crack upon standing. Specimens used for data collection were coated with an epoxy cement. Even so, of the 2873 reflections measured out to $2\theta = 100°$ for the calcium salt, only 1226 were "observed." In view of the limited data, refinements were terminated ($R = 0.18$) after a few cycles of isotropic least-squares analysis (anisotropic Ca and O atoms). Somewhat larger crystals ($0.3 \times 0.34 \times 0.18$ mm) were obtained for the similarly unstable orthorhombic Cd salt. Of the 3481 reflections measured out to $2\theta = 115°$, 2554 were "observed." Only the 1729 most intense reflections were used for least-squares refinements. Program limitations also precluded simultaneous refinement of all atomic parameters in any least-squares cycle. At early stages of refinement only the coordinates were refined; thereafter the coordinates and temperatures factors of different halves of the molecule alternately were refined, then fixed during consecutive cycles.

The R factor mentioned above and throughout the text is the conventional index $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. The quantity minimized during least-squares analysis was $\Sigma w(|F_o|^2 - |F_c|^2)^2$ with $w = 1/\sigma^2$. Atomic scattering factors, including the anomalous components for cadmium, were taken from the International Tables for X-ray Crystallography.[7]

Spectroscopy. The 70-eV electron-impact low-resolution mass spectra were obtained on an AEI MS-902 double-focusing mass spectrometer using a direct insertion solid probe with a source temperature ~160 °C above ambient. Data were acquired via frequency-modulated analog tape, which was subsequently processed on a PDP-11 computer using published programs.[8] Manual peak matching of masses was done with the wide-range accessory of the MS-902.

^{1}H NMR spectra were measured on a Varian Associates XL-100-15 100-MHz spectrometer. Dr. G. Gray of Varian Associates obtained the 25.16-MHz proton-decoupled and off-resonance ^{13}C NMR spectra on a XL-100-12 Fourier transform spectrometer. We are grateful to Professor Joseph Fried of the University of Chicago for obtaining the 270-MHz ^{1}H NMR spectrum.

Supplementary Material Available: Tables of atomic coordinates for the Cd monoclinic crystal structure, temperature factors, and observed and calculated structure factors for each of the three crystal structures (49 pages). Ordering information is given on any current masthead page.

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A Novel Zwitterionic Structure and an Unusual Sugar Ring Conformation in 5-Iodo-5′-amino-2′,5′-dideoxyuridine, an Antiviral Nucleoside[1]

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Abstract: The three-dimensional structure of 5-iodo-5′-amino-2′,5′-dideoxyuridine, a potent inhibitor of herpes simplex virus, was determined by X-ray crystallography. The crystals belong to the orthorhombic space group $P2_12_12_1$ and the cell dimensions are $a = 7.892$ (1), $b = 9.332$ (1), and $c = 15.749$ (2) Å. Intensity data were measured with a diffractometer and the structure was solved by the heavy-atom method. Least-squares refinement, which included hydrogen atoms, converged at $R = 0.047$. The structure is zwitterionic, with a protonated 5′-NH_2 group and a negative charge on N(3) in the pyrimidine ring. The glycosyl bond is in the anti conformation ($\chi_{CN} = 53.6°$) and the exocyclic -$CH_2NH_3^+$ group is gauche-trans. The deoxyribose ring has the unusual O(1′) endo pucker. ^{1}H NMR spectroscopy was used to determine the conformation in solution. The spectra indicate an anti conformation about the glycosyl bond and equal contributions of the three staggered side-chain rotamers. The sugar ring may consist of a 36:64 equilibrium mixture of ^3E and ^2E conformers. It is pointed out, however, that the conventional interpretation may be inadequate, and that there may be a significant contribution of an O(1′) endo puckered ring.

Modification of the structure of nucleosides has produced anticancer, antiviral, and antibacterial agents.[3a-e] Examples of alterations of the base moiety include 5-fluoro-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, 5-ethyl-2′-deoxyuridine, 5-(2-iodovinyl)-2′-deoxyuridine, 6-azauridine, and 5-azacytidine, whereas alteration of the pentose moiety has produced compounds such as 1-β-D-arabinofuranosylcytosine, 9-β-D-arabinofuranosyladenine, 1-β-arabinofuranosylthymine, (*S*)-9-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA), and 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine).

