Crystal Structure and Molecular Conformation of Formycin Monohydrates. Possible Origin of the Anomalous Circular Dichroic Spectra in Formycin Mono- and Polynucleotides†

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ABSTRACT: The crystal structure of formycin monhydrate, a *C*-glycosyl antibiotic, has been determined using 937 intensities measured on a diffractometer. The compound crystallized in the orthorhombic space group $P2_12_12_1$ with unit cell dimensions $a = 17.962$, $b = 9.217$, and $c = 7.512$ Å. The structure was solved by direct methods and refined by full-matrix least squares to a final R index of 0.035. Formycin adopts a glycosyl torsion angle χ of 109.8°, which is in a range intermediate between the classical anti and syn regions (Donohue, J., and Trueblood, K. N. (1960), *J. Mol. Biol. 2*, 363), while the χ (212°) in formycin hydrobromide (N(1) protonated) (Koyama, G., Maeda, K., Umezawa, H., and Iitaka, V. (1966), *Tetrahedron Lett. 6*, 592) was in the classical syn region. The ribose is in the C(2′)-endo–C(1′)-exo ($^2T_1$) twist conformation which is commonly observed in nucleosides exhibiting large χ angles or the syn conformation. The conformation about the C(4′)–C(5′) bond is gauche–trans. The latter conformation and the intermediate χ angle in formycin are mainly a result of electrostatic repulsion between the negatively charged base nitrogen atom N(8) and the sugar O(5′) atom. This is in contrast to the stabilization of the anti and gauche–gauche conformation in the *common* 5′ nucleotides by electrostatic attraction between the phosphate and base H(8). In 5′-formycin monophosphate (FMP) and poly(F) the conformational features found in formycin are expected to predominate. The observed negative Cotton effect of FMP and poly(F) has been attributed to the syn conformation by Ward and Reich (Ward, D. C., and Reich, E. (1968), *Proc. Nat. Acad. Sci. U. S. 61*, 1494). The intermediate (anti–syn) χ angle may also explain the anomalous spectral properties of these compounds in addition to allowing them to be substrates to enzymes which require the anti conformation. With the exception of N(8) all of the potential hydrogen bonding sites are involved in hydrogen bonding. Interestingly, O(1′) is involved in an unusually strong hydrogen bond to the amino group of an adjacent nucleoside. The bases are stacked with considerable overlap, but the stacking interactions are probably very weak owing to the fairly large interplanar separation (3.755 Å).

Formycin is a *C*-ribosyl nucleoside antibiotic which is cytotoxic and its biological properties have been intensely investigated ever since its discovery by Hori *et al.* (1964). It has been shown to inhibit several aspects of purine metabolism (Henderson *et al.*, 1967). Specifically, it inhibits phosphoribosyl pyrophosphate synthesis presumably at the level of phosphoribose pyrophosphokinase. Ward and coworkers (Ward and Reich, 1968; Ward *et al.*, 1969) have demonstrated that formycin can substitute effectively for adenosine in a wide variety of enzyme reactions (*e.g.*, adenosine kinase, adenosine deaminase, hexokinase, myokinase, phosphoenolpyruvate kinase, etc.). They have also shown that tRNA molecules with formycin (F) at the 3′ termini are aminoacylated and can transfer amino acids into growing polypeptide chains in protein synthesis. These workers have also pointed out several paradoxical properties of formycin nucleotides and polynucleotides. Specifically, copolymers containing F and adenosine (A) or uridine (U) can effectively code for polypeptides whereas poly(F) fails to code for any polypeptide. Secondly, FTP exhibits anomalous behavior in several reactions catalyzed by RNA polymerase. FTP is poorly utilized for homopolymer synthesis and unlike ATP, FTP is not polymerized with poly(U) or poly(dT) as templates. Thirdly, poly(F) is found to be degraded 100–10,000 times more slowly by nucleases specific for poly(A), whereas it is degraded by minute amounts of pancreatic RNase which is known to be specific for C or U. Ward and coworkers (1968, 1969) have attributed the anomalous properties of formycin to its tendency to adopt the syn conformation. It may be noted that Koyama *et al.* (1966) found that in the crystal structure of formycin hydrobromide, the molecules exhibit the syn conformation with a glycosyl torsion angle χ_{CN} of 212°. Our interest in investigating the crystal structure of formycin monohydrate was not only to elucidate the conformation of neutral formycin but also to provide detailed information on the molecular geometry. This information will be of help in understanding the rotational barriers about the C–C glycosyl bond.

