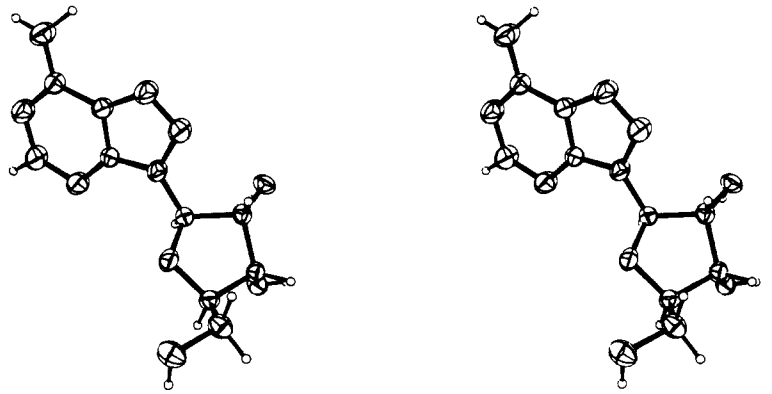


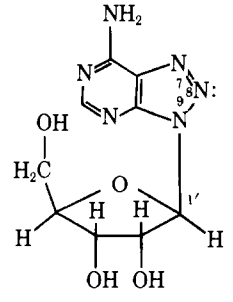
Figure 1. A stereoscopic drawing of 8-azaadenosine showing 50% probability thermal ellipsoids for nonhydrogen atoms. Hydrogen atoms are shown as small open circles.

Table I. Crystallographic Data for 8-Azaadenosine Monohydrate

Chemical formula: $C_9N_6O_4H_{12}\cdot H_2O$
Mol wt = 286.3
System: orthorhombic
Space group: $P2_12_12_1$, from systematic absences of $h00$ for h odd, $0k0$ for k odd, and $00l$ for l odd
a = 17.125 (2), b = 9.830 (1), c = 7.428 (1) Å
μ = 1250.42 Å^3
D_m = 1.51 (2) g cm^{-3} by flotation in CCl_4-benzene
D_c = 1.521 g cm^{-3} assuming Z = 4
R = 0.033, R_w = 0.052
Total no. of observations > 3σ = 1136

	Copper data	Molybdenum data
Take-off angle	1.4°	1.0°
2θ limit	123°	$47.74 \leq 2\theta \leq 52.50°$
No. of observations	1013	123
Scan range	$1.8° + \Delta 2\theta(K_{\alpha 2} - K_{\alpha 1})°$	$1.6° + \Delta 2\theta(K_{\alpha 2} - K_{\alpha 1})°$
Scan rate	1° min^{-1}	0.5° min^{-1}
Background time	40 s each side of peak	40 s each side of peak

velling a structure-function relationship which is essential for a rational approach to the design of more potent chemotherapeutic nucleosides.



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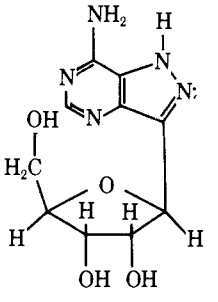

II

The 8 position (I) in purine nucleosides is unique in that it is next to the glycosyl bond which links the base and the sugar moieties in these molecules. The replacement of CH by N in this position can affect the rotation around the glycosyl bond owing to the absence of the steric barrier to rotation which is caused by the hydrogen atom. This may in turn allow a facile interconversion between the syn and anti conformations.[6] This hypothesis has been used[4] to explain the peculiar behavior of polymers containing 8-azapurine nucleosides, such as formycin A (8-aza-9-deazaadenosine) (II) and 8-azaadenosine (I and Figure 1). In addition to relieving the steric interaction caused by the hydrogen atom 8-aza substitution also provides an additional basic site on the heterocyclic ring and can, in principle, affect the basicity of the other nitrogen atoms and the nucleophilicity of the carbon atoms. Furthermore, the lone pair of electrons on N(8) (I and II) is situated close enough to the ribose to interact with it electrostatically. In an effort to better understand the effect of these interactions on the structure and conformation of this important class of nucleosides, we have determined the crystal structure of 8-azaadenosine monohydrate, and used the precise structural information thus obtained to calculate a conformational energy map for rotation around the glycosyl bond by the CNDO/2 self-consistent field molecular orbital method. To assess the importance of the altered electronic environment we have also compared the net atomic charges on 8-azaadenosine with those on the naturally occurring nucleoside adenosine. A brief communication on the structure of 8-azaadenosine has been published.[7]

Experimental Section

X-ray Data Collection. 8-Azaadenosine was obtained from Dr. J. A. Montgomery of the Southern Research Institute, Birmingham, Ala. Slow evaporation of a concentrated aqueous solution at room temperature yielded crystals of rather marginal quality in the beginning. After several attempts, however, crystals of much better size and shape were obtained. Two data sets were, therefore, collected. The first set was collected using a long and thin crystal of dimensions 1 × 0.15 × 0.05 mm with graphite monochromatized Mo Kα radiation on an automatic Picker FACS-I diffractometer with its associated electronics. The structure was refined to a conventional R index of 0.049 using this data set (1029 reflections above 3σ), and the results were reported in a preliminary communication.[7] When better quality crystals became available a second data set was collected using a crystal of dimensions 0.63 × 0.40 × 0.33 on the same diffractometer, but this time using nickel-filtered (0.5 mil foil) Cu Kα radiation. Both data sets were collected using the $\theta/2\theta$ scan technique. The unit cell constants were obtained by a least-squares refinement procedure using the setting angles of 12 accurately centered high angle reflections at a take-off angle of 0.5°. The relevant crystallographic data are presented in Table I. The intensity data were processed as described elsewhere.[8] No correction for absorption errors in either data set was applied.