Nucleosides in which a sugar hydroxyl had been replaced by an amino group had been shown to possess biological activity.[4] 5′-Amino-5′-deoxythymidine and 3′-amino-3′-deoxythymidine were found to have antiviral and antineoplastic activity, respectively.[5] Since the thymidine analogue 5-iodo-2′-deoxyuridine is a potent antiviral agent, the corresponding 5′-amino analogue of 5-iodo-2′-deoxyuridine (5-iodo-5′-amino-2′,5′-dideoxyuridine; AIU, AIdUrd) as well as a number of other 5- and 5′-substituted analogues were synthesized by Lin et al.,[5,6] and AIU was found to have good antiviral activity with an unusual lack of toxicity to mammalian cells and experimental animals. Therefore, it is of considerable interest to obtain details of the structure and conformation of AIU, both in the crystal as well as in solution.

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Table I. Final Atomic Parameters and Their Standard Deviations

A. Nonhydrogen Atoms[a]

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
N(1)	4565 (11)	5076 (6)	4917 (4)	32 (4)	11 (2)	18 (3)	5 (2)	−2 (3)	−1 (3)
C(2)	4432 (13)	4849 (8)	5807 (5)	40 (6)	11 (3)	19 (4)	9 (3)	0 (4)	−4 (4)
O(2)	4488 (12)	3594 (7)	6062 (4)	73 (6)	22 (3)	29 (3)	6 (3)	−5 (4)	9 (4)
N(3)	4286 (9)	5978 (8)	6324 (4)	22 (5)	23 (3)	21 (3)	−3 (3)	−1 (3)	−6 (3)
C(4)	4305 (10)	7348 (9)	6027 (5)	17 (5)	29 (4)	19 (4)	−7 (3)	1 (3)	4 (3)
O(4)	4114 (11)	8330 (8)	6559 (4)	71 (6)	29 (3)	22 (3)	−13 (3)	1 (4)	2 (4)
C(5)	4494 (14)	7556 (8)	5126 (5)	37 (5)	14 (3)	21 (4)	8 (3)	6 (4)	2 (4)
I(5)	4649.7 (10)	9651.7 (6)	4675.8 (4)	62.3 (3)	17.9 (2)	33.4 (3)	5.0 (3)	5.8 (4)	0.6 (3)
C(6)	4658 (13)	6440 (6)	4614 (5)	39 (4)	7 (2)	19 (4)	2 (3)	4 (5)	−3 (3)
C(1′)	4738 (14)	3868 (9)	4338 (6)	37 (5)	21 (4)	27 (4)	−8 (3)	−5 (5)	−8 (4)
O(1′)	6287 (8)	4064 (7)	3880 (4)	21 (3)	31 (3)	24 (3)	−12 (3)	−1 (3)	3 (3)
C(2′)	3371 (13)	3718 (12)	3681 (6)	17 (5)	50 (6)	36 (5)	−16 (5)	9 (4)	−4 (4)
C(3′)	4321 (14)	3304 (9)	2853 (6)	48 (7)	20 (3)	26 (4)	−5 (3)	6 (5)	−3 (4)
O(3′)	3795 (11)	1950 (8)	2511 (5)	55 (5)	31 (4)	48 (4)	−27 (4)	−3 (4)	−4 (4)
C(4′)	6124 (13)	3158 (9(	3159 (5)	42 (5)	15 (3)	16 (4)	−1 (3)	−6 (4)	−2 (4)
C(5′)	7437 (11)	3621 (10)	2526 (6)	18 (5)	24 (4)	26 (4)	−3 (3)	−8 (4)	7 (3)
N(5′)	9124 (9)	3771 (5)	2907 (5)	21 (4)	20 (3)	22 (3)	−2 (3)	3 (3)	−1 (3)

B. Hydrogen Atoms[b]

atom	x	y	z	U	atom	x	y	z	U
H(6)	445 (23)	653 (21)	400 (13)	15 (7)	H(5′)	696 (15)	451 (15)	218 (7)	6 (4)
H(1′)	481 (16)	327 (15)	463 (8)	6 (4)	H(5″)	744 (15)	320 (16)	194 (8)	6 (4)
H(2′)	308 (18)	471 (18)	354 (10)	8 (5)	H(N5′)	1010 (24)	407 (23)	249 (13)	11 (7)
H(2″)	259 (13)	305 (13)	385 (7)	4 (3)	H(N5″)	916 (20)	477 (20)	316 (11)	9 (6)
H(3′)	405 (14)	422 (13)	237 (7)	4 (3)	H(N5‴)	958 (22)	279 (22)	318 (13)	11 (7)
H(4′)	637 (10)	226 (9)	322 (5)	1 (2)					

[a] All coordinates were multiplied by 10^4 and all thermal parameters by 10^3. The thermal parameters are expressed as $\exp[-2\pi^2(U_{11}h^2a^{*2} + \ldots + 2U_{23}klb^*c^* + \ldots)]$. [b] All coordinates were multiplied by 10^3 and all thermal parameters by 10^2.