Experimental Section

Crystals of formycin monohydrate were obtained by slow evaporation of an aqueous ethanol solution. Weissenberg and oscillation photographs established the crystal to be orthorhombic. The systematic absences $h00$, $h = 2n + 1$, $0k0$, $k = 2n + 1$, $00l$, $l = 2n + 1$, indicated the space group to be $P2_12_12_1$. The unit cell dimensions were found to be $a = 17.962$ (3), $b = 9.217$ (2), and $c = 7.512$ (1) Å as determined

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by a least-squares refinement of the angles 2θ, ω, and χ of 12 reflections measured in the 2θ range of 40–60° on a Picker FACS1 diffractometer. The measured density of 1.523 g cm^{-3} by flotation in CCl_4–cyclohexane agrees well with the calculated value of 1.523 g cm^{-3} for four formycin monohydrate molecules in the unit cell.

Three-dimensional intensity data were collected using the θ–2θ scan technique up to $2\theta = 127°$ using Ni-filtered Cu Kα radiation. The crystal used had the following dimensions: $0.15 \times 0.15 \times 0.3$ mm^3 and it was mounted parallel to the c axis. The intensities of 1119 reflections were measured and these were corrected for Lorentz and polarization effects. The standard deviation $\sigma(I)$ was computed using counting statistics and an electronic instability factor of 0.02 (Stout and Jensen, 1968). A reflection was considered observed if $I > 1.5\sigma(I)$. This criterion yielded 937 reflections which were used in the structure analysis.

Structure Determination. The structure was solved by the application of direct methods as applied to the noncentrosymmetric case (Karle and Karle, 1966). The Σ_2 relations were set up for 168 reflections with $|E| > 1.4$ where E values are the normalized structure amplitudes. The reflections which were chosen to define the origin and the enantiomorph are given in Table I in the microfilm edition (see title footnote). The phases of seven two-dimensional and four three-dimensional reflections (Table I) were obtained employing the symbolic addition procedure. The above 15 phases were then used in the tangent formula (Karle and Hauptman, 1956) to generate the phases of 85 additional reflections whose E values were greater than 1.6. The resultant $R(E)$ $[=(|\Sigma|E_{obsd}| - |E_{calcd}||)/\Sigma|E_{obsd}|]$ was 0.24. The E map calculated with these phases revealed the purine ring and the atoms N(6) and C(1′) attached to it. Next the phases of 168 reflections with $|E| > 1.4$ derived from the partial structure were refined using the tangent formula. The refinement converged in nine cycles giving an $R(E)$ of 0.18. The E map calculated at this stage gave the 20 nonhydrogen atoms of the structure among the 22 strongest peaks in the map.

Structure Refinement. The positional coordinates of these 20 atoms and their isotropic temperature factors were subjected to two cycles of a full-matrix least-squares refinement using the program written by Busing *et al.* (1962) and modified for our use on the Univac 1108 computer by Rao (1968).[1] This, followed by two cycles of refinement using anisotropic temperature factors, lowered the R $[=(\Sigma||F_{obsd}| - |F_{calcd}||)/\Sigma|F_{obsd}|]$ value to 0.06. A difference electron density map computed at this stage revealed the positions of all the 15 hydrogen atoms in the structure. Two cycles of isotropic refinement of the hydrogen atoms lowered the R value to 0.055. The initial weighting scheme based on counting statistics was replaced at this point by a Hughes (1941) type weighting scheme, where $\sigma(F_{obsd}) = 2.35$ for $|F_{obsd}| < 36.17$ and $\sigma(F_{obsd}) = 0.023 \times |F_{obsd}| + 1.55$ for $|F_{obsd}| > 36.17$. The latter scheme was based on the error function, $||F_{obsd}| - |F_{calcd}||$ *vs.* $|F_{obsd}|$. Convergence of all positional and thermal parameters was achieved after two more cycles of isotropic refinement of the hydrogen atoms and two cycles of anisotropic refinement of the nonhydrogen atoms. The final R value was 0.035 for 937 observed reflections and 0.044 for all the 1119 measured reflections. The final average shift/σ ratios in the atomic parameters were 0.14 and 0.19 for the nonhydrogen and hydrogen atoms respectively, with corresponding maximum values of 0.40 and 0.78.

[1] Rao, S.T. (1968), unpublished work.

TABLE IIA: Positional Parameters of Atoms in Formycin.[a]

