of oxyhemoglobin which suggested the presence of low-lying excited states. They postulated that these excited states were related to a rotation of the O_2 molecule. In a later Mössbauer study of crystals of the picket fence iron porphyrin dioxygen complex,[35,36] similar temperature dependence in ΔE_Q was observed and explained on the basis of rotation of the O–O moiety between the two possible orientations of the Fe–O_2 and Fe–imidazole planes observed in the crystals. However, this interpretation did not appear to be applicable to the protein data.[37] It would be interesting to reconsider the Mössbauer data in light of the possibility of H bonding from a distal residue of the protein or an amide "picket" of the model compound.

While the results presented herein do not rule out the possibility that O_2 rotation is prevented by the steric restrictions imposed by the internal sculpture of the protein pocket, rather than by H bond donation from the distal histidine, the weight of X-ray and neutron diffraction data, along with the present EPR results, argues strongly in favor of the H-bonding explanation. It further provides a new technique for observing this H bonding in solution at room temperature. Further investigation of the room-temperature EPR spectra of $CoMbO_2$ and $CoHbO_2$ is underway.

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Registry No. (*p*-$NHCOCH_3$)TPPCo(*N*-MeIm)O_2, 98859-46-2; (*o*-$NHCOCH_3$)TPPCo(*N*-MeIm)O_2, 98859-47-3; O_2, 7782-44-7.

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X-ray and ^{1}H NMR Analyses of the Structure and Conformation of 2-Azacoformycin, a Potent Inhibitor of Adenosine Deaminase[1]

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Abstract: The three-dimensional structure of 2-azacoformycin was determined by X-ray crystallography. The crystals belong to the orthorhombic space group $P2_12_12_1$, and the cell dimensions are a = 7.022 (1) Å, b = 8.491 (1) Å, and c = 20.773 (1) Å. Intensity data were measured on a diffractometer, and the structure was determined by direct methods. Least-squares refinement, which included all hydrogen atoms, converged at R = 0.043 for 1502 observed reflections. The diazepine ring is in a somewhat distorted and flattened sofa conformation, C(7) being displaced from the mean plane. The distribution of electrons in the aglycon can be inferred from its geometry. The glycosyl torsion angle is in the high-anti range (χ_{CN} = 72.4°), the furanose ring has a C(2′) endo/C(3′) exo ($^2T_3$) pucker, and the conformation of the –CH_2OH side chain is gauche$^-$. The solution conformation was determined by high-resolution ^{1}H NMR spectroscopy. The conformation of the diazepine ring in solution is essentially the same as that in the solid state. It is shown that the interpretation of NMR data on the basis of average furanose conformations may lead to inaccurate results.

A number of adenosine analogues are known to possess significant antiviral and antileukemic activities.[3] However, a major problem encountered in the use of such compounds is the facility with which they undergo intracellular deamination by the enzyme adenosine deaminase (ADA), since the products (inosine analogues) exhibit lower or no activity. The inhibition of this enzyme is not only therapeutically attractive,[4,5] but it is also thought to cause immunosuppression which is desirable in tissue transplants.[6] In cases of lymphoblastic leukemia, coformycin, a potent inhibitor of ADA, has been shown to induce a remission when used alone.[7] Biological testing of coformycin has also demonstrated[8] that the inhibitor can be incorporated into cellular DNA and that it has different effects on the cell cycle in normal and virally transformed cells.[9]

Of the known inhibitors of ADA, coformycin (**1a**) and its 2′-deoxy derivative, pentostatin (**1b**), are the most potent. Furthermore, they are noteworthy because they exhibit anomalous binding kinetics characteristic of transition-state analogues.[10] Heterocyclic analogues of these inhibitors had not been reported and therefore a structure–activity study of the relationship of inhibition by this class of transition-state analogues could not be accomplished. This prompted us to apply some previously developed methodology from our laboratory to synthesize[11-14] several

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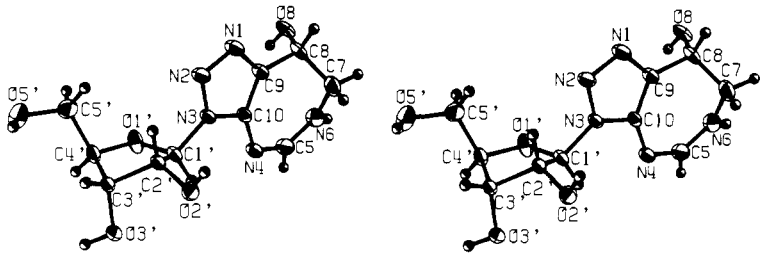
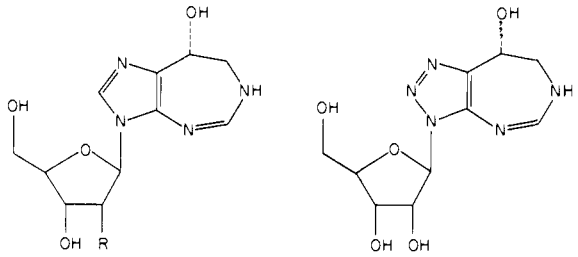