Solution and Refinement of the Structure. The crystal structure of 8-azaadenosine monohydrate is isomorphous with the previously published structure of formycin A monohydrate.[9] The positional parameters of the nonhydrogen atoms of the formycin A monohydrate crystal were, therefore, taken as the starting point for the refinement of the structure of 8-azaadenosine monohydrate. A structure factor calculation on 1013 Cu Kα data, using the formycin A monohydrate coordinates, assuming a B of 3.0 Å^2 for all atoms, and changing the chemical type of the atom at the 9 position from C to N, yielded the residual, R ($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$) of 0.33 and the weighted residual R_w ($= [\Sigma w(|F_o| - |F_c|)^2/\Sigma w(F_o)^2]^{1/2}$) of 0.45. Three cycles of full-matrix least-squares refinement of the positional and individual isotropic thermal parameters and a scale factor reduced R to 0.12 and

Table III

(a) Fractional Coordinates and Anisotropic Temperature Factors and Their esd's × 10^4 for the Nonhydrogen Atoms in 8-Azaadenosine Monohydrate

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	1554 (1)	4 551 (2)	3825 (4)	23 (1)	75 (2)	228 (8)	−6 (1)	5 (2)	6 (3)
C(2)	1418 (1)	5 888 (3)	3696 (6)	14 (1)	82 (3)	295 (10)	0 (1)	5 (2)	13 (5)
N(3)	1924 (1)	6 906 (2)	3637 (4)	20 (1)	65 (2)	244 (7)	2 (1)	1 (2)	8 (3)
C(4)	2657 (1)	6 439 (3)	3678 (4)	18 (1)	59 (2)	106 (6)	−1 (1)	0 (2)	6 (3)
C(5)	2889 (1)	5 090 (2)	3760 (4)	20 (1)	57 (2)	138 (7)	1 (1)	5 (2)	5 (3)
C(6)	2289 (2)	4 097 (3)	3881 (4)	21 (1)	62 (2)	125 (7)	−3 (1)	2 (2)	−1 (4)
N(6)	2438 (1)	2 778 (2)	4026 (4)	30 (1)	63 (2)	205 (7)	−6 (1)	8 (2)	18 (3)
N(7)	3685 (1)	5 031 (2)	3764 (4)	22 (1)	63 (2)	257 (8)	1 (1)	5 (2)	5 (4)
N(8)	3957 (1)	6 257 (2)	3680 (4)	17 (1)	69 (2)	271 (8)	2 (1)	8 (2)	3 (3)
N(9)	3333 (1)	7 145 (2)	3634 (3)	16 (1)	57 (2)	152 (6)	−1 (1)	6 (1)	0 (3)
C(1′)	3444 (1)	8 605 (2)	3594 (4)	16 (1)	57 (2)	104 (7)	−1 (1)	−3 (2)	8 (3)
C(2′)	4270 (1)	9 081 (3)	4058 (4)	15 (1)	58 (2)	107 (7)	2 (1)	2 (2)	−6 (4)
O(2′)	4458 (1)	8 995 (2)	5871 (3)	26 (1)	70 (2)	104 (5)	5 (1)	−15 (1)	9 (2)
C(3′)	4245 (1)	10 532 (2)	3292 (4)	19 (1)	56 (2)	100 (7)	−5 (1)	−3 (2)	−3 (3)
O(3′)	3918 (1)	11 440 (2)	4535 (3)	24 (1)	56 (2)	121 (5)	3 (1)	−11 (1)	−16 (2)
C(4′)	3703 (1)	10 392 (2)	1662 (4)	22 (1)	53 (2)	90 (7)	−3 (2)	−3 (2)	4 (3)
O(4′)	3007 (1)	9 087 (2)	1840 (3)	33 (1)	81 (2)	128 (5)	−24 (1)	−22 (1)	22 (3)
O(5′)	4121 (1)	10 407 (3)	−105 (4)	24 (1)	84 (3)	105 (7)	3 (1)	−1 (2)	3 (4)
O(5′)	3598 (1)	10 254 (2)	−1579 (3)	35 (1)	98 (2)	97 (5)	9 (1)	−6 (2)	−8 (3)
OW	191 (1)	2 880 (2)	3590 (4)	27 (1)	97 (2)	254 (7)	−9 (1)	31 (2)	−80 (4)

(b) Fractional Coordinates ×10^3 and Isotropic Temperature Factors and Their esd's for the Hydrogen Atoms in 8-Azaadenosine Monohydrate

HW(1)	61 (4)	335 (7)	407 (9)	1 (2)
HW(2)	25 (2)	241 (4)	260 (7)	5 (1)
HC(2)	90 (2)	608 (3)	367 (5)	4 (1)
H(62)	299 (2)	252 (3)	434 (5)	4 (1)
H(61)	211 (2)	217 (4)	408 (5)	4 (1)
HC(1′)	303 (2)	903 (3)	443 (5)	3 (1)
HC(2′)	459 (1)	852 (2)	336 (3)	1 (1)
HO(2′)	399 (3)	962 (5)	667 (7)	8 (1)
HC(3′)	481 (1)	1083 (2)	288 (3)	1 (1)
HO(3′)	425 (3)	1186 (5)	530 (8)	7 (1)
HC(4′)	330 (1)	1112 (3)	175 (4)	2 (1)
H1C(5′)	449 (2)	966 (3)	−8 (4)	3 (1)
H2C(5′)	434 (2)	1135 (4)	−30 (5)	4 (1)
HO(5′)	332 (2)	1111 (5)	−155 (6)	4 (1)

R_w to 0.16. Four cycles of anisotropic refinement reduced R to 0.076 and R_w to 0.108. A difference Fourier calculation at this stage revealed the positions of all the 14 hydrogen atoms unambiguously. For the final round of refinement, 123 intensity data which were beyond the Cu Kα limit of our instrument (2θ = 123°) and which had been collected previously with Mo Kα radiation (vide supra) were merged with the rest of the data with a separate scale factor. The hydrogen atom positions were refined with isotropic and the nonhydrogen atoms with anisotropic temperature factors. Inspection of the data near the end of the refinement suggested an error due to secondary extinction in the Cu Kα data set. A correction of the type suggested by Zachariasen[10] for secondary extinction was, therefore, applied. The final residuals R and R_w are 0.033 and 0.052, respectively. The refined value of the Zachariasen extinction parameter is 1.5 (1) × 10^{-7}.

The scattering factors for C, N, and O were taken from the International Tables[11] and those for H from Stewart, Davidson, and Simpson.[12] The full-matrix least-squares program was that of Busing, Martin, and Levy[13] as modified by Ibers and Doedens, and the Fourier program was Zalkin's FORDAP as modified by Dellaca and Robinson. Other programs used were Doedens' PLANET, Baur's SADIAN, Busing, Martin, and Levy's ORFFE, and Johnson's ORTEP.[14] Programs for data processing and manipulation were written locally.

The observed and calculated structure amplitudes are presented in Table II (see paragraph at end of the paper regarding supplementary material).