Atom	*X*	*Y*	*Z*
N(1)	1556 (2)	4,388 (4)	3741 (6)
C(2)	1444 (2)	5,835 (5)	3659 (8)
N(3)	1936 (2)	6,887 (4)	3505 (5)
C(4)	2654 (2)	6,390 (4)	3655 (6)
C(5)	2829 (2)	4,937 (4)	3784 (6)
C(6)	2252 (2)	3,887 (5)	3865 (6)
N(6)	2380 (2)	2,468 (3)	4055 (6)
N(7)	3584 (2)	4,846 (4)	3884 (5)
N(8)	3901 (2)	6,189 (3)	3820 (5)
C(9)	3350 (2)	7,135 (4)	3680 (6)
C(1′)	3487 (2)	8,742 (4)	3662 (6)
O(1′)	3339 (2)	9,301 (3)	1910 (4)
C(2′)	4295 (2)	9,203 (4)	4012 (5)
O(2′)	4516 (1)	9,122 (3)	5806 (4)
C(3′)	4317 (2)	10,730 (4)	3208 (5)
O(3′)	4044 (1)	11,796 (3)	4430 (4)
C(4′)	3774 (2)	10,612 (4)	1633 (5)
C(5′)	4169 (2)	10,505 (5)	−120 (6)
O(5′)	3659 (2)	10,291 (3)	−1555 (4)
O(W)	202 (2)	2,820 (3)	3453 (5)
H(W-1)	53 (3)	339 (6)	365 (8)
H(W-2)	27 (2)	233 (5)	255 (6)
H(2)	88 (2)	613 (5)	343 (7)
H(61)	280 (2)	218 (4)	440 (6)
H(62)	195 (3)	189 (6)	437 (9)
H(7)	385 (2)	409 (4)	366 (5)
H(1′)	311 (2)	922 (4)	455 (5)
H(2′)	463 (2)	856 (4)	329 (5)
H(O-2′)	422 (2)	941 (4)	628 (5)
H(3′)	484 (2)	1,095 (3)	273 (5)
H(O-3′)	444 (2)	1,196 (4)	518 (5)
H(4′)	346 (2)	1,157 (4)	153 (5)
H(51′)	453 (2)	976 (5)	−16 (6)
H(52′)	451 (2)	1,139 (4)	−23 (5)
H(O-5′)	344 (2)	1,101 (4)	−177 (5)

[a] Positional parameters of nonhydrogen atoms have been multiplied by 10^4. Positional parameters of hydrogen atoms have been multiplied by 10^3. Standard deviations in parentheses refer to the least significant digits.

The scattering factors for carbon, nitrogen, and oxygen were those of Cromer and Waber (1965) while those for hydrogen were from Stewart *et al.* (1965). The positional coordinates are listed in Table IIA while the anisotropic thermal parameters for the nonhydrogen atoms and the isotropic thermal parameters for the hydrogen atoms are given in Table IIB of the microfilm edition of this volume of the journal. The observed and calculated structure factors (Table III) are published in the microfilm edition of this volume of the journal. The thermal ellipsoids of the atoms including the hydrogen-bonded water molecule with the atom numbering are shown in Figure 1 which was drawn using the program written by Johnson (1965).

Results and Discussion

Geometry of the Base. The bond distances and angles are shown in Figure 2. The bond lengths and angles in the py-

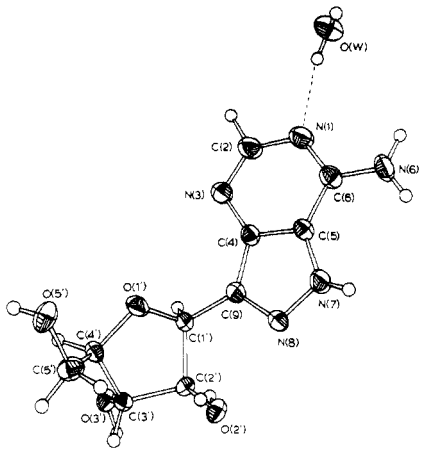

FIGURE 1: The ellipsoids of thermal vibrations of the nonhydrogen atoms. We have used here a numbering system analogous to the adenine base in order to facilitate comparisons with the normal purine nucleosides. See also other numberings (Patterson *et al.*, 1960).

rimidine portion of the purine ring compare favorably with those found in other unprotonated adenine derivatives: *e.g.*, 3′-*O*-acetyladenosine (Rao and Sundaralingam, 1970), 2′-amino-2′-deoxyadenosine (Rohrer and Sundaralingam, 1970), deoxyadenosine monohydrate (Watson *et al.*, 1965; Lin and Sundaralingam[2]), adenosine (Lai and Marsh, 1972), and tubercidin (Stroud, 1972; Abola and Sundaralingam, 1972). The largest differences occur around N(3). In formycin, the C(2)–N(3) bond is shorter by 0.03 Å and hence exhibits a greater amount of double bond character, while the N(3)–C(4) bond is longer by 0.03 Å. The C(2)–N(3)–C(4) angle in formycin is about 1.0° greater than those of the normal nucleosides.

The molecular dimensions of the diazole ring of formycin are considerably different from those found for the imidazole ring in adenine derivatives. Many adenine analogs are modified in the five-membered ring and it is of interest to compare the geometry of the five-membered ring in some of these analogs. In Table IV, the bond distances and angles in the diazole ring of formycin (**1**) are compared to the diazole ring of allopurinol (**2**) (Prusiner and Sundaralingam, 1972), the triazole ring of 8-azaguanine (**3**) (Sletten *et al.*, 1968), the pyrrole ring of tubercidin (**4**) (Stroud, 1972; Abola and Sundaralingam, 1972), the neutral imidazole ring of 3′-*O*-acetyladenosine (**5**) (Rao and Sundaralingam, 1970), and the protonated imidazole ring system of hypoxanthine-HCl (**6**) (Sletten and Jensen, 1969). It is seen that there are significant differences in bond angles and distances in these various five-membered heterocyclic rings with positional variations in the