Figure 1. Stereoscopic view of 2-azacoformycin. The ellipsoids correspond to 50% probability.

heterocyclic analogues of coformycin. One of these analogues, 2-azacoformycin (**2**), proved[15] to have a K_i of 2×10^{-9}, less than an order of magnitude higher than the K_i reported for coformycin.[10] Compound **2** also exhibited the similar slow binding kinetics of coformycin, which would suggest that it is also acting as a transition-state analogue.



1a: R = OH
1b: R = H

2

A study[16] of some heterocyclic analogues of purine riboside and *erythro*-hydroxynonyladenine (EHNA), the former a ground-state inhibitor and the latter a semitight inhibitor having biphasic inhibition kinetics, provided an interesting observation that a nitrogen in the one position of the purine ring in EHNA was required for biphasic inhibition kinetics. A recent study of isotope and pH kinetic effects on the inhibition of ADA by ground- and transition-state inhibitors[17] delineated some of the requirements of the transition state attained during the enzymatic reaction. In particular, the suggestion that N(1) is likely to be protonated in the transition state was made. Because 2-azacoformycin retains all the features of coformycin, while displaying major differences in electronic properties due to the substitution of the triazole ring for imidazole ring in coformycin, it appeared to be a good candidate for a more detailed study. We were very interested in being able to determine the electronic and steric differences between coformycin and the 2-aza analogue **2**. By determining the bond geometries of the molecule by single-crystal X-ray diffraction and the solution conformation by ^{1}H NMR, we felt that certain inferences could be made as to the distribution of charge in the molecule. These findings should allow comparisons to be made between the observed electronic properties and inhibitory behavior of these compounds.

Experimental Section

The title compound was crystallized from aqueous ethanol to give colorless prisms. Precession photographs indicated space group $P2_12_12_1$. A crystal fragment measuring $0.35 \times 0.40 \times 0.50$ mm was mounted on an Enraf-Nonius CAD-4 diffractometer; it provided the following data: $a = 7.022$ (1) Å, $b = 8.491$ (1) Å, $c = 20.773$ (1) Å, $V = 1238.56$ Å^3, $\rho_c = 1.53$ g cm^{-3}, $Z = 4$ (20 °C; Cu Kα_1, $\lambda = 1.54056$ Å); $F(000) = 600$, μ(Cu Kα) = 10.2 cm^{-1}.

Cell dimensions were determined from angular settings of 22 high-order ($\theta > 62°$) reflections. Intensities were measured with Ni-filtered Cu Kα radiation, using $\omega/2\theta$ scans with variable scan ranges and speeds. There were 1518 unique reflections accessible to the diffractometer ($2\theta \leq 152°$); only 9 of them had $I \leq 3\sigma(I)$ and were considered unobserved.

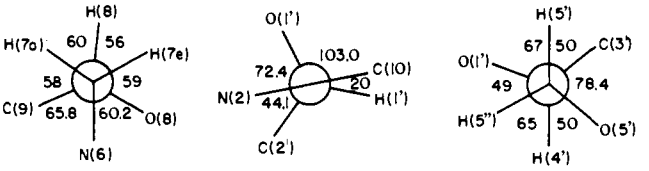


Figure 2. Newman projections along C(7)–C(8), N(3)–C(1'), and C(4')–C(5').

The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary.

The structure was determined by direct methods with the aid of the computer program MULTAN78.[18] Of the 12 starting sets subjected to tangent refinement, the solution with the highest combined figure of merit yielded an E map on which all 20 non-hydrogen atoms were located. Atomic parameters were refined by block-diagonal least squares. All hydrogen atoms were located on difference Fourier maps and refined with isotropic temperature parameters. The scattering factors were taken from the "International Tables for X-ray Crystallography"[19] and the oxygen curve was corrected for anomalous dispersion with both the real and the imaginary components. Throughout the refinement the function $\sum w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1w_2$, where $w_1 = 1$ for $|F_o| \leq 8.0$, $w_1 = 8.0/|F_o|$ for $|F_o| > 8.0$; and $w_2 = \sin^2\theta/0.9$ for $\sin^2\theta < 0.9$, $w_2 = 1$ for $\sin^2\theta \geq 0.9$. This scheme made the average values of $w(\Delta F^2)$ independent of $|F_o|$ and $\sin^2\theta$. After the final cycle the average parameter shift equalled 0.1σ and the largest one 0.3σ. The conventional residual index R is 0.043 and the weighted index R' is 0.047 for 1502 reflections (seven low-order reflections suffered from secondary extinction and were given zero weights). A final difference Fourier map showed no significant features. The coordinates are listed in Table I. Lists of anisotropic temperature parameters and of observed and calculated structure factors are available (see supplementary material paragraph at the end of the paper).