Experimental Section

The title compound, $C_9H_{12}N_3O_4I$, was prepared as described by Lin et al.[6] and crystallized from water. The colorless prisms have a rhombic cross-section and belong to the orthorhombic space group $P2_12_12_1$. There are four molecules in a unit cell with dimensions a = 7.892 (1), b = 9.332 (1), and c = 15.749 (2) Å. The observed and calculated crystal densities are 2.00 and 2.02 g cm^{-3}, respectively. A crystal measuring 0.15 × 0.20 × 0.22 mm was mounted along the prism (b) axis on a Picker four-circle diffractometer, and intensities were measured with Nb-filtered Mo Kα radiation. There were 1544 unique reflections with $2\theta < 55°$, of which 1341 (87%) were considered observed. The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary in view of the regular shape of the crystal (μ = 27.4 cm^{-1}).

The structure was determined by the heavy-atom method and the parameters of the nonhydrogen atoms were refined with anisotropic temperature parameters by block-diagonal least squares. All hydrogen atoms, except the one attached to O(3′), were located on difference Fourier maps and refined with isotropic temperature parameters. It should be emphasized that *three* hydrogen atoms were found attached to N(5′), with reasonable distances and bond angles, while no hydrogen atom was found within bonding distance of N(3). The refined positions and temperature parameters confirmed the presence of a protonated amino group. All scattering factors were taken from the "International Tables for X-Ray Crystallography"[7] and the iodine curve was corrected for anomalous dispersion. A weighting scheme was chosen which 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 0.16σ, the largest one (for a hydrogen atom) was 0.83σ, and the final value of R was 0.047. The precision of our results, as expressed by the estimated standard deviations (esd's) from the least-squares refinement, is, to our knowledge, higher than that achieved in previous X-ray analyses of 5-iodo or 5-bromo nucleosides. The coordinates and temperature parameters, as well as their standard deviations, are listed in Table I.

The 270-MHz ^{1}H NMR spectra of Me_2SO-d_6 solutions (10 mg/cm^3) were obtained using the Bruker HX-270 system of the Southern New England High Field Facility at New Haven, Conn., which is equipped with a BNC data system and is capable of performing 16K transforms. The spectra were recorded at 25 °C and chemical shifts are reported relative to internal Me_4Si with an accuracy of ±0.001 ppm. A local version of the LAOCOON program was used to analyze the spectra.

Results

X-ray Analysis. Previous crystal-structure analyses have shown nucleotides to exist in zwitterionic form, with a positively charged base and a negative phosphate group.[8] The structure of AIU also turned out to be zwitterionic, but with a positive charge on N(5′) and a negative charge on N(3). To our knowledge, this is the first crystal structure of a uridine derivative in which a negative charge resides within the pyrimidine ring. Consequently, it is of considerable interest to examine what effect this has on the geometry of that ring. While the presence of the heavy iodine atom precludes a detailed analysis, some general features emerge which are in agreement with theoretical predictions. A formal negative charge at N(3) would be expected to increase the delocalization of electrons from the nitrogen to the adjacent carbonyl groups. This should result in shorter C—N and longer C=O bonds. In uridine[9] (two independent molecules) and in 5-methyluridine[10] these C—N bonds are in the range 1.37–1.39 Å, while the range for the C=O bonds is 1.20–1.23 Å. In our structure (Figure 1) we found the C—N bonds to be 1.34 and 1.36 Å, while the lengths of the C=O bonds are 1.24 and 1.25 Å. In cytosine derivatives it has been observed[11] that protonation at N(3) causes the ring bond angle at that atom to increase by 4–5°, while the angles at C(2) and C(4) decrease by the same amount. By analogy, one would expect deprotonation of a uracil derivative to have the opposite effect, and this is indeed what we observe. The angle at N(3) is 4–5° smaller than the corresponding angles in uridine and 5-methyluridine, while the angles at C(2) and C(4) are 3–5° larger. Although some individual deviations are statistically not significant, the combination of all observed changes leaves no doubt about the validity of our conclusions.