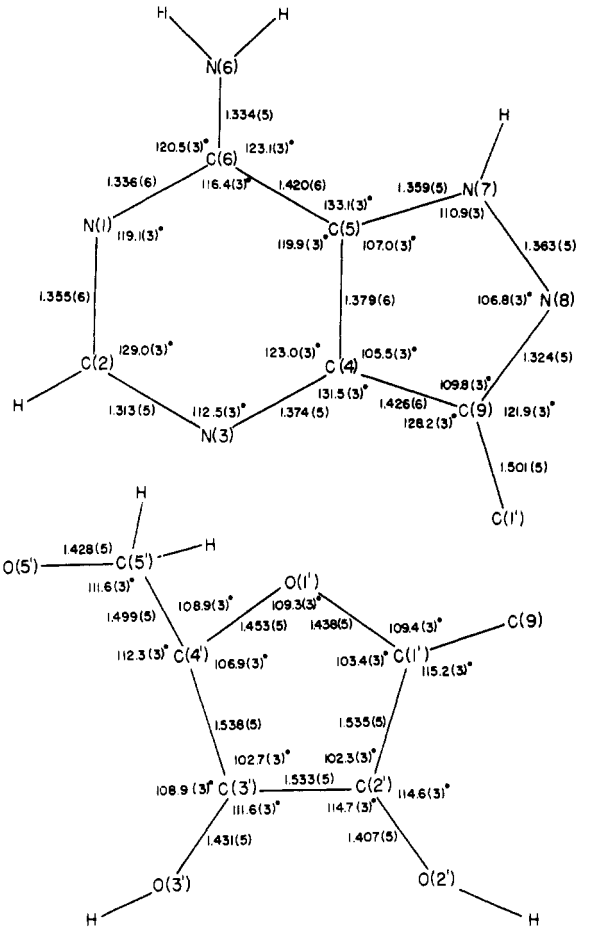

FIGURE 2: The bond lengths and bond angles involving the nonhydrogen atoms.

TABLE IV: Comparison of the Bond Distances and Angles in the Modified Imidazole Rings of Formycin Monohydrate (**1**), Allopurinol (**2**), 8-Azaguanine (**3**), Tubercidin (**4**), the Neutral Imidazole Ring of 3′-*O*-Acetyladenosine (**5**), and the Protonated Imidazole Ring of Hypoxanthine HCl (**6**).[a]

	Å					
Bond	1	2	3	4	5	6
C(4)–C(5)	1.379	1.395	1.383	1.403	1.378	1.368
C(5)–X(7)	1.359	1.414	1.361	1.433	1.406	1.376
X(7)–X(8)	1.363	1.325	1.303	1.359	1.314	1.318
X(8)–X(9)	1.324	1.374	1.359	1.400	1.367	1.333
C(4)–X(9)	1.426	1.338	1.350	1.370	1.380	1.375
σ bond	0.005				0.006	
	Degrees					
Angle	1	2	3	4	5	6
C(4)–C(5)–X(7)	107.0	104.6	109.2	107.1	110.7	107.7
C(5)–X(7)–X(8)	110.9	110.4	108.1	106.8	103.7	107.7
X(7)–X(8)–X(9)	106.8	106.4	108.2	109.9	113.5	110.0
C(5)–C(4)–X(9)	105.5	107.8	103.9	108.4	105.5	106.3
C(4)–X(9)–X(8)	109.8	110.7	110.3	107.8	106.6	108.4
σ angle	0.3				0.3	

[a] Note:

Compd	X(7)	X(8)	X(9)
1	N–H	N	C–ribose
2	C–H	N	N–H
3	N	N	N–H
4	C–H	C–H	N–ribose
5	N	C–H	N–ribose
6	N–H	C–H	N–H

[2] Lin, G. H-Y., and Sundaralingam, M. (1972), manuscript in preparation.

nitrogen atoms. The endocyclic angles C(5)–N(7)–N(8) and C(4)–C(9)–N(8) of formycin are larger by 2–3° while the N(7)–N(8)–C(9) angle is smaller by 3° when compared to the imidazole ring of hypoxanthine-HCl (**6**). A similar trend is exhibited in allopurinol (**2**) and 8-azaguanine monohydrate (**3**) in which C(8)–H is also replaced by N(8). Thus, in compounds **1**, **2**, and **3** there is a decrease in the endocyclic angle at N(8) with a concomitant increase in the two adjacent endocyclic angles at positions 7 and 9.