A 3-mg crystal was dissolved in 0.4 mL of 99.8% D_2O containing a trace of acetone which was used as the internal reference set at δ 2.225. The ^{1}H NMR spectrum was obtained on a Bruker AM-500 spectrometer operating in the Fourier mode with quadrature detection. Two hundred transients of 16K data points were accumulated with use of a 45° pulse. The temperature of the sample was 310 K. The HDO resonance was reduced with homonuclear decoupling. Prior to Fourier transformation a resolution enhancement function was used and the data set was zero-filled to 32K data points to increase the digital resolution to 0.18 Hz/pt.

The ^{1}H NMR spectrum was assigned by performing a two-dimensional homonuclear shift correlated experiment which identifies proton resonances that are scalar-coupled to each other. The chemical shifts and coupling constants of the proton resonances were obtained from a spin simulation of the observed spectrum. The calculations were performed on an Aspect 3000 computer with the Bruker program PANIC. A line width of 0.1 Hz was used in all cases. The root-mean-square error of the frequency differences between the experimental and theoretical lines was 0.07 Hz for the ribose resonances and 0.2 Hz for the diazepine resonances.

Results and Discussion

A stereoscopic view of 2-azacoformycin, showing the overall conformation in the crystal and the atomic numbering scheme, is presented in Figure 1. Details of the conformation can be seen in the Newman projections (Figure 2) and in Figure 3 which also

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Table I. Final Atomic Parameters and Their Standard Deviations[a]

atom	x	y	z	U_{eq}/U_{iso}
N(1)	8118 (3)	36 166 (21)	54 318 (8)	294
N(2)	8418 (3)	32 544 (20)	48 243 (8)	301
N(3)	8787 (3)	46 211 (18)	45 186 (7)	227
N(4)	8996 (3)	73 852 (19)	47 096 (8)	265
C(5)	8839 (4)	85 765 (25)	51 020 (10)	316
N(6)	8749 (3)	86 299 (23)	57 403 (9)	361
C(7)	9349 (4)	74 057 (32)	61 890 (10)	368
C(8)	8075 (3)	59 646 (26)	61 601 (8)	272
O(8)	6141 (2)	63 330 (21)	63 165 (6)	319
C(9)	8282 (3)	51 970 (23)	55 169 (8)	237
C(10)	8740 (3)	58 724 (22)	49 312 (8)	210
C(1′)	9050 (3)	46 169 (21)	38 201 (8)	218
O(1′)	7257 (2)	43 700 (20)	35 278 (7)	327
C(2′)	10310 (3)	32 620 (20)	35 911 (8)	202
O(2′)	12265 (2)	35 806 (17)	36 599 (7)	255
C(3′)	9602 (3)	30 435 (21)	29 022 (8)	202
O(3′)	10398 (2)	42 374 (18)	25 046 (6)	284
C(4′)	7453 (3)	32 592 (24)	29 962 (9)	252
C(5′)	6401 (3)	17 544 (32)	31 695 (12)	377
O(5′)	6073 (3)	7 770 (22)	26 337 (10)	413
H(5)	871 (4)	955 (3)	489 (1)	3 (5)
H(6)	847 (6)	944 (5)	590 (2)	25 (9)
H(7a)	1062 (5)	712 (4)	612 (2)	12 (8)
H(7e)	935 (6)	789 (54)	661 (2)	26 (9)
H(8)	851 (5)	528 (4)	649 (2)	16 (8)
H(O8)	564 (6)	681 (5)	601 (2)	20 (9)
H(1′)	964 (4)	566 (4)	374 (1)	4 (6)
H(2′)	989 (4)	238 (3)	328 (1)	1 (5)
H(O2′)	1271 (5)	294 (4)	395 (2)	16 (8)
H(3′)	987 (5)	206 (4)	273 (2)	13 (8)
H(03′)	999 (5)	403 (4)	211 (2)	14 (8)
H(4′)	683 (5)	379 (4)	262 (1)	8 (6)
H(5′)	702 (4)	116 (3)	355 (1)	3 (6)
H(5″)	521 (6)	199 (4)	331 (2)	18 (8)
H(O5′)	717 (7)	20 (6)	253 (2)	34 (11)

[a] The x coordinates and the U_{eq} values of the non-hydrogen atoms were multiplied by 10^4 and the y and z coordinates by 10^5. All hydrogen atom parameters were multiplied by 10^3.

shows the bond lengths and bond angles.