The six atoms of the pyrimidine ring are coplanar within

Table II. Least-Squares Planes and Deviations of Atoms from Them[a]

plane 1[b]		plane 2[b]	
atom	Δ, Å[c]	atom	Δ, Å[c]
N(1)	0.020	C(1′)	0.016
C(2)	−0.015	C(2′)	−0.028
N(3)	−0.002	C(3′)	0.023
C(4)	0.009	C(4′)	−0.013
C(5)	0.004	O(1′)*	0.543
C(6)	−0.024	C(5′)*	0.790
C(1′)*	−0.002		
O(2)*	−0.081		
O(4)*	0.058		
I(5)*	−0.077		

[a] Atoms marked with an asterisk were not included in the calculation of the plane. [b] Plane 1: $0.9945X + 0.0165Y + 0.1030Z - 4.4798 = 0$. Plane 2: $0.1464X + 0.9598Y - 0.2396Z - 2.3581 = 0$. [c] Esd's are 0.001 Å for I(5) and 0.007–0.011 Å for other atoms.

Table III. Torsion Angles (Degrees)

C(1′)-C(2′)-C(3′)-C(4′)	+4.1
C(2′)-C(3′)-C(4′)-O(1′)	−26.9
C(3′)-C(4′)-O(1′)-C(1′)	+40.5
C(4′)-O(1′)-C(1′)-C(2′)	−37.2
O(1′)-C(1′)-C(2′)-C(3′)	+19.6
O(1′)-C(1′)-N(1)-C(6)	+53.6
C(2′)-C(1′)-N(1)-C(6)	−64.7
C(2′)-C(1′)-N(1)-C(2)	+120.3
O(1′)-C(1′)-N(1)-C(2)	−121.4
O(3′)-C(3′)-C(4′)-C(5′)	+94.9
O(1′)-C(4′)-C(5′)-N(5′)	+49.0
C(3′)-C(4′)-C(5′)-N(5′)	+167.0

0.02 Å (Table II). In contrast to many other nucleosides,[12] C(1′) lies in the same plane. On the other hand, O(2), O(4), and I(5) are significantly displaced.

The conformation of the deoxyribose ring is an almost perfect envelope with an O(1′) endo pucker.[13] The phase angle of pseudorotation[14] (P) is 84.2°. The displacement of O(1′) from the mean plane through the other four ring atoms amounts to 0.543 Å (Table II). The glycosyl bond is, as usual, in the anti conformation, the χ_{CN} angle of 53.6° being within the normally observed range. The conformation of the exocyclic $-CH_2NH_3^+$ is gauche-trans. Although the gauche-gauche conformation is favored by $-CH_2OH$ groups, a substantial decrease of this rotamer was observed in 5′-NH analogues of thymidine nucleosides.[15] The gauche-trans conformation has been frequently observed in crystal structures, including the structures of 5-chloro-2′-deoxyuridine[16] and 5-bromo-2′-deoxyuridine,[17] and may be stabilized by intermolecular hydrogen bonds. A stereoscopic view of the molecule is shown in Figure 2 and the torsion angles are shown in Table III.

There are four protons in the molecule capable of forming hydrogen bonds and there are, in fact, four intermolecular hydrogen bonds in the crystal structure. The protonated amino group donates two protons to the two carbonyl groups and the third one to the negatively charged N(3). O(4) also accepts a proton from O(3′). The distances are given in Table IV.

It has been noted before[18] that 5-halogenated uracils and uridines have increased propensities for base stacking, an iodine substituent having the largest effect. As shown in Figure 3, stacking interactions do occur between molecules related by twofold screw axes parallel to x.

NMR Analysis. The assignments of the various resonances in the NMR spectra were made by homonuclear decouplings, comparison with previously published data,[19] and computer

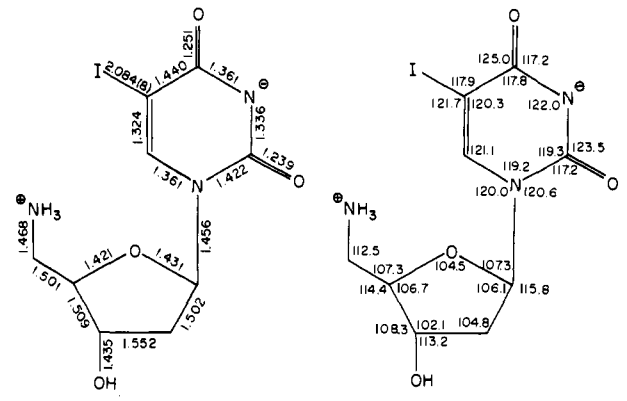

Figure 1. Molecular geometry: (left) bond lengths; eds's are 0.008–0.015 Å, (right) bond angles; esd's are 0.6–0.8°.