Molecular Orbital Calculations. The MO calculations were performed by the CNDO/2 self-consistent field method of Pople and coworkers[15] using Quantum Chemistry Program Exchange Program No. 141 (University of Indiana, Bloomington, Ind.). The molecular geometry of 8-azaadenosine was taken from the x-ray diffraction results described in this paper and that for adenosine from Lai and Marsh.[16] The C-H, N-H and O-H bond lengths, however, were changed to 1.08, 1.01, and 0.97 Å, respectively, keeping their bond directions the same as those observed in the x-ray work. For the conformational energy calculations, new coordinates for the ribose were generated at intervals of 30° as it was rotated around the glycosyl bond, N(9)-C(1′) (I), keeping the base fixed, except near the two minima where two extra calculations were performed, one on each side of the minimum. These coordinates were used as input to the CNDO/2 program.

The electronic charge densities were calculated using the CNDO/2 method for both 8-azaadenosine and adenosine in their crystallographically observed conformation. The net atomic charge is defined as the number of valence shell electrons (e.g., 4 for carbon, etc.) minus the nuclear charge as calculated by the sum of the diagonal density matrix elements for the atom in question.

Results and Discussion

The fractional coordinates and anisotropic thermal parameters for the nonhydrogen atoms in the asymmetric unit are presented in Table III, part a. The fractional coordinates and isotropic thermal parameters for the hydrogen atoms are presented in Table III, part b. The overall shape of the nucleoside is depicted in Figure 1 as a stereoscopic pair. The bond distances and bond angles are shown in Figure 2, except for those involving hydrogen atoms; the latter are presented in

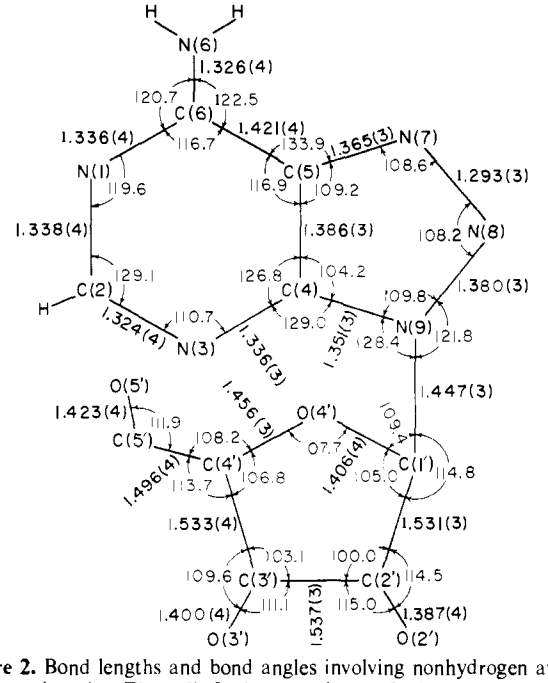

Figure 2. Bond lengths and bond angles involving nonhydrogen atoms in 8-azaadenosine. The esd's for bond angles are 0.3°.

Tables IVa and IVb (see paragraph at end of the paper regarding supplementary material).

Dimensions of the Base and the Glycosyl Bond. The bond distances and bond angles in the nine-membered heterocyclic ring system of 8-azaadenosine (Figure 2) are, as expected, similar to those in adenosine[16] with the most significant differences occurring in the five-membered ring near N(8), the site of substitution. Except for the bond C(4)-C(5), which is practically unaffected, presumably because it is the farthest away from the site of substitution, all other bond distances in the triazole moiety have changed significantly. Thus, bonds C(4)-N(9) and C(5)-N(7) in 8-azaadenosine are shorter by 0.024 and 0.020 Å, respectively, than in adenosine, indicating more double bond character in these bonds in the 8-aza derivative. Of the other two bonds in this moiety the formally double bond N(7)=N(8) in 8-azaadenosine is, as expected, smaller by 0.015 Å than the formally double bond N(7)=C(8) in adenosine, but the formally single bond N(9)—N(8) in 8-azaadenosine is unexpectedly larger by 0.018 Å than the formally single bond N(9)—C(8) in adenosine, which would indicate that the N(9)—N(8) bond in 8-azaadenosine has much less double bond character than the N(9)—C(8) bond in adenosine. It may be pointed out that a number of 6-azapyrimidines and their nucleosides, e.g., 6-azauracil,[17,18] 6-azauridine,[19] and 6-azacytidine,[20,21] when compared with their natural counterparts, also show a similar diminution of double bond character in their N(1)—N(6) bond (N(1) is the site of attachment of the sugar residue in these nucleosides).

A comparison of bond angles in the triazole moiety of 8-azaadenosine, Figure 2, with those in the imidazole moiety of adenosine[16] shows considerable distortions in the ring angles. The main reason for these distortions is the lack of a substituent at the 8 position in 8-azaadenosine, which brings about a decrease in the endocyclic angle at N(8) compared to that at C(8) in adenosine, the latter having a proton at this site. A *decrease* of 6.1° at N(8) is accompanied by fairly symmetrical compensating changes at the remaining four triazole endocyclic angles. Thus, the two endocyclic angles nearest to N(8), i.e., those at N(7) and N(9), *increase* by 5.1 and 4.1°, respectively, and those at C(5) and C(4) *decrease* by 1.6 and 1.5°, respectively, compared to those in adenosine, bringing the total change naturally to zero, since both the triazole ring in 8-azaadenosine and the imidazole ring in adenosine are planar.

Perhaps the most interesting angle distortions occur in the exocyclic bond angles at N(9), the site of attachment of the glycosyl bond to the base. In 8-azaadenosine, the "inside" exocyclic bond angle, C(4)-N(9)-C(1′) (θ_1 hereinafter) with a value of 128.4° is 6.6° larger than the "outside" exocyclic bond angle C(8)-N(9)-C(1′) (θ_2 hereinafter) with a value of 121.8°, while in adenosine the reverse is true, θ_1 (124.3°) being 5.7° smaller than θ_2 (130.0°). Moreover, the exocyclic angle at C(4) (129.0°) in 8-azaadenosine is larger than that in adenosine (126.7°) by 2.3°. The net effect of these angle distortions would be a reduction in the barrier to rotation around the glycosyl bond. The relative magnitudes of θ_1 and θ_2 in 8-azaadenosine are quite similar to those observed in formycin A[9] but not in formycin B.[22,23] This point is further discussed in more detail in a later section.