Aglycon Moiety. In contrast to purine nucleosides, the aglycon in the present structure is not planar. A mean plane was calculated for all ring atoms except C(7). Most atoms are within 0.03 Å of this plane, although for C(5) and N(6) the deviations amount to 0.081 and 0.073 Å, respectively. The seven-membered ring is puckered, with C(7) at a distance of 0.751 Å from the mean plane. This conformation is known as "sofa", but, as shown in Figure 3, three torsion angles, which would be 0° in an undistorted sofa, are in the range 1.5–12.1°. Such distortions suggest some delocalization of electrons (see below). A detailed analysis by the method of Cremer and Pople[20] yielded the following parameters: $q_2 = 0.46$, $q_3 = 0.35$, $\phi_2 = 27.2$, $\phi_3 = 27.5$. In an ideal sofa the puckering amplitudes (q_2 and q_3) are 0.47 and the phases (ϕ_2 and ϕ_3) are 28.0.[21] In such a sofa the torsion angles would be 0, ±35.1, and ±92.5°. The diazepine ring in the present structure can therefore be described as a somewhat distorted and flattened sofa.

The five atoms of the triazole ring are coplanar within experimental error (≤0.006 Å). A comparison of this ring's geometry with that in related systems provides significant information about the distribution of electrons. The C(9)–C(10) bond is 0.019 Å longer than the corresponding one in coformycin[22] and 0.006 Å longer than the mean value of the C(4)–C(5) bond in guanine residues.[23] The N(1)–C(9) bond is 0.047 Å shorter than in coformycin and 0.031 Å shorter than in guanines. These two bond lengths as well as that of N(1)–N(2) correspond to bond orders 1.4–1.6.[24] We can conclude, therefore, that there is appreciable

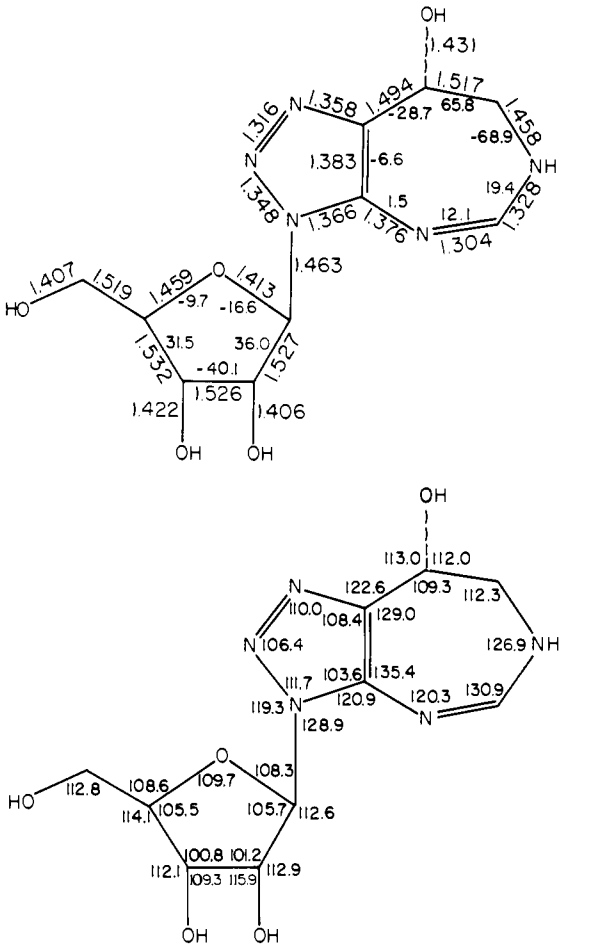
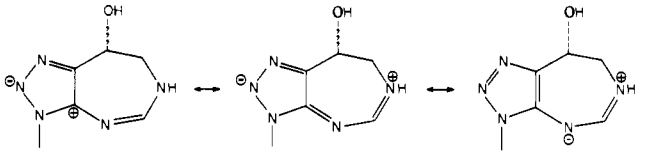


Figure 3. (Top) Bond distances (Å) and endocyclic torsion angles (deg). Their estimated standard deviations (esd's) are 0.002–0.003 Å and 0.2–0.4°, respectively. (Bottom) Bond angles (deg); their esd's are 0.14–0.22°.

delocalization of electrons within the triazole ring. The N(4)–C(5) and N(4)–C(10) bond lengths correspond to bond orders 1.7 and 1.3, respectively, indicating additional, albeit smaller, delocalization within the seven-membered ring. Thus, on the basis of these bond lengths we can write three charged resonance forms, the first of which apparently contributes more to the structure than the fully conjugated one.