Table IV. Distances and Angles for Hydrogen Bonds

	distances, Å		angles, deg	
	D···*A*	H···*A*	*D*-H···*A*	H-*D*···*A*
N(5′)-H···O(2)[a]	2.76	1.8	152	17
N(5′)-H···N(3)[b]	2.80	1.9	142	24
N(5′)-H···O(4)[c]	2.83	1.8	171	6
O(3′)-H···O(4)[d]	2.76			

[a] At $1/2 + x$, $1/2 - y$, $1 - z$. [b] At $3/2 - x$, $1 - y$, $-1/2 + z$. [c] At $1/2 + x$, $3/2 - y$, $1 - z$. [d] At $1/2 - x$, $1 - y$, $-1/2 + z$.

Table V. Proton Chemical Shifts and Coupling Constants for AlU[a]

$\delta_{1'}$	6.06	$J_{1'2'}$	6.7
$\delta_{2'}$	2.19	$J_{1'2''}$	6.4
$\delta_{2''}$	2.08	$J_{2'2''}$	−13.5
$\delta_{3'}$	4.19	$J_{2'3'}$	6.7
$\delta_{4'}$	3.69	$J_{2''3'}$	3.3
$\delta_{5'}$	2.75	$J_{3'4'}$	3.8
$\delta_{5''}$	2.75	$J_{4'5'}$	4.9[b]
δ_6	8.43	$J_{4'5''}$	4.9
		$J_{5'5''}$	−12.6[c]

[a] Chemical shifts in parts per million from Me_4Si (Me_2SO); coupling constants in hertz. [b] Due to chemical-shift equivalence only the sum of coupling constants is significant. [c] Arbitrary; when $\delta_i \simeq \delta_j$, the spectrum is independent of J_{ij}.

line-shape simulations. The results are presented in Table V. The assignments for H(2′) and H(2″) were based on the observations of the near equalities $J_{1'2''} \simeq J_{2'3'}$ and $J_{2''3'} \simeq J_{3'4'}$,[20] and the inequality $J_{1'2'} > J_{1'2''}$.[21] Owing to the near magnetic equivalence of H(5′) and H(5″), only the sum of the coupling constants ($\Sigma = J_{4'5'} + J_{4'5''}$) could be derived from the spectrum. As shown in Figure 4, the computer-simulated spectrum is a good reproduction of its experimental counterpart.

The conformation about the glycosyl bond is predominantly anti, as inferred from the value of $\delta_{2'}$. According to Schweizer et al.,[22] the H(2′) shift in pyrimidine nucleosides constrained in the syn conformation (e.g., 6-methyluridine) is displaced downfield by about 0.6 ppm relative to its resonant position in anti nucleosides. Our value of 2.19 ppm is very similar to that in 2′-deoxythymidine in Me_2SO (2.10 ppm), found to be in anti conformation.[23] On the other hand, in 6-methyl-2′-deoxyuridine (in Me_2SO) the value of $\delta_{2'}$ was found to be 2.71 ppm.[23,24]

The conformation of the deoxyribose ring may be assessed by making the usual assumption of a C(2′) endo ⇌ C(3′) endo equilibrium. The percentage of the 3′-endo conformer in the equilibrium mixture can be estimated with the formula %

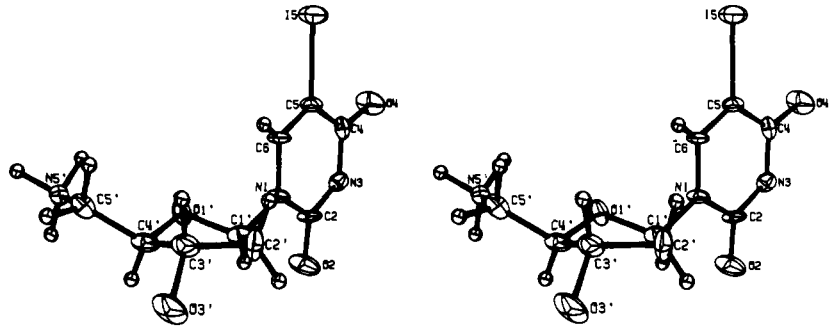

Figure 2. Stereoscopic view of AIU; the ellipsoids include 50% probability.

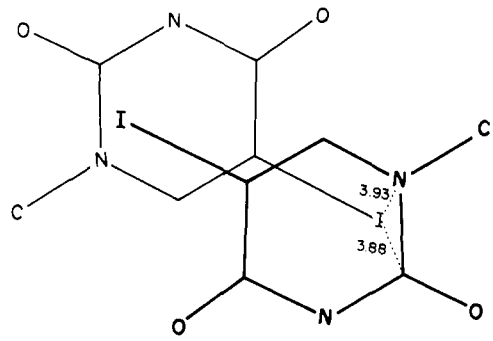

Figure 3. Stacking of uracil rings.

