Molecular and Crystal Structure of 6-Methyluridine. A Pyrimidine Nucleoside in the Syn Conformation[1]

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Abstract: 6-Methyluridine crystallized from water in the monoclinic space group $P2_1$ with cell dimensions a = 19.895, b = 8.201, and c = 6.786 Å, and β = 92.70°, and two molecules per asymmetric unit. The crystal structure has been determined from 2723 three-dimensional diffractometer measured data by direct methods and refined to a reliability index R = 4.9%. 6-Methyluridine exhibits the syn conformation which seems to be stabilized mainly by the influence of the bulky methyl group in the 6 position of the base. The C(2′)-endo pucker of the ribose units offers optimal steric conditions for this syn conformation nucleoside, which is obvious from the bond distances and angles showing no significant distortion compared to the corresponding values in nucleosides in the normal anti conformation. Due to the different orientation of the C(5′)–O(5′) bond in the two crystallographic independent molecules (gauche, gauche and trans, gauche, respectively) only one of the 6-methyluridine molecules shows an intramolecular hydrogen bond between O(5′) of the sugar and O(2) of the base. The 6-methyluracil bases are stacked along the c axis parallel to each other at interplanar spacings of 3.24 Å.

Nucleosides can assume two conformations, syn or anti,[2] depending upon the relative position of the base with respect to the sugar residue, *i.e.*, depending upon the rotation about the glycosidic C(1′)–N(1) bond. Analysis of the rotational barriers and calculations of the energy profile of this rotation indicate that the anti conformation is favored slightly over the syn conformation and that the pucker of the sugar ring influences the stability of the respective rotamer.[3-6] X-Ray structural investigations of several purine nucleosides indicate that, in the crystalline state, the syn conformation occurs almost as frequently as the anti conformation, but in the pyrimidine series only 4-thiouridine, a naturally occurring tRNA nucleoside, has been found crystallized in the syn conformation.[7] It may be argued that in this special case the unusual packing and hydrogen bonding scheme and the previously unobserved C(3′)-endo–C(4′)-exo conformation of the sugar residue could contribute to the stabilization of the syn conformation.

According to recent nmr studies[8] 6-methyluridine (Figure 1) exists in syn conformation even in aqueous solution. Therefore this nucleoside appeared suitable for a detailed X-ray structural study to obtain more insight into the correlation between the sugar conformation and the conformation about the glycosidic bond in pyrimidine nucleosides.

Experimental Section

We wish to thank Dr. H. Vorbrüggen, Schering AG, who generously provided us with the thick, colorless, tabular 6-methyluridine crystals grown from an aqueous solution. The space group and cell constants of these crystals were determined from X-ray photographs and diffractometer measurements and are gathered in Table I. According to the measured density of 1.54 g/cm³ there are two nucleoside molecules per assymmetric unit.

Table I. Crystallographic Data

a = 19.895 ± 0.003 Å
b = 8.201 ± 0.002 Å
c = 6.786 ± 0.002 Å
β = 92.70 ± 0.03°
Monochromatized Mo radiation, λ = 0.70926
Space group $P2_1$
Molecules/cell Z = 4
Density ρ_{obsd} = 1.540 g/cm³
ρ_{calcd} = 1.543 g/cm³
Chemical formula $C_{10}H_{14}N_2O_6$

Intensity data were collected from a crystal of dimensions 0.15 × 0.25 × 0.4 mm using a Stoe four circle automatic diffractometer equipped with a Mo tube and a graphite monochromator (λ 0.70926 Å). Altogether 2723 reflections (up to a glancing angle θ = 29°) were measured in the $\theta/2\theta$ step scanning mode with a scan width of 80 steps (0.01° θ/0.02° 2θ per step, 1 sec counting time); the backgrounds were measured by the stationary counter–stationary crystal method for 20 sec on each side of a peak. The data were corrected for Lorentz and polarization factors but not for absorption due to the small linear absorption coefficient (μ = 1.4 cm⁻¹).