As mentioned before, the central bond in coformycin is shorter (1.364 Å) than C(9)–C(10) in the present structure, indicating more double bond character. The other bonds in the diazepine ring are equal in both structures (within one standard deviation). It appears, therefore, that the charged resonance form shown below contributes to the structure of coformycin.

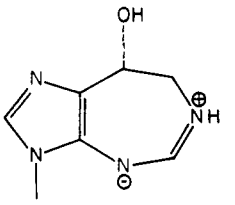


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Table II. Distances and Angles for Hydrogen Bonds

D A at	A at	distances, Å D···A	H···A	H···A_{corr}	angles, deg, D—H···A
N(6)—H···O(2′)	$-^1/_2 + x, {}^3/_2 - y, 1 - z$	2.872 (3)	2.10 (4)	1.85	168 (3)
O(2′)—H···N(1)	$^1/_2 + x, {}^1/_2 - y, 1 - z$	2.720 (2)	1.86 (4)	1.77	166 (3)
O(5′)—H···O(3′)	$x, -^1/_2 + y, {}^1/_2 - z$	2.816 (2)	1.90 (5)	1.86	168 (4)
O(3′)—H···O(8)	$^1/_2 - x, 1 - y, -^1/_2 + z$	2.738 (2)	1.86 (4)	1.77	173 (3)
O(8)—H···N(4)	$-^1/_2 + x, {}^3/_2 - y, 1 - z$	2.828 (2)	2.01 (4)	1.87	167 (4)

The bond angles in the seven-membered ring are also the same, within experimental error, as the corresponding ones in coformycin, but there are significant differences between the five-membered rings. They stem from the presence of the unsubstituted N(2) in the ring instead of the C(–H) atom in coformycin. As a result, the angle at N(2) is 4.2° smaller, the angles at N(1) and N(3) are 3.5–3.7° larger, while the angles at C(9) and C(10) are each 1.4° smaller. These values as well as the relationships between them are in good agreement with the results of a recent exhaustive analysis of the geometry of triazole rings.[25]

Glycosyl Linkage. The glycosyl torsion angle χ_{CN} [N(2)–N(3)–C(1′)–O(1′)] is 72.4° which is in the upper range of the anti conformation or the lower range of the high-anti conformation. This value, although identical with that in coformycin, is nevertheless surprising, because nucleosides with a nitrogen atom ortho to the glycosyl bond tend to have larger values of χ_{CN}.[26] With one exception,[27] χ_{CN} in 8-azapurine nucleoside analogues has been found in the range 102–114°.[27–29] The smaller torsion angle in the present structure can be attributed to the short N(2)···H(2′) distance of 2.39 Å. This distance, considerably shorter than the sum of van der Waals radii, would have been even shorter if χ_{CN} was larger. The N(2)···O(1′) distance of 2.969 Å is acceptable.[26] In coformycin the H(2)···H(2′) distance of 2.10 Å is somewhat short while the H(2)···O(1′) distance of 3.08 Å is quite long. In retrospect, one might be surprised that the angle χ_{CN} in coformycin is not smaller. In fact, the identical values of χ_{CN} in the two structures may be related to their activities as inhibitors of adenosine deaminase.

The relative sizes of the exocyclic angles at N(9) in purine and in 8-azapurine nucleosides are known to depend on χ_{CN}.[28] There is a large difference between the angles at N(3) in the present structure while in coformycin, with the same χ_{CN}, the angles are essentially equal. These observations can be explained on the basis of intramolecular interactions. First, the van der Waals radius of N(2) is smaller than that of C(2) in coformycin. Moreover, the large C(1′)–N(3)–C(10) angle increases the N(4)···H(1′) distance to an acceptable value of 2.50 Å. A similarly large angle in coformycin would push H(2′) too close to H(2). On the other hand, the N(4)···H(1′) contact is diminished by a longer N(3)–C(10) bond (1.391 Å).