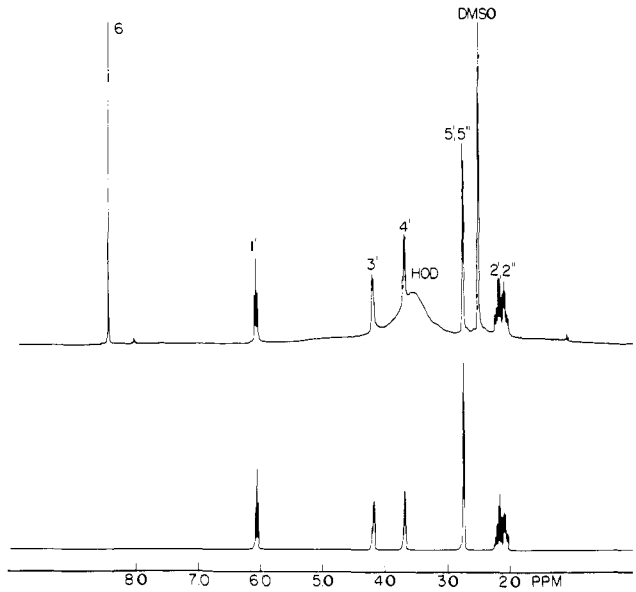

Figure 4. NMR spectrum of AIU (top) and computer-simulated spectrum (bottom).

3′-endo = $100J_{3'4'}/(J_{1'2'} + J_{3'4'})$,[26] which yields a value of 36%. It should be noted that the coupling constants (and hence the conformation[27]) in AIU are almost identical with those in deoxyuridine.[19a] While this may, at first, appear reasonable, it is not in agreement with the expected effect of 5-halo substitution. It has been observed that substitution at C(5) by a halogen tends to increase the population of the C(3′) endo conformer, the iodine substituent having the largest effect.[28] In the case of uridine, iodination increases the percentage of the 3′-endo conformer from 44 to 66%. We would, therefore, expect an ~60% contribution of the 3′-endo conformer in the solution conformation of AIU. The ring conformation will be further discussed below.

The conformation about the exocyclic C(4′)-C(5′) bond may be estimated by the use of the expressions:

$$\text{gauche-gauche} = (13 - \Sigma)/10$$

$$\text{gauche-trans} = (J_{4'5''} - 1.5)/10$$

which have been derived for $-CH_2OH$ side chains.[20] These equations indicate that each of the three staggered rotamers is equally represented in solution.[29] Thus, the concentration of the gauche-gauche rotamer is relatively low (in view of its usual predominance), and it is not surprising that the gauche-trans rotamer was found in the crystal structure.

Discussion

The presence of a uracil ring with a negative charge on N(3) is of interest not only because of its novelty in a crystal structure, but also because such a species has been implicated in the mechanism by which 5-halogenated uracil nucleosides are incorporated into DNA. Such incorporation gives rise to AT-GC transitions, i.e., the ionized uracil base mispairs with a guanine residue rather than with an adenine (Figure 5a). The loss of the proton from N(3) would be facilitated by the presence of the electronegative halogen atom at C(5). This scheme, originally suggested by Lawley and Brookes,[30] is one of several which have been put forward to explain the mispairing of 5-halogenated uracils with guanine. Another popular model involves a tautomeric shift in the uracil base.[31] A transition from the usual keto tautomer to an enol would enable a uracil to mispair with guanine (Figure 5b), again aided by 5-halogenation.

Bugg and Sternglanz[32] cite supporting evidence for these two models as well as experimental results which contradict them. Bugg's model[18,32,33] invokes stacking patterns of 5-halogenated uracils that have been observed in numerous crystal structures. A conformational change within the strand is required to permit this pattern between the 5-halogenated uracil and its nearest intrastrand neighbor to be established. Such distortion would then permit the uracil to pair with a guanine, albeit via a single hydrogen bond (Figure 5c). It is probably fair to state that none of the proposed models has been proven beyond any doubt. Consequently, the present structure analysis which provides details of an ionized 5-halogenated uracil should be useful in future discussions of this subject.