The presence or absence of hydrogen atoms at the 7, 8, and/or 9 positions also influences the values of the corresponding endocyclic angles. It has already been shown (Sundaralingam and Jensen, 1965; Singh, 1965; Rao and Sundaralingam, 1970) that protonation of N(1) in adenine systems results in a significant increase in the C(2)–N(1)–C(6) bond angle. A comparison of hypoxanthine and 3′-*O*-acetyladenosine indicates that protonation of N(7) in hypoxanthine results in an increase of 4° in the C(5)–N(7)–C(8) bond angle and a decrease of 4° in the adjacent C(4)–C(5)–N(7) and N(7)–C(8)–N(9) angles. These changes in the imidazole ring angles are accompanied by small changes in bond distances. In contrast, when the protonated N(7) atom of hypoxanthine is replaced by a C(7)–H as in tubercidin only small changes occur in the ring angles, but significant differences occur in the bond distances.

The exocyclic angles C(4)–C(9)–C(1′) (128.2° *vs.* 124.3° and 126.6°), N(8)–C(9)–C(1′) (121.9° *vs.* 130.0° and 127.2°), and N(3)–C(4)–C(9) (131.5° *vs.* 126.7° and 126.4°) show important differences between formycin and the normal purine nucleosides adenosine (Lai and Marsh, 1972) and guanosine (Thewalt *et al.*, 1970). These differences in angles and the increase in the bond distances C(1′)–C(9) and C(9)–C(4) in formycin would reduce the barrier to rotation about the C–C glyosyl bond (Yathindra and Sundaralingam, 1973[3]).

In general the difference in the molecular dimensions between the purine analogs and the normal purine nucleosides is a consequence of the combined effect of the number of N atoms and their positional distribution as well as the differences in protonation sites.

The bond distances and angles involving the hydrogen atoms are in the usual range and they are summarized in Table V in the microfilm edition.

Deviations from Planarity of the Base. Table VI (published in microfilm edition) shows the deviations of the atoms from the least-squares planes through the base atoms. The pyrimidine ring atoms N(1), C(6), and N(3) show significant displacements (0.028, 0.033, and 0.038 Å, respectively) from the base plane; the former two atoms are displaced on the same side of the ring while the third is on the opposite side. The substituent atoms N(6) and C(1′) are also displaced significantly (0.084 and 0.052 Å, respectively) and lie on the same side of the ring plane as N(1) and C(6). The dihedral angle between the five-membered diazole ring and the six-membered pyrimidine ring is 1.4°.

Geometry of the Sugar. The bond angles and distances in the ribose moiety of formycin are generally in agreement with those normally found in nucleosides and nucleotides having C(2′) endo puckering (Sundaralingam, 1965, 1972; Sundaralingam and Jensen, 1965). It may be noted that even for the *C*-glycosyl nucleoside the C(1′)–O(1′) bond distance is significantly shorter than the C(4′)–O(1′) bond distance. In this regard, perhaps a major difference between formycin and the normal *N*-glycosyl derivatives is that the C(1′)–O(1′) bond

[3] N. Yathindra and M. Sundraralingam, to be published.

TABLE VIII: Torsional Angles in Formycin Monohydrate.[a,b]

		Deg
Glycosyl angle	χ	109.8
	τ_0	−30.6
Sugar	τ_1	39.4
Ring	τ_2	−33.1
Angles	τ_3	15.7
	τ_4	9.4
Phase angle of pseudorotation	P	148.3
Maximum amplitude of pseudorotation	τ_{max}	39.6

[a] Sugar puckering C(2′)-endo–C(1′)-exo ($^2T_1$). Conformation about C(4′)–C(5′) is gauche–trans. [b] For definitions and notations of torsion angles see Sundaralingam (1969).

distance in formycin (1.438 Å) is close to or slightly larger than the standard C–O bond distance (1.427 Å) (Venkateswarlu and Gordy, 1955), whereas in the *N*-glycosyl derivatives the C(1′)–O(1′) bonds exhibit some double bond character (Sundaralingam, 1965, 1968; Sundralingam and Jensen, 1965). Similar trends in these so called anomeric bonds have been observed for showdomycin (Tsukuda and Koyama, 1970) and α-pseudouridine monohydrate (Rohrer and Sundaralingam, 1970), both of which are *C*-glycosyl nucleosides.

It is well known that the bond angles in particular (to a less extent the bond distances) in the sugar are influenced by the mode of puckering (or conformation) of the furanose ring (Sundaralingam, 1965; Sundaralingam and Jensen, 1965). In formycin, the endocyclic angle O(1′)–C(1′)–C(2′) is 103.4° which is smaller than the value observed in other nucleosides. This difference is attributed to both the ring conformation ($^2T_1$) which is slightly different from the other commonly observed modes of C(2′) endo puckering, *viz.* $^2T_3$ (see below), and the *C*-glycosyl linkage. Apart from the angles around C(1′), the largest variation between the endocyclic and exocyclic angles is associated with the atom (C(2′)) that shows the greatest puckering (Sundaralingam, 1965).