Ribose Moiety. The furanose ring is in a half-chair conformation, corresponding very closely to a C(2′) endo/C(3′) exo ($^2T_3$) pucker. The phase angle of pseudorotation (P) is 174.7° and the maximum amplitude of puckering (τ_m) is 40.3°.[30] In contrast, the ribose ring in coformycin was found to have a C(1′) exo/C(2′) endo ($_1T^2$) pucker with P = 138.6°. The conformation of the side chain is also different in the two molecules: it is gauche$^+$ in coformycin and gauche$^-$ in this structure. The gauche$^-$ rotamer is the least stable of the three staggered ones found in nucleosides. While a gauche$^+$ rotamer would be destabilized in this structure by a repulsion between the electronegative atoms N(2) and O(5′) (see below), the side chain in coformycin could easily rotate to the gauche$^-$ conformation. Some evidence supporting the existence of this conformation in an enzyme-inhibitor complex is presented elsewhere.[26]

Table III. ^{1}H Chemical Shifts (in ppm) and ^{1}H–^{1}H Coupling Constants (in Hz)

H	δ(H)	H,H	$J_{H,H}$(exptl)	$J_{H,H}$(calcd)[a]	$J_{H,H}$(calcd)[b]
H(5)	7.350	5,7a	1.0		
H(7a)	3.463	7a,7e	–13.7		
H(7e)	3.597	7a,8	1.3		
H(8)	5.343	7e,8	4.7		
H(1′)	6.129	1′,2′	4.8	4.6	4.8
H(2′)	4.890	2′,3′	5.0	4.6	4.9
H(3′)	4.508	3′,4′	4.9	4.8	4.9
H(4′)	4.236	4′,5′	3.3		
H(5′)	3.823	4′,5″	5.0		
H(5″)	3.716	5′,5″	–12.5		

[a] Assuming an equilibrium mixture of 48% of C(3′) endo (P = 9°) and 52% of C(2′) endo (P = 162°) conformations. [b] Assuming a mixture of 48% of C(3′) endo/C(4′) exo (P = 36°) and 52% of C(2′) endo/C(3′) exo (P = 175°) conformations.

All bond lengths have values commonly found in ribonucleosides. The observed endocyclic bond angles can be compared with calculated ones for P = 174.7° and τ_m = 40.3°.[31] The angle at C(3′) differs from the calculated value (101.9°); the other angles are within 0.6° of calculated ones.

Hydrogen Bonding and Packing. Each molecule has five potential proton donors, and all of them participate in intermolecular hydrogen bonds. The hydrogen bond network can be represented schematically as follows:

N(6)—H···O(2′)—H···N(1)

O(5′)—H···O(3′)—H···O(8)—H···N(4)

The distances and angles are shown in Table II. As commonly observed in X-ray analyses, the N–H and O–H bonds appear shorter than their real values. By extending the covalent bond lengths to their nominal values (1.04 and 0.97 Å, respectively) one obtains corrected H···A distances which reflect more accurately the strengths of these hydrogen bonds.

With eight potential acceptors of the five protons, it is interesting to examine which of them are not being used in the crystal structure. Of those, O(1′) rarely participates in hydrogen bonds, being an ether oxygen. N(2) is shielded by hydrogen atoms attached to C(2′) and C(5′). The fact that N(4) acts as a hydrogen bond acceptor, rather than O(5′), may reflect its partial negative charge, as shown (above) in the resonance structure.

The packing of the molecules in the crystal is shown in Figure 4. There are distinct hydrophobic and hydrophilic regions parallel to x, consisting respectively of the aglycon and sugar moieties. The bases are seen to be stacked perpendicular to x at an approximate distance of 3.5 Å, and there is partial overlap between adjacent ones.

Solution Conformation. The results of the NMR analysis are shown in Table III. A simulated spectrum is indistinguishable from the experimental (Figure 5). The chemical shifts of the ribose protons are very similar (within 0.1 ppm) to those recorded for 8-azaguanosine in D_2O.[32] However, compared to guanosine,

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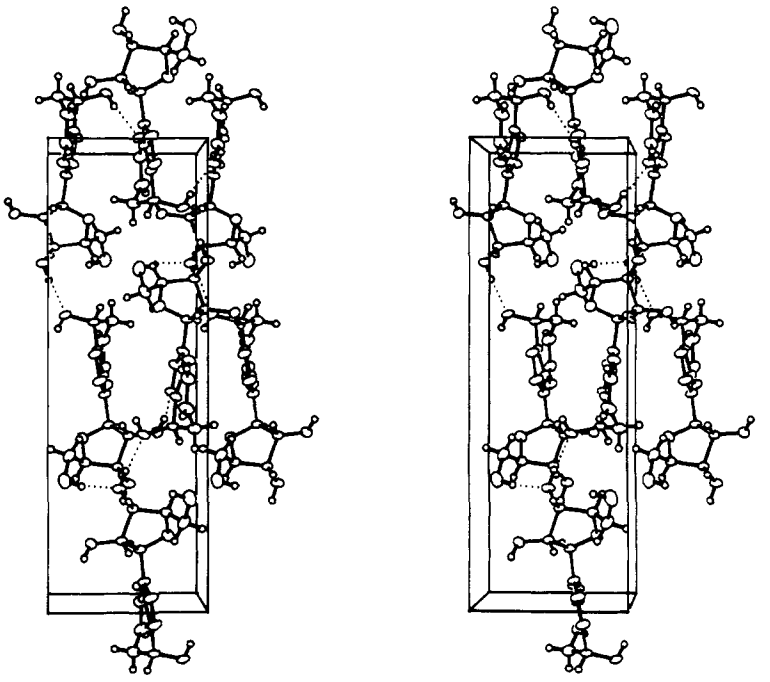

Figure 4. Stereoscopic view of the molecular packing in the crystal. Some hydrogen bonds are indicated by dotted lines.