The triazole ring angles in 8-azaadenosine are in excellent agreement with those in 8-azaguanine[24,25] which, with a proton at N(9), is the only other triazole ring, to our knowledge, with a substituent at N(9), and which, therefore, resembles the triazole ring in 8-azaadenosine. It is interesting and probably quite significant (vide infra) that the "inside" and "outside" exocyclic bond angles θ_1 and θ_2, respectively, subtended by the glycosyl bond at N(9) in 8-azaadenosine, 128.4 (2)° and 121.8 (2)°, respectively, are very close to the similar angles subtended by the *proton* at N(9) in 8-azaguanine,[25] 128.2 and 121.5° (σ = 1°), respectively.

The bond distances and bond angles in the pyrimidine moiety of 8-azaadenosine have not changed much from those in adenosine. The bond distances N(1)-C(6) and N(3)-C(4), however, do exhibit significant shortening, and there are significant changes in the bond angles at C(4) and C(6).

The glycosyl bond, N(9)-C(1′), with a distance of 1.447 (3) Å, is about 0.02 Å shorter than the glycosyl bond distance reported for adenosine[16] and for other purine nucleosides.[26] As documented elsewhere,[7] this may be indicative of a general shortening of the glycosyl bond in ortho azanucleosides of both the purine and the pyrimidine type.

It may be noted that the C–C "glycosyl" bond in the various formycin derivatives (which possess an 8-aza-9-deazapurine base) has a mean length of less than 1.50 Å: formycin A,[9] 1.501 (5) Å; formycin A·H$^+$, 1.492 (17) Å;[27] formycin B, 1.488 (4) Å,[22] 1.493 Å;[23] oxoformycin B, 1.501 (6) Å, 8-methylformycin A,[28] 1.494 (2) Å. There is, therefore, some double bond character in the C–C "glycosyl bond" in 8-aza-9-deazapurine nucleosides similar to that observed in the C–N glycosyl bond in the other ortho azanucleosides cited above.

Planarity of the Base. As seen from Table V, the 9-atom azapurine base, plane 1, is quite planar with rms and maximum deviations of 0.011 and 0.019 Å, respectively. The exocyclic atoms N(6) and C(1′) are displaced significantly from this plane, in the same direction, by 0.051 and 0.068 Å, respectively. The 6-atom pyrimidine moiety, plane 2, and the 5-atom triazole moiety, plane 3, are also planar, with rms and maximum deviations of 0.011 and 0.016 Å, respectively, for the former, and 0.002 and 0.003 Å, respectively, for the latter. The dihedral angle between the 9-atom azapurine plane and the 6-atom primidine plane is 0.9°.

The Ribose. The bond distances and especially the bond angles in the sugar moiety of nucleosides and nucleotides are determined, to some extent, by the mode of puckering of the furanose ring.[26] Their values in 8-azaadenosine, therefore, are quite similar, with few exceptions, to those in formycin A[9] which has the same mode of puckering (vide infra). The main differences lie in the bond distance C(1′)-O(4′), which is shorter in 8-azaadenosine by 0.032 Å, and in the endocyclic bond angles at O(4′), C(1′), and C(2′) where the differences (8-azaadenosine–formycin A) are: −1.6, +1.6, and −2.3°.

Table V. Deviations, in Å, of Atoms from the Least-Squares Planes in 8-Azaadenosine

Plane 1: 9-atom azapurine plane
Plane 2: 6-atom pyrimidine plane
Plane 3: 5-atom triazole plane
Planes 4 and 5: two of the best 4-atom ribose planes

	Base moiety				Ribose moiety	
	1	2	3		4	5
N(1)	−0.003	−0.001	−0.018	C(1′)	−0.044	−0.60
C(2)	0.015	0.012	0.017	C(2′)	0.603	0.052
N(3)	0.000	−0.007	0.012	C(3′)	0.040	−0.081
C(4)	−0.005	−0.007	0.001	C(4′)	−0.066	0.085
C(5)	0.012	0.016	0.001	O(4′)	0.070	−0.056
C(6)	−0.019	−0.013	−0.041	C(5′)	0.998	1.417
N(7)	0.011	0.018	−0.002			
N(8)	0.003	0.005	0.003	Ribose pucker: C(2′)-endo-C(1′)-exo		
N(9)	−0.014	−0.018	−0.002			
N(6)	−0.051	−0.039	−0.040			
C(1′)	−0.068					
rms deviation	0.011	0.011	0.002			

Since these differences in the dimensions of the ribose occur near the glycosyl bond they can be attributed to the difference in the type of the glycosyl bond in the two nucleosides, 8-azaadenosine having a normal N-C glycosyl bond and formycin A an anomalous C-C bond at C(1′). The exocyclic bonds, C(2′)-O(2′) and C(3′)-O(3′), in 8-azaadenosine are shorter than those in formycin A by 0.020 and 0.031 Å, respectively, the latter possessing values close to those normally observed in other nucleosides. The diminution in the C(2′)-O(2′) bond length in 8-azaadenosine is, probably to a large extent, an artifact of the refinement since the proton on O(2′) has refined to a position slightly too far away from it giving an O(2′)-H distance of 1.17 Å (Table IVa, see paragraph at the end of paper regarding supplementary material). The diminution in the C(3′)-O(3′) bond length in 8-azaadenosine compared to that in formycin A can be attributed to O(3′) accepting no hydrogen bond in the former but participating in an O(3′)···H-N(7) hydrogen bond in the latter (vide infra).

As seen from the least-squares planes 4 and 5 in Table V, the five-membered furanose ring of the ribose is puckered in the twisted conformation C(2′)-endo-C(1′)-exo ($^2T_1$)[29] similar to that observed in formycin A.

The conformation of the C(5′)-O(5′) bond about the C(4′)-C(5′) bond is gauche-trans[30,31] (gt), again in agreement with that observed in formycin A.[9] The occurrence of the gt conformation in 8-azaadenosine and formycin A is believed to be stabilized by a repulsive interaction between the negatively charged atoms N(8) on the base and O(5′)[9,22,32] on the sugar; alternatively, the gg conformer is destabilized by the absence of any attraction between the 8 position (which is electropositive in adenosine) and O(5′).