Table VII (published in the microfilm edition) shows the deviations of the atoms from the five-atom and the "best" four-atom least-squares planes. The mode of puckering belongs to the general C(2′) endo category. The puckering with respect to the three-atom (O(1′)–C(4′)–C(3′)) plane is C(2′)-

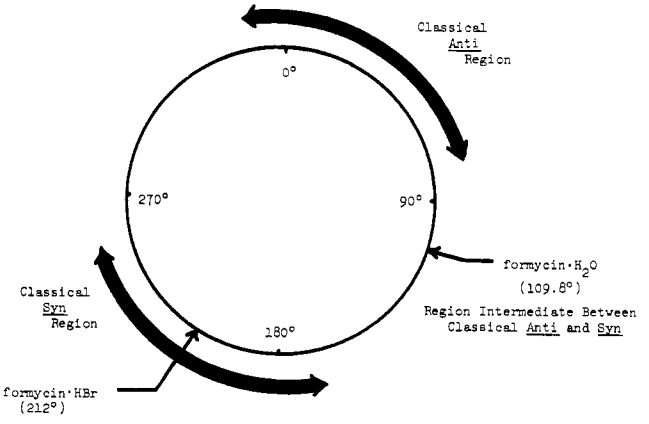

FIGURE 3: A conformation circle showing the classical ranges for the χ angles.

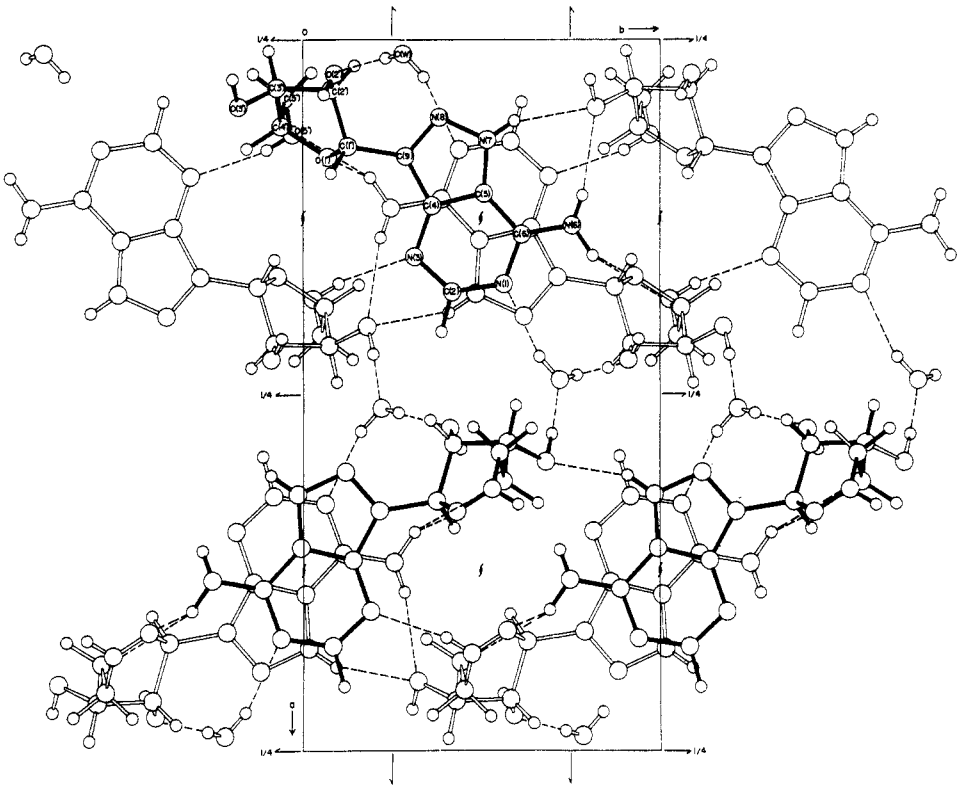

FIGURE 4: A view along the *c* axis showing the crystal packing and hydrogen bonding.

endo-C(1′)-exo ($^2T_1$) (Sundaralingam, 1965, 1972). The ring torsion angles together with the pseudorotation parameters P (phase angle) and τ_{max} (maximum amplitude of puckering) for the ribose ring are shown in Table VIII. Similar values for the latter parameters have been found in other β nucleosides (Altona and Sundaralingam, 1972).

The conformation about the C(4′)–C(5′) bond is gauche–trans (Sundaralingam, 1965; Shefter and Trueblood, 1965), $\phi_{OO} = 57.6$, $\phi_{OC} = 175.8°$. Although this is one of the plausible conformations for the nucleosides, apparently it is not preferred for the common 5′ nucleotides (Sundaralingam, 1972; Rubin *et al.*, 1972).