H(1′) and H(2′) are shifted downfield by 0.3–0.4 ppm and H(3′) and H(4′) by 0.1–0.2 ppm. This effect has been shown to reflect a shift in the syn ⇌ anti equilibrium in purine nucleosides.[33] However, the substitution of C(8) in a purine by a nitrogen atom also affects the chemical shifts of the sugar protons, and it is difficult to evaluate quantitatively the extent of each influence. It should be pointed out that no 8-azapurine nucleoside has ever been found in a syn conformation in the solid state.

The conformation of the diazepine ring was determined from the coupling constants listed in Table III. We used a relationship, derived by Haasnoot et al.,[34] which takes into account the electronegativities of substituents. From the $^3J_{7a,8}$ value we calculated a torsion angle of 64° while the value of $^3J_{7e,8}$ yielded a torsion angle of −51°. The agreement with the torsion angles found in the X-ray analysis (60° and −56°, respectively) is remarkably good, and we can state with confidence that the seven-membered ring assumes the same conformation in solution and in the solid state.

The solution conformation of the ribose ring was analyzed with the help of coupling constants calculated by de Leeuw and Altona.[35] In view of the observed τ_m (40.3°, see above), the values calculated for τ_m = 40° were used. The first approach was based on the usual assumption that the conformational equilibrium consists of C(3′) endo (P = 9°) and C(2′) endo (P = 162°) ring puckers.[35] The mole fraction of the C(3′) endo conformer was calculated to be 0.46 from $J_{1'2'}$ and 0.49 from $J_{3'4'}$. The near-equality of $J_{2'3'}$ for the two conformations gives rise to large errors in this calculation, resulting in an impossible value of −1. In the second approach it was assumed that one of the conformers in the equilibrium is that found in the solid state (P = 175°) and the second one is C(3′) endo/C(4′) exo (P = 36°). Given the high value of χ_{CN} in this structure (72.4°), a C(3′) endo pucker would be associated with a rather short distance between N(2) and H(3′). This interaction can be alleviated by a pseudorotation of the ring toward higher P values. The mole fraction of the C(3′) endo/C(4′) exo conformation was calculated to be 0.48 from both $J_{1'2'}$ and $J_{3'4'}$; the result from $J_{2'3'}$ (0.6) was within experimental error of the other values. Coupling constants calculated on the basis of each of the above assumptions are shown in Table III. The results show clearly that the second assumption (equilibrium of P = 36° and 175°) is in excellent agreement with experimental data while the first one is not. Two conclusions can be drawn from this analysis: (1) interpretation of NMR data on the basis of "standard" or "average" conformations may lead to inaccurate results; (2) a combination of X-ray and NMR analyses may be

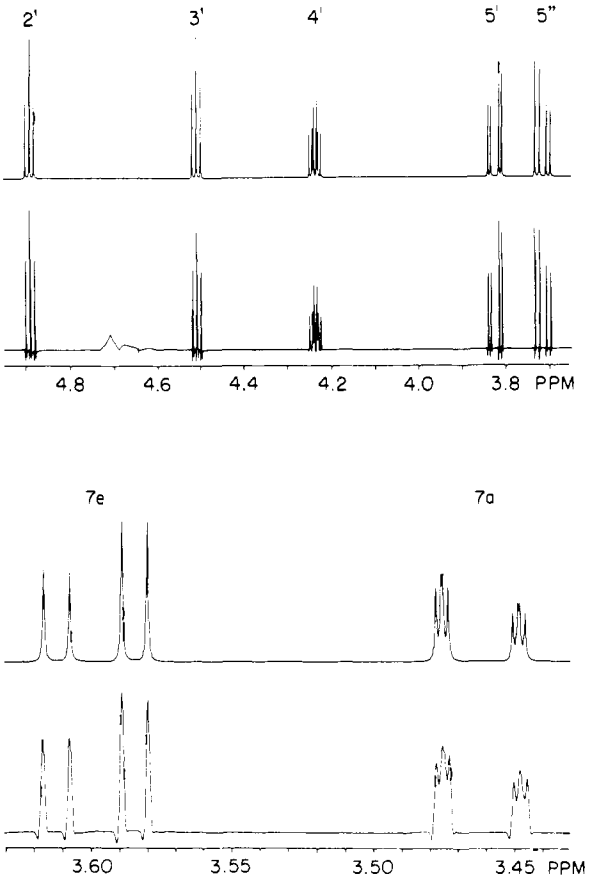

Figure 5. Experimental (lower) and simulated (upper) ^{1}H NMR spectra of 2-azacoformycin in D_2O (310 K). The HDO resonance at 4.65 ppm was saturated.