The Glycosyl Conformation: Experimental and Theoretical. The glycosyl torsion angle, χ[33] [O(4′)-C(1′)-N(9)-N(8)], describing[6] the relative orientation of the sugar and the base, is 103.7° in the crystal of 8-azaadenosine; the value for the isomorphous nucleoside formycin A is 109.5.[9] These χ values are intermediate between the classical anti ($\chi \simeq 30 \pm 45°$) and syn ($\chi \simeq 210 \pm 45°$) ranges and are sometimes referred to as high anti[7,9] or syn B.[34] Apparently, this is a preferred conformation for ortho azanucleosides[7] and is distinguished by a rather close intramolecular contact between the ortho nitrogen atom on the base and the C(2′) atom on the sugar.[7,20,21] The closeness of the intramolecular contact between N(8) and C(2′) in the 8-azaadenosine molecule, Figure 1, can be visualized by the fact that the N(8)···C(2′) distance of 2.84 Å at the observed conformation, χ = 103.7°, is only slightly larger than the minimum possible distance, 2.82 Å, that these two atoms approach at $\chi \simeq 117°$, Figure 3, during a complete rotation around the glycosyl bond.

The NMR spectrum of 8-azaadenosine in solution at pH 8.0 has been interpreted[32] on the basis of a syn $\rightleftharpoons$ anti equilibrium or as a single nonequilibrium conformation in which on a time-averaged basis the molecules adopt a glycosyl conformation intermediate between syn and anti. The latter interpretation is consistent with the observed solid state conformation, Figure 1. Another indirect line of evidence for the anti rather than the classical syn conformation in solution for 8-azaadenosine may be cited by extrapolating the similarity between 8-azaadenosine and formycin A, observed in the solid state (vide supra), to solution, since the circular dichroism spectra of formycin A, in aqueous solution at neutral pH, have been interpreted in terms of an anti conformation for formycin A.[35]

The conformational energy diagram for rotation around the glycosyl bond, N(9)-C(1′), calculated by the CNDO/2 molecular orbital method, Figure 4, seems at first sight to contradict the crystallographically observed conformation, χ = 103.7°, since the global minimum in energy occurs at χ = 265° (or −95°) which is in the syn region. It should be noted, however, that the observed conformation lies very near the trough of the local minimum which is around χ = 100° in the high-anti region, and is only 0.5 kcal mol^{-1} higher in energy than the global minimum (Figure 4). The energy barrier for the syn $\rightleftharpoons$ anti interconversion is fairly small, about 1.25 kcal mol^{-1}, and occurs around χ = 0° (Figure 4). By comparison, the energy barrier for the syn $\rightleftharpoons$ anti interconversion for adenosine has been calculated[36] to be approximately 6 kcal mol^{-1} and is quoted to be in agreement with solution studies of the temperature dependence of relaxation time, which yields a value of 6.2 kcal mol^{-1}.[37] The highest energy barrier occurs near χ = 190° and is about 1.5 kcal mol^{-1} higher in energy than the global minimum.

The MO theory, therefore, predicts that the most stable glycosyl conformation for 8-azaadenosine is syn, but the high-anti conformation is almost equally probable. The latter conformation, of course, is that observed in the crystal and also in solution at nonacidic pH (vide supra). The MO results are, therefore, quite consistent with experiment. It is particularly gratifying to note that they pinpoint the high-anti region as the

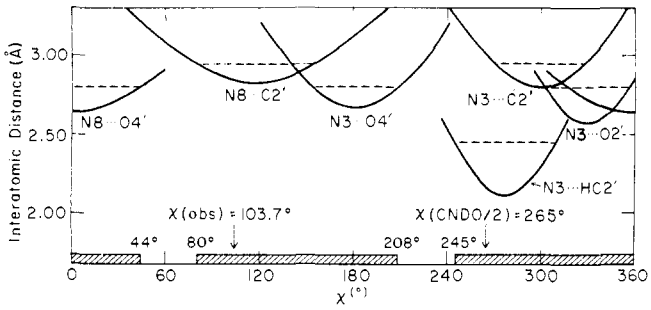

Figure 3. Nonbonded contact search diagram showing interatomic distances between selected pairs of atoms on the base and the sugar as a function of the torsion angle, χ. The horizontal dashed line on each curve indicates the distance below which severe contact between the two atoms is assumed, as judged by the following intramolecular contact radii: H = 1.0, C = 1.50, N = 1.45, and O = 1.35 Å.[38] Disallowed χ regions are shown cross-hatched near the bottom of the drawing.

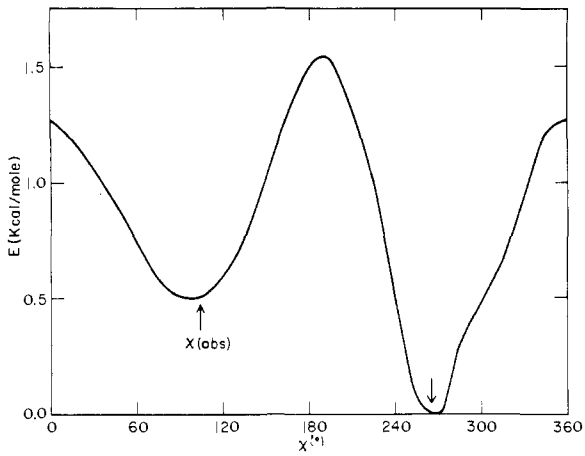

Figure 4. Conformational energy map calculated by the CNDO/2 self-consistent field MO method, showing the energy as a function of the torsion angle χ as defined by Sundaralingam.[33] All other structural and conformational parameters were kept fixed at the crystallographically observed values. The energy of the global minimum, indicated by an arrow near the bottom, is taken as zero.

stable anti conformation which confirms our earlier[7] contention from the results of x-ray crystallography that high-anti conformation is the stable anti conformation for ortho azanucleosides.

Nonbonded Interactions for Rotation around the Glycosyl Bond vs. the Maxima and the Minima in the MO Conformational Energy Map. A perusal of the nonbonded contact search diagram, Figure 3, indicates that the two conformational energy maxima in Figure 4, one near $\chi = 0°$ and the other near $\chi = 190°$, both lie in regions of close intramolecular contacts between atoms carrying *similar* charges. Thus, the instability of the low-anti region in this nucleoside can reasonably be ascribed to the close contact which would result between N(8) and O(4′) (with charges of −0.04 and −0.24 e, respectively (Table VII)) near $\chi = 0°$. This intramolecular interaction extends approximately 44° on both sides of $\chi = 0°$, Figure 3. Similarly, the other energy barrier (near $\chi = 190°$) in the MO map is primarily due to the close contacts between the negatively charged atoms N(3) and O(4′) with charges of −0.26 and −0.24 e, respectively.