Conformation of Formycin. One of the interesting features in formycin monhydrate is the *C*-glycosyl torsion angle χ (109.8°) which lies in the range intermediate (anti–syn) to the classical anti and syn conformations (Donohue and Trueblood, 1960) (Figure 3). A low value for the χ angle in formycin is apparently not favored because of electrostatic repulsion between the negatively charged nitrogen atom N(8) (which replaces the C(8) of adenine) and the sugar O(5′) atom. This repulsion can be alleviated if the *C*-glycosyl torsion angle assumes a high or intermediate value or the C(5′)–O(5′) bond rotates away from the generally favored gauche–gauche conformation to the gauche–trans or trans–gauche. It seems, therefore, that base modification influences not only the favored conformation about the glycosyl bond but also the sugar–phosphate backbone. The above conformational features in the formycin nucleoside can be extrapolated to formycin 5′-phosphate and polyformycin. Thus, the favored conformation for 5′-FMP and the individual residues of formycin in poly(F) would be expected to be different from the standard anti and gauche–gauche conformations favored for the common nucleotides and nucleic acids (Sundaralingam, 1972, 1973; Rubin *et al.*, 1972; Yathindra and Sundaralingam, 1973).

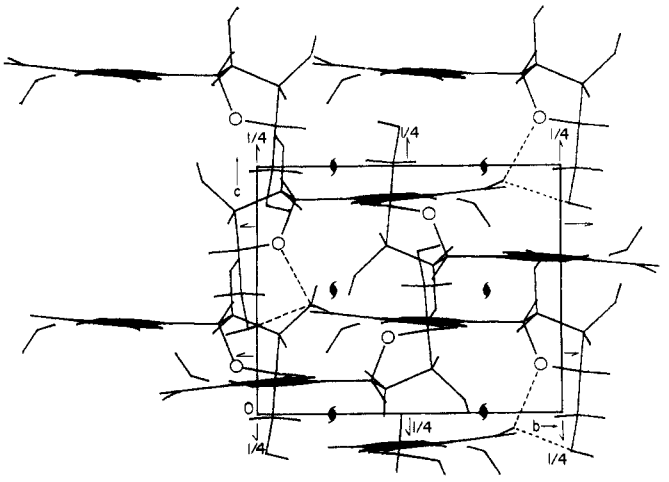

FIGURE 5: A view along the *a* axis showing the base stacking and the hydrogen bond involving O(1′).

Optical rotatory dispersion (ORD) studies on poly(F) have revealed a curious "inverted spectra" for poly(F) relative to poly(A). It is known that circular dichroic (CD) and ORD spectra are very sensitive to the orientation of the chromophore relative to the sugar ring. Thus, Ward and Reich (1968) have attributed the reversed Cotton effects to the adoption of the classical syn conformation by the individual formycin residues in poly(F). It may be possible that the favored intermediate (anti–syn) χ angle for formycin found in the present work (and possibly also the change in the base dipole orientation arising from interchanging the N(9) and C(8) of adenine by C(9) and N(8) in formycin) can provide an explanation for

the anomalous spectral properties of formycin mono- and polynucleotides. Furthermore, the fact that formycin can assume the high anti or intermediate (anti–syn) χ angles renders it also susceptible to the action of adenosine deaminase which is found to be inactive on systems in the classical syn conformation (Ogilive *et al.* 1971).

It is interesting that reversed Cotton effects have also been observed for polynucleotides containing inosine and guanosine residues (Mitsui *et al.*, 1970; Warshaw and Cantor, 1970), and both of them have also been found to adopt these intermediate χ values in crystal structures (Thewalt *et al.*, 1970; Sundaralingam, 1969).

Hydrogen Bonding and Crystal Packing. Figure 4 shows a view along the *c* axis displaying the crystal packing and hydrogen bonding pattern. Nine independent hydrogen bonds are observed in the crystal structure of formycin monohydrate and their bond angles and distances are given in Table IX. All potential hydrogen bond donors and acceptors have been found to be involved except N(8). This is in contrast to allopurinol (Prusiner and Sundaralingam, 1972) and 8-azaguanine (Sletten and Jensen, 1969) both of which utilize N(8) in hydrogen bonding.

Of interest is the fact that O(1′) is engaged in a hydrogen bond with the amino function of a screw-related molecule shown in Figure 5 which is a projection diagram along the *a* axis. The N(6)···O(1′) distance is 2.988 Å while the H(62)···O(1′) distance is 2.26 Å. The angle N(6)–H(63)···O(1′) is 131°. O(1′) hydrogen bonding has been observed in only a few nucleoside crystals so far (Sundaralingam, 1968). Its observance in formycin is most likely due to the balance of crystal packing and conformation energies. The amino hydrogen atom H(62) is in van der Waals contact with O(5′). The N(6)···O(5′) and H(62)···O(5) distances are 3.188 and 2.39 Å, respectively, and the O(5′)···H(62)···O(1′) angle is 74°.

A stereoview of the molecular packing is shown in Figure 6 in the microfilm edition. It may be seen that the bases tend to stack in columns along the screw axis and there is a substantial degree of overlap (see also Figure 4), but the interplanar distance (3.755 Å) between adjacent bases is greater than the normal van der Waals contacts, indicating that the stacking interactions are not strong.