Another unusual result of this structure analysis is the O(1′) endo conformation of the deoxyribose ring in the crystal. On the basis of X-ray analyses, Sundaralingam showed long ago[34] that the preferred conformations of ribose and deoxyribose rings in nucleosides and nucleotides are C(2′) endo and C(3′) endo. A subsequent compilation showed that of 98 ribo- and deoxyribonucleosides and nucleotides, only 7 have sugar puckers outside the ranges P = 0-36° (^3E) and P = 144-180° (^2E).[35] Theoretical calculations have confirmed the presence of energy minima in these ranges of the pseudorotational phase angle and have also indicated an energy barrier of ca. 4 kcal mol^{-1} at P = 90°.[35] Thus, it is not surprising that to this date

Table VI. Observed and Calculated Conformational Parameters[a]

protons	J_{obsd}	ϕ_{NMR}[b]	$\phi_{X\text{-}ray}$[c]	$J_{X\text{-}ray}$[d]	$J_{36:64}$[e]	$J_{25:55:20}$[e]
1′2′	6.7	138	142	7.6	6.5	7.1
1′2″	6.4	41	20	10.0	6.2	6.9
2′3′	6.7	39	3	11.3	5.3	6.5
2″3′	3.3	121	125	4.1	4.0	3.5
3′4′	3.8	124	148	8.8	3.8	4.4

[a] The following Karplus equation was used in all calculations: $J_{ij} = 11.7 \cos^2 \phi - 0.4 \cos \phi$. All coupling constants in hertz, all dihedral angles in degrees. [b] Dihedral angles calculated from J_{obsd}. [c] Dihedral angles from X-ray analysis. [d] Coupling constants calculated from $\phi_{X\text{-}ray}$. [e] Calculated coupling constants for indicated populations of ^3E, ^2E, and ^0E conformations (see text).

only few crystal structures are known in which the sugar ring has an O(1′) endo pucker,[36] and in only one of them[36b] is the furanose ring attached to a normal base (guanine). The suggestions of Altona and Sundaralingam[37] have been generally adopted in NMR analyses, and the spectra have been interpreted as stemming from equilibrium mixtures of C(3′) endo (type N) and C(2′) endo (type S) conformers. More recently, however, it was pointed out[19c] that 8 out of the 23 deoxynucleosides and mononucleotides compiled by Sundaralingam[38] have sugar ring puckers outside the usual ^3E and ^2E ranges. This indicates a flatter potential energy well for the deoxyribose conformers than had been previously assumed. NMR studies of 6-methyluracil nucleosides and nucleotides have led Hruska and his colleagues to the conclusion that in this series of compounds the 2′-deoxyribose ring assumes conformations which differ from the pure type N and type S conformers.[23] Based on the results of their X-ray analysis of the tetranucleotide d-pApTpApT, Viswamitra et al. concluded that the sugar pucker is a "soft" parameter with a low energy barrier between different conformations.[39] Finally, Levitt and Warshel[40] recently calculated the total energies of the ribose and deoxyribose rings, using the consistent force field method. Their results showed that the energy barrier between the C(3′) endo and C(2′) endo conformers amounts to only 0.6 kcal mol^{-1}. Consequently, it appears that NMR analyses should not a priori exclude all conformers other than the two most stable ones. It is therefore pertinent to consider whether the O(1′) endo conformation, which was found in the crystal structure of AIU, contributes to the conformational equilibrium in solution.

In Table VI we show the observed coupling constants and the dihedral angles which can be derived from them by the application of the formula $J_{HH} = 11.7 \cos^2 \phi - 0.4 \cos \phi$.[21] A comparison with the torsion angles obtained from the X-ray analysis[41] shows that, while the differences in the angles are not large, clearly the solution conformation is not exclusively O(1′) endo. If it was, the expected coupling constants would differ substantially from the observed ones, up to 5.0 Hz for $J_{3'4'}$. As noted above, the formula generally used for estimating the relative contributions of C(3′) endo and C(2′) endo conformers to the N $\rightleftharpoons$ S equilibrium indicates an equilibrium mixture of 36% C(3′) endo and 64% C(2′) endo. But how good is this estimate? Cheng and Sarma[19c] recently calculated coupling constants for pentose rings in various envelope conformations. Using their values for ^3E and ^2E envelopes and the 36:64 ratio, we obtain the J_{HH} values listed in the sixth column. These values differ by an average of 0.5 Hz from the observed ones, the largest discrepancy being 1.4 Hz.[42] On the other hand, let us assume a 25:55 ratio for the ^3E and ^2E envelopes as well as a 20% contribution of the O(1′) endo conformation which was found in the crystal structure. The J_{HH} values for the latter are those calculated from our HH torsion angles. The coupling constants for this equilibrium mixture are shown in the last column of Table VI. Their average deviation from observed values is 0.4 Hz and the largest deviation is only 0.6 Hz. This equilibrium mixture thus appears at least as likely as the conventionally derived one.[43] We do not wish to claim, however, that this postulated equilibrium necessarily represents the true conformation in solution. Our aim rather is to suggest that the conventional interpretation of the NMR spectra of pentose rings may be inadequate to detect unusual ring conformations. Such conformations should be borne in mind, however, particularly when their presence is indicated by an X-ray analysis.