Explanations of the occurrence of either of the two energy minima in the MO map, Figure 4, in terms of allowed nonbonded contacts are not possible since both of them fall in regions of close contacts as seen in Figure 3. Even if one allows for the arbitrarily chosen *rare* contacts of Haschemeyer and Rich,[38] which would permit a pair of interacting atoms to come 0.20 Å closer than is allowed by the normal contacts as shown by the horizontal lines in Figure 3, the syn conformation calculated by the MO theory ($\chi = 265°$) is disallowed since the N(3)···HC(2′) distance at $\chi = 265°$ would be 2.17 Å, which is less than that allowed by the rare contacts (2.25 Å). Another possible stabilizing force in the syn region for purine nucleosides is an intramolecular hydrogen bond usually observed between N(3) and O(5′).[39] This, however, is not possible here since the N(3)···O(5′) distance for a complete rotation around the glycosyl bond is never less than 4.0 Å,[40] the latter being the maximum distance surveyed in these calculations. A satisfactory, albeit, qualitative, explanation for the occurrence of both the low-energy minima in the MO diagram probably lies in the fact that the interacting atoms are oppositely charged: $N(8)^{\delta-}\cdots C(2')^{\delta+}$ in the high-anti region, Figure 1, and $N(3)^{\delta-}\cdots HC(2')^{\delta+}$ in the syn region, Figure 3, and would, therefore, tend to stabilize conformations with these χ values by attractive interactions.

It is interesting that the two regions calculated to be completely free of any intramolecular contacts in 8-azaadenosine, namely, $44 \leq \chi \leq 80°$ in the anti region and $209 \leq \chi \leq 245°$ in the syn region, Figure 3, are in much better agreement with those calculated[38] for 2′-deoxyguanosine[41] which has an observed syn conformation than with those calculated for adenosine[42] which has an observed anti conformation (see Tables 1 and 3 of ref 38 for allowed χ values for deoxyguanosine and adenosine).

The above comparisons between these three nucleosides can be explained by assuming that base-sugar interactions are dependent on the relative values of the exocyclic angles θ_1 and θ_2 at N(9). Thus, 8-azaadenosine and 2′-deoxyguanosine (vide supra) have $\theta_1 > \theta_2$, while adenosine has $\theta_2 > \theta_1$. It is noteworthy that, in the absence of large steric constraints, all syn nucleosides have $\theta_1 > \theta_2$.[39,43] Examination of the structures of the bases themselves[44-46] shows that in 9-alkylpurines θ_1 is (on the average) approximately 2.5° smaller than θ_2.[47] It is probable that this trend carries over to the structures of 9-H adenine and 9-H guanine; no data, however, are available to support this speculation.[48] In the case of 8-azapurines, however, accurate structures of 8-azaguanine[25] and 8-aza-7-deazahypoxanthine[49] (also called allopurinol), both of which have a proton on N(9), show that θ_1 and θ_2 involving the proton on N(9) in these 8-azapurines followed the same trend as that observed in the nucleosides 8-azaadenosine and formycin A (Table VI, category II), i.e. θ_1 is larger than θ_2.

The above data seem to suggest that the high-anti conformation of the 8-azapurine nucleosides and the low energy of activation for the syn $\rightleftharpoons$ anti interconversion are largely a function of the geometry of the aglycon itself, which has the exocyclic bond at N(9) directed in such a way that the inside exocyclic bond angle θ_1 is larger than the outside bond angle θ_2. Similarly, the preponderance of anti conformation for the normal purine nucleosides is correlated with θ_1 being smaller than θ_2 as shown above for the 9-alkylpurines.

Electronic Charge Density. A comparative study of the electronic charge density on a series of related purines can provide important clues as to their chemical and, therefore, physiological properties.[50,51] Table VII lists the net atomic charge density, as calculated by the CNDO/2 method for the nucleoside analogue 8-azaadenosine and the normal nucleoside adenosine. It is apparent that the substitution of an electronegative nitrogen atom for the methine group at the 8 position on the base causes withdrawal of electron density from both N(7) and N(9) leaving them with considerably less negative charge (−0.08 and −0.08 e, respectively) than they possess in adenosine (−0.21 and −0.13 e, respectively). The extra nitrogen atom N(8) in 8-azaadenosine possesses a rather small charge of −0.04 e. The remaining three nitrogen atoms, N(1), N(3), and N(6), in 8-azaadenosine possess approximately the same charge as in adenosine, but the four carbon atoms possess

Table VI. A Comparison of the Exocyclic Bond Angles at N(9) in 8-Azapurine Nucleosides with Those in C(8)-Purine Nucleosides Exhibiting High-Anti Glycosyl Conformation with $70 \leq \chi \leq 125°$

Nucleoside	χ	$\angle(4\text{-}9\text{-}1')^a = \theta_1$	$\angle(8\text{-}9\text{-}1')^a = \theta_2$	$\theta_1 - \theta_2$	esd
I. C(8)-Purine Nucleosides; High-Anti					
Guanosine, mol. A[b]	122.5	126.2	128.2	−2.0	0.3
Inosine, mol. A[b]	120.1	125.1	128.9	−3.8	0.3
Tubercidin[c]	71.8	125.2	126.8	−1.6	0.2
Tubercidin[d]	71.3	124.9	128.3	−3.4	0.8
2′-Deoxyguanosine, mol. 1[e,f]	88.5	124.6	128.4	−3.8	0
2′-Deoxyguanosine, mol. 2[e,f]	93.0	123.2	131.4	−8.2	0
II. N(8)-Purine Nucleosides; High-Anti					
8-Azaadenosine[g]	103.6	128.4	121.8	+6.6	0.2
Formycin A[h,i]	109.5	128.2	121.9	+6.3	0.3
III. N(8)-Purine Nucleosides; Low-Anti					
Formycin B[i,j]	29.7	124.5	125.3	−0.8	0.2
Formycin B[i,k]	30.4	124.8	124.8	0.0	
IV. N(8)-Purine Nucleosides; Syn					
Formycin A·H$^+$ [i,l]	210.7	130.6	122.7	+7.9	1.1
Formycin B·H$^+$ [i,m]	221.1	128.0	122.7	+5.3	0.9
Oxoformycin B[i,k]	195.9	131.1	119.6	+11.5	0.4
8-Methylformycin A[n]	154.8	132.0	122.8	+9.2	0.2

[a] The numbers in parentheses refer to the atom numbers given in Figure 2. [b] U. Thewalt, C. E. Bugg, and R. E. Marsh, *Acta Crystallogr., Sect. B*, **26**, 1089 (1970). [c] J. Abola and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **29**, 697 (1973). [d] R. M. Stroud, *Acta Crystallogr., Sect. B*, **29**, 690 (1973). [e] S. C. Jain and H. M. Sobell, *J. Mol. Biol.*, **68**, 1 (1972). [f] This structure is different from the syn conformer of this molecule used in the calculations of ref 38. [g] This work. [h] Reference 9. [i] A C nucleoside. [j] Reference 22. [k] Reference 23. [l] Reference 27. [m] P. Singh and D. J. Hodgson, unpublished observation. [n] Reference 28.