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Added in Proof

The negative Cotton effect similar to poly(F) observed in dimers and oligomers of 8,2′-*S*-cycloadenosine 5′-monophosphate and 8,2′-*O*-cycloadenosine 5′-monophosphate (Ikehara *et al.* (1972), which have a fixed glycosyl conformation of $\chi \simeq 108°$ as a result of covalent linkage between the sugar and the base, has been attributed to a left-handed helical conformation. This in conjunction with the observed glycosyl conformation in formycin ($\chi \simeq 109.8°$) suggests that a helical conformation with a left-handed chirality with bases having a glycosyl conformation in the intermediate anti–syn range can also account for the observed "inverted spectra" in poly(F).

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Structure and Activity in Chemical Carcinogenesis. Comparison of the Reactions of 7-Bromomethylbenz[*a*]anthracene and 7-Bromomethyl-12-methylbenz[*a*]anthracene with Deoxyribonucleic Acid *in Vitro*†

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ABSTRACT: 7-Bromomethyl-12-methylbenz[*a*]anthracene is a more effective carcinogen than 7-bromomethylbenz[*a*]-anthracene. It is shown that the more carcinogenic bromo compound reacts less extensively with DNA *in vitro* than does 7-bromomethylbenz[*a*]anthracene. Comparison of the products of reaction of each bromo compound with DNA indicates that similar products are produced in each case, through reaction of these compounds on the amino groups of the DNA bases, and that the products of reaction of the 12-methyl derivative with DNA are more light sensitive than those derived from the less carcinogenic compound. No correlation between the amounts of any hydrocarbon–DNA product formed and carcinogenic potency was found.

The precise nature of the initiating event in chemical carcinogenesis has not yet been defined for any chemical carcinogen. In order to approach this problem, detailed comparative studies of similar chemical agents which exhibit different carcinogenic potencies have been undertaken.

For example, it has been shown that 7-bromomethyl-12-methylbenz[*a*]anthracene has a shorter half-life and reacts less extensively with the nucleophile 4-(*p*-nitrobenzyl)pyridine than 7-bromomethylbenz[*a*]anthracene, and that the former compound is far more carcinogenic than the latter either after subcutaneous injection in the rat (Dipple and Slade, 1970), or topical application to mouse skin (Dipple and Slade, 1971). Since initiation may involve the interaction of a carcinogen with DNA (Brookes and Lawley, 1964), the major products of reaction of 7-bromomethylbenz[*a*]anthracene with DNA were identified (Dipple *et al.*, 1971). In the present studies the reactions of the above two bromo compounds with DNA *in vitro* have been carefully compared in order to define any differences which might possibly relate to their different carcinogenic activities.

Experimental Section

Radioactive 7-Bromomethylbenz[a]anthracene and 7-Bromomethyl-12-methylbenz[a]anthracene. [^{3}H]7-Bromomethylbenz[*a*]anthracene (specific activity 165 Ci/mol) was prepared as previously described (Dipple *et al.*, 1971). [^{3}H]7-Bromomethyl-12-methylbenz[*a*]anthracene (specific activity 687 Ci/mol) was prepared by bromomethylation of radioactive 12-methylbenz[*a*]anthracene using the procedure described by Dipple and Slade (1970). Tritium-labeled 12-methylbenz-[*a*]anthracene was prepared by catalytic exchange methods at the Radiochemical Centre, Amersham, and purified in our laboratory (Duncan *et al.*, 1969). In some experiments preparations of higher specific activities than those listed above were used. 7-Bromo[^{14}C]methyl-12-methylbenz[*a*]-anthracene (specific activity 5.6 Ci/mol) was prepared by bromomethylation of unlabeled 12-methylbenz[*a*]anthracene using [^{14}C]paraformaldehyde (Radiochemical Centre, Amersham), again following the procedure of Dipple and Slade (1970).

Reaction of [^{3}H]7-Bromomethyl-12-methylbenz[a]anthracene with Synthetic Polyribonucleotides. Solutions of the potassium salt of poly(A) (Boehringer), the sodium salt of poly(G) (Miles) and the potassium salt of poly(C) (Miles) each at 2 mg/ml in 0.01 M sodium phosphate buffer (pH 7) were separately treated in a darkened room with a tenth volume of a dry acetone solution of the [^{3}H]bromo compound (0.2 mg/ml). After 15 min the polynucleotides were precipitated and washed as previously described (Dipple *et al.*, 1971). Portions of these polynucleotides were then hydrolyzed to mononucleotides by incubation at 37° overnight in 0.33 N potassium hydroxide (0.2 ml) and acetic acid was then added to lower the pH of these solutions to pH 8–9. These nucleotide solutions were then treated with 0.05 ml of an *Escherichia coli* alkaline phosphomonoesterase solution (1 mg/ml) and incubated at 37° for 10 hr.

Reaction of Unlabeled 7-Bromomethyl-12-methylbenz[a]-

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