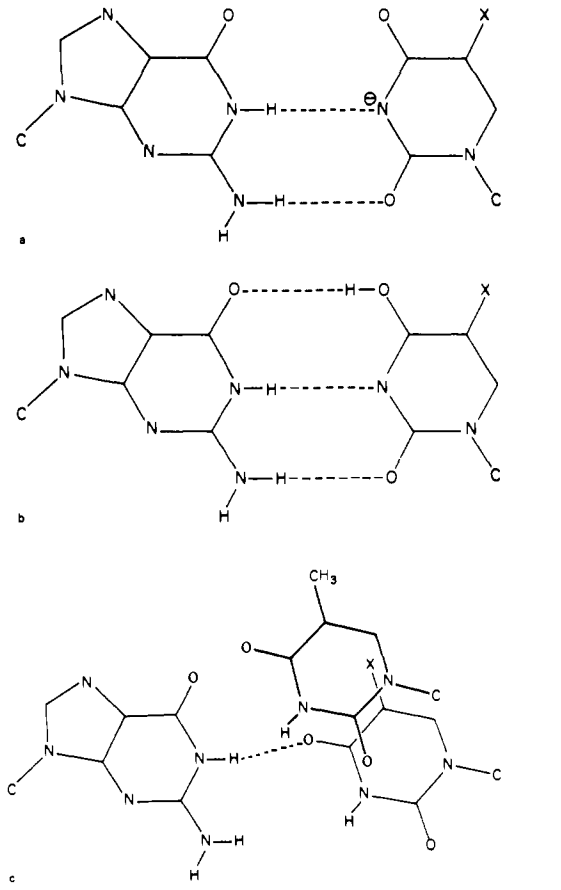

Figure 5. Models for mispairing between 5-halogenated uracil derivatives and guanine residues: (a) involving an ionized uracil ring; (b) involving an enol tautomer of the uracil ring; (c) involving stacking interactions of 5-halogenated uracil rings.

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Supplementary Material Available: A listing of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

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Biosynthesis of Camptothecin. 3. Definition of Strictosamide as the Penultimate Biosynthetic Precursor Assisted by ^{13}C and ^{2}H NMR Spectroscopy

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Abstract: The biosynthesis of camptothecin (**1**) is shown to involve tryptamine (**2**) and secologanin (**3**) by radioactive precursor feeding experiments with *Camptotheca acuminata* apical cuttings. The incorporation of radioactive strictosidine (**4**), but not vincoside (**16**), into **1** is consistent with the results of other studies that have proven **4** to be the key biogenetic precursor of monoterpene indole alkaloids found in other higher plants. Strictosamide (**5**) is the penultimate biosynthetic precursor of **1** in vivo as demonstrated by validation of the stereo- and regiospecific incorporation of [5-^{13}C]- and [14-^{2}H]**5a** with ^{13}C and ^{2}H NMR spectroscopic analysis. These two experiments serve to define the lower limits for the successful use of stable isotopes in biosynthetic studies in plants. Three possible explanations for the unexpected low loss of ^{3}H during the incorporation of [14-^{3}H,5-^{14}C]**5a** into **1** in vivo are examined experimentally. Since **1** is shown to be labeled only at C-14 by [14-^{2}H,^{3}H]**5a**, and since only a 5–9% loss of ^{3}H from C-14 of **5a** is observed regardless of the labeling stereochemistry of the C-14 diastereotopic hydrogens, it is concluded that the loss of ^{3}H from C-14 is by a nonstereospecific process. Several literature precedents and the results of oxidation of [14-^{3}H,5-^{14}C]**5** with DDQ to the [^{3}H,^{14}C]indolopyranoquinolizinones, **25** and **26**, in vitro are consistent with the low tritium loss being due to a significant isotope effect associated with a nonenzymatic step during the D ring aromatization of **5** in vivo.

A study of the biosynthesis of camptothecin (**1**), a pyrrolo[3,4-*b*]quinoline alkaloid found originally in the mainland China tree, *Camptotheca acuminata* Decne, and later in *Mappia foetida* Miers[4] and *Ophiorrhiza mungos* L.,[5] has been underway in our laboratory since 1971. The results of several initial feeding experiments with isotopically labeled precursors

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