Table VII. Net Electronic Charge Density in e ($\times 10^3$) Associated with Each Atom in 8-Azaadenosine[a] (8-AzAdo) and Adenosine[b] (Ado)

Atom	8-AzAdo	Ado	Atom	8-AzAdo	Ado
N(1)	−0.287	−0.281	O(4′)	−0.243	−0.242
C(2)	0.238	0.223	C(5′)	0.131	0.138
N(3)	−0.256	−0.259	O(5′)	−0.246	−0.248
C(4)	0.226	0.201	HC(2)	−0.037	−0.039
C(5)	−0.088	−0.060	H1N(6)	0.116	0.118
C(6)	0.286	0.267	H2N(6)	0.122	0.119
N(6)	−0.223	−0.226	HC(1′)	−0.024	−0.014
N(7)	−0.082	−0.211	HC(2′)	0.008	−0.010
N(8)[c]	−0.044	0.171	HO(2′)	0.147	0.141
N(9)	−0.075	−0.127	HC(3′)	−0.026	0.024
C(1′)	0.227	0.227	HO(3′)	0.146	0.145
C(2′)	0.111	0.112	HC(4′)	0.001	0.010
O(2′)	−0.249	−0.260	H1C(5′)	0.007	−0.013
C(3′)	0.132	0.098	H2C(5′)	−0.014	−0.010
O(3′)	−0.263	−0.261	HO(5′)	0.140	0.137
C(4′)	0.117	0.140			

[a] Coordinates from Table III, parts a and b. [b] Coordinates from ref 16. [c] C(8) for adenosine.

significantly different charges, C(2), C(4), and C(6), becoming more positive by 0.02, 0.03, and 0.02 e, respectively, and C(5) becoming more negative by 0.03 e.

The charge density on the ribose atoms remains unaltered, except at C(3′), HC(3′), and C(4′); C(3′) becomes more positive by 0.03 e, its proton HC(3′) more negative by a surprising 0.05 e, and C(4′) more negative by 0.02 e. It may be noted that the conformation of the ribose in the two nucleosides under comparison here is different: it is C(3′)-endo-C(2′)-exo in adenosine and C(2′)-endo-C(1′)-exo in 8-azaadenosine. Whether this has an effect on the charge density is not clear since 3-deazaadenosine[52] has a C(3′)-endo envelope conformation which is closer to that of the adenosine than to 8-azaadenosine but the charges on its ribose atoms are quite similar to those on the latter.

Hydrogen Bonding and Packing. The distances and angles associated with the hydrogen bonds are presented in Table VIII. The hydrogen bonding pattern in the crystal of 8-azaadenosine monohydrate is quite similar to that in the isostructural crystal of formycin monohydrate,[9] except, of course, at N(7), where the two nucleosides differ in their molecular structure. Atom N(7) in 8-azaadenosine is basic and is, therefore, a potential acceptor, whereas in formycin A it has a proton attached to it and is, therefore, a potential donor. In the formycin monohydrate crystal[9] the N(7)-H···O(3′) hydrogen bonding distance is 2.96 Å whereas in 8-azaadenosine monohydrate the corresponding N(7)···O(3′) distance is 3.60 Å, indicating no interaction.

The lack of hydrogen bond acceptor capacity of atom N(7) in 8-azaadenosine seems to be related to its small net negative charge of −0.08 e, since in the adenosine crystal[16] (where it is also a basic nitrogen atom and where it does participate in a hydrogen bond) it has a substantially higher charge of −0.21 e. Atom N(8) does not participate in a hydrogen bond in either the 8-azaadenosine monohydrate crystal (Table VIII) or the formycin monohydrate crystal.[9] This, again, is consistent with

Table VIII. Lengths and Angles Associated with the Possible Hydrogen Bonds in 8-Azaadenosine Monohydrate

A-H···B	A-H, Å	H···B, Å	A···B, Å	∠A-H···B, deg
N(6)-H1N(6)··· O(3′)	1.00	1.92	2.88	159
N(6)-H2N(6)··· O(1′)	0.82	2.50	3.06	127
O(2′)-H···O(5′)	1.17	1.59	2.70	156
O(3′)-H···OW	0.90	1.83	2.67	155
O(a′)-H···N(3)	0.97	2.00	2.94	163
OW-HW(1)··· N(1)	0.93	2.01	2.86	152
OW-HW(2)··· O(2′)	0.87	1.95	2.80	163

low net negative charges on N(8) of −0.04 and −0.10 e, respectively, in the two nucleosides. It has been shown elsewhere[22,53] that there is a correlation between the net negative charge on the ortho nitrogen atom and its ability to form a hydrogen bond in ortho azanucleosides and their bases. These correlations, we think, provide a more plausible explanation of an important experimental observation than that obtained by invoking the presence of packing forces.

Some Biochemical Implications. The results obtained in the present investigation by means of x-ray crystallography, conformational analysis, and charge density calculations for 8-azaadenosine can be used to shed some light on aspects of its chemical and biochemical action.

The explanation by Ward and Reich[4] of the curious behavior of polymers containing 8-azapurine nucleosides is based on the assumption of increased conformational mobility in these nucleosides around their glycosyl bond as compared to the normal nucleosides. The present evaluation of 0.5 kcal mol^{-1} for the activation energy of this process in 8-azaadenosine compared to 6 kcal mol^{-1} for adenosine supports this assumption. Recent results, however, on the structure of protonated 8-azaadenosine[54] suggest that the protonation of 8-azaadenosine does not perturb its structure enough to provide the needed activation for it to switch over to the syn conformation.