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necessary to produce reliable results.

The rotamer populations of the $-CH_2OH$ side chain were calculated according to equations 4.11-4.13 of Davies.[36] The results are as follows: 45% gauche$^+$, 36% trans, and 19% gauche$^-$. As usual, the gauche$^+$ conformation predominates, but its contribution is not as large as in purine nucleosides. These results can be attributed to electrostatic repulsion between N(2) and O(5'). The distance between these two atoms would be very short (<3.0 Å) if the furanose ring was in the C(2') endo/C(3') exo and the side chain in the gauche$^+$ conformation. Consequently, the gauche$^+$ rotamer can occur only when the ring pucker is C(3') endo/C(4') exo.

Acknowledgments. Apart from MULTAN78,[18] all crystallographic computations were carried out with programs written by Ahmed et al.[37] Figures 1 and 4 were drawn with the ORTEP program of Johnson.[38] Haasnoot's program CAGPLUS, which relates vicinal coupling constants to torsion angles between protons,[34] was used to determine the conformation of the diazepine ring.

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Supplementary Material Available: Tables of anisotropic temperature parameters and a list of observed and calculated structure amplitudes (8 pages). Ordering information is given on any current masthead page.

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βI- and βII-Turn Conformations in Model Dipeptides with the Pro-Xaa Sequences

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Abstract: Model dipeptides *t*-BuCO-L-Pro-Xaa-NHMe (Xaa = L- or D-Leu, Val, Cys, Met, Phe, and Tyr) with an aliphatic, aromatic, or weakly polar Xaa side chain have been investigated in solution by IR and ^1H NMR spectroscopies and in the solid state by X-ray diffraction. The heterochiral dipeptides L-Pro-D-Xaa are found to accommodate the same βII-turn conformation in both states. The homochiral dipeptides L-Pro-L-Xaa experience more conformational freedom since the β-turn conformation is shown to be of the βI type in solution and of the βII type in the crystal. This conformational change probably arises from the molecular packing forces and essentially from an intermolecular hydrogen bond between the Xaa NH and C'O groups of two neighboring molecules. This shows that the βII-turn conformation is a stable disposition of a LL-dipeptide sequence provided there is some energy compensation through an intermolecular interaction in oligopeptides or a long-range interaction in larger peptides.

β-Folded regions or β-turns are conformational units of primary importance for the three-dimensional structure of peptides and proteins.[2,3] Four consecutive amino acid residues indexed from i to $(i + 3)$ are arranged in such a way that the peptide chain is folded back on itself, giving rise to the globular character of the proteins.

Ten types of peptide chain reversals have been found in crystallized proteins.[4] Three of them containing an intramolecular hydrogen bond of the $(i + 3) \rightarrow i$ type are denoted βI-, βII-, and βVI-turns, and they differ by the rotational angles associated with the $(i + 1)$ and $(i + 2)$ residues.[5] The βVI-turn is also characterized by the cis disposition of the amide bond between the $(i + 1)$ and $(i + 2)$ residues, and it exclusively concerns L-Xaa-L-Pro[3] and L-Xaa-Me-L-Yaa sequences.[6,7]

The biological importance[2] and the frequent occurrence of β-turns in proteins[3] (32% residues are involved in a β-turn) and peptides[8] have justified the considerable amount of statistical, theoretical, and experimental work dealing with this particular conformational unit.[2] After the first experimental characterization of a β-turn in the crystal structures of cyclohexaglycine[9] and ferrichrome A,[10] Venkatachalam performed pioneering conformational calculations in which atoms were taken as hard spheres.[11] He concluded that different stereochemical sequences defined by the $(i + 1)$ and $(i + 2)$ residues should correspond to different β-turns. Homochiral LL sequences and heterochiral LD sequences should prefer the so-called βI and βII dispositions, respectively. The achiral glycyl residue G was predicted to equally accomodate both the L and D dispositions. The enantiomeric sequences should of course favor the symmetrical dispositions denoted as βI'- (DD) and βII'-turns (DL). These conclusions were then corroborated by more sophisticated calculations using empirical,[12-17] semi-

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