A second important biochemical reaction of 8-azaadenosine where the present results may have relevance is its deamination by calf intestine deaminase. It has been reported[55] that this enzyme has a lowered binding affinity for 8-azaadenosine ($K_m = 9.6 \times 10^{-5}$) as compared to that for adenosine ($K_m = 2.9 \times 10^{-5}$). However, once the analogue nucleoside is bound to the enzyme it is deaminated much faster than adenosine itself (relative $V_{max} = 2.17$). Since it has been shown[56] that this adenosine deaminase does not bind nucleosides in the syn conformation a lowered affinity for 8-azaadenosine (which has a stable conformation intermediate between syn and anti) is understandable. As for the increased rate of deamination, it is very probably related to the increased positive charge on atom C(6) of 8-azaadenosine as compared to that of adenosine (+0.29 e vs. +0.27 e, Table VI) since, as shown by Wolfenden,[57] the mechanism of deamination involves a nucleophilic attack on C(6) which would be enhanced by an increased positive charge on the latter.

An important chemical reaction which all nucleosides, except those possessing a C-C "glycosyl" bond, undergo is the acid hydrolysis of the N-C glycosyl bond which cleaves them into their constituent base and sugar moieties. The mechanism of this reaction seems to involve a fast protonation of the base followed by a rate-determining cleavage of the glycosyl bond.[58] It has been suggested[50,51] that under these circumstances a lowered electron density on N(9) would favor hydrolysis, since N(9) would then easily pick up the released pair of electrons. On this premise, therefore, 8-azaadenosine with a charge of −0.08 e would undergo glycosyl hydrolysis much more readily than would adenosine itself with a charge of −0.12 e. We are aware of no data on the rate of hydrolysis of 8-azaadenosine to verify this prediction.

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Supplementary Material Available: Tables II (observed and calculated structure amplitudes for 8-azaadenosine monohydrate), IVa (bond distances involving the hydrogen atoms), and IVb (bond angles involving the hydrogen atoms) (9 pages). For ordering information, consult any current masthead page.

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Reaction of Carbon Monoxide with Ferrous Porphyrins. Kinetics and Equilibria for the Binding of Carbon Monoxide to Octamethyltetrabenzoporphyriniron(II) Derivatives

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Abstract: The six-coordinate complexes $L_2Fe(OMBP)$, where OMBP = the dianion of octamethyltetrabenzoporphyrin and L = 1-methylimidazole (1-MeIm), pyridine (py), or piperidine (pip), reversibly bind carbon monoxide in toluene solution. The equilibria and kinetics of these reactions are compared to analogous data obtained previously for carbonylation of other ferrous porphyrins and phthalocyanines. The intermediary structure of OMBP with respect to phthalocyanine (Pc) and porphyrins such as protoporphyrin IX (PpIX) and tetraphenylporphyrin (TPP) presents an opportunity to compare reactivity within a series of closely related tetradentate nitrogen macrocycles. Equilibrium constants, K, for the reaction $L_2Fe(OMBP) + CO \rightleftharpoons LFe(OMBP)(CO) + L$ are intermediate between those found for corresponding PpIX and TPP systems on one hand and Pc on the other. Large rate constants for dissociation of L indicate a substantial cis effect for the OMBP ligand, and a possible mechanism is presented for the porphyrin-induced lability of axial ligands. The carbonyl compounds, LFe(OMBP)(CO) and LFePc(CO), have been isolated.

A well-known[1,2] and important, but poorly understood, property of metalloporphyrins is the enhanced lability of axial ligands, and this undoubtedly accounts for the incorporation of such compounds into biological systems. For example, iron(II) complexes are generally substitution inert, and yet the lability of heme centers such as Fe(PpIX) is clearly critical for biological activity in many protein systems, especially those involving binding and activation of dioxygen.

Considerable inorganic chemistry can be interpreted in terms of kinetic and thermodynamic trans effects,[3,4] while in contrast cis effects are poorly characterized.[4,5] Biological chemistry appears to require subtle balances in the contributions from both of these properties[6] and it is essential to clarify the consequences of porphyrin substitution on the axial coordination properties of metalloporphyrins.

Studies of amine binding to four-coordinate ferrous porphyrins[7] and substitution reactions of bisamine ferrous porphyrins with RNC,[8] CO,[9] and O_2[2,10] have not explained unequivocally how porphyrins mediate the properties of a metal center. Our objective has been to gain further insight into this problem by examining the binding properties of a series of ferrous porphyrins. Octamethyltetrabenzoporphyriniron(II), Fe(OMBP), completes a series in which it is a structurally intermediate complex between more typical porphyrins, such as Fe(PpIX) and Fe(TPP), and a tetraazaporphyrin, FePc (Figure 1). The equilibria and kinetics defining the reaction of CO with derivatives of all of these systems are discussed in this paper.

Experimental Section

Inert atmosphere techniques were employed for all of the work described herein. The $(py)_2Fe(OMBP)$ complex was provided by J. R. Sams and T. B. Tsin of this department. Toluene was distilled from CaH_2 and stored under nitrogen. Pyridine (Fisher), piperidine (Fisher), and 1-methylimidazole (Aldrich) were distilled under anaerobic conditions from KOH and stored under nitrogen. Carbon monoxide was Matheson C.P. grade. Visible spectra were recorded on Perkin-Elmer 202 or Cary 14 spectrophotometers equipped with circulating constant-temperature baths. Infrared spectra were obtained in the solid state as Nujol mulls using a Perkin-Elmer 457. Carbonyl stretching frequencies were calibrated against the 1602-cm^{-1} peak of polystyrene and ν(CO) of CO gas and are considered accurate to ± 2 cm^{-1}.

Equilibrium Constant Measurements. Samples of $(py)_2Fe(OMBP)$ were weighed into a 1-mm path length spectrophotometric cuvette which was attached by a side arm to a reservoir bulb, fitted with a Teflon stopcock (Kontes Glass Co.) and an O-ring joint for attachment to a vacuum/gas-handling line. Toluene and appropriate quantities of amine required to give a reasonable variation in $[LFe(OMBP)(CO)]/[L_2Fe(OMBP)]$ with variation in CO pressure (0-1 atm) were placed in the reservoir bulb and degassed by four freeze-pump-thaw cycles. Solutions (~ 5-8×10^{-5} M) were prepared by dissolving the compound in the solvent in vacuo. All solutions used for spectrophotometric measurements were prepared in a similar manner. For K_{CO}