Solution and Refinement of the Structure

Normalized structure factors were computed from $E_h = (F_h^2/\langle F^2\rangle)^{1/2}$ with $\langle F^2\rangle$ the observed magnitude of F^2 averaged over a range of sin θ/λ values appropriate to F_h.[9] The structure was solved by direct methods using the program MULTAN[10] which combines the cyclic application of the tangent formula[9] with multisolution techniques.[11] The start phase set shown in Chart I, chosen automatically by the program, served to obtain the phases of the 199

Chart I

h	k	l	E	Phase, deg	
15	3	0	3.15	360	to define the origin
2	7	3	3.28	45, 315	
13	3	5	3.13	45	
15	4	1	2.94	45, 135, 225, 315	
15	4	2	2.93	45, 135, 225, 315	
2	0	4	3.32	0, 180	
0	0	2	2.98	180	from Σ_1 relationships with probabilities >80%
10	0	4	2.08	360	
0	0	6	2.11	180	

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Table II. Fractional Atomic Coordinates and Anisotropic Temperature Factors in the Form $T = \exp -(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)$[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
				Molecule A					
N(1)	−0.1834/1	0.1670/3	−0.2137/4	0.0009/1	0.0060/3	0.0146/5	−0.0001/1	0.0003/1	0.0003/4
C(2)	−0.2192/1	0.0209/4	−0.2266/4	0.0012/1	0.0065/4	0.0156/7	−0.0006/1	0.0001/2	0.0008/4
O(2)	−0.1936/1	−0.1123/3	−0.2389/4	0.0015/1	0.0063/3	0.0269/7	−0.0004/1	0.0002/1	−0.0007/4
N(3)	−0.2878/1	0.0367/4	−0.2191/4	0.0011/1	0.0084/4	0.0224/7	−0.0008/1	−0.0001/2	0.0026/5
C(4)	−0.3239/1	0.1804/4	−0.2126/4	0.0012/1	0.0105/5	0.0145/6	0.0002/2	−0.0003/2	0.0031/5
O(4)	−0.3849/1	0.1755/4	−0.1954/4	0.0011/0	0.0151/5	0.0266/6	0.0004/1	−0.0000/1	0.0074/5
C(5)	−0.2840/2	0.3250/4	−0.2278/4	0.0015/1	0.0074/4	0.0127/6	0.0008/1	0.0003/2	0.0020/4
C(6)	−0.2162/1	0.3162/4	−0.2240/4	0.0013/1	0.0063/4	0.0098/6	0.0001/1	0.0004/2	0.0012/4
C(7)	−0.1750/2	0.4706/4	−0.2312/5	0.0017/1	0.0052/4	0.0181/7	−0.0002/1	0.0005/2	0.0017/4
C(1′)	−0.1093/1	0.1576/4	−0.1928/4	0.0010/1	0.0061/4	0.0126/5	−0.0005/1	0.0004/1	−0.0003/4
C(2′)	−0.0796/1	0.0465/4	−0.0290/4	0.0011/1	0.0063/4	0.0112/6	−0.0005/1	0.0005/1	0.0006/4
O(2′)	−0.0766/1	0.1231/3	0.1558/3	0.0014/0	0.0132/4	0.0123/4	−0.0001/1	0.0009/1	−0.0027/3
C(3′)	−0.0093/1	0.0165/3	−0.1049/4	0.0011/1	0.0051/4	0.0122/6	0.0000/1	0.0005/1	−0.0005/4
O(3′)	0.0356/1	0.1460/3	−0.0550/3	0.0012/0	0.0083/3	0.0180/5	−0.0006/1	0.0008/1	−0.0031/3
C(4′)	−0.0213/1	0.0175/4	−0.3288/4	0.0012/1	0.0054/4	0.0128/6	−0.0001/1	0.0012/1	0.0009/4
C(5′)	−0.0246/2	−0.1479/4	−0.4270/4	0.0019/1	0.0079/4	0.0118/6	0.0001/2	0.0013/1	−0.0012/4
O(5′)	−0.0709/1	−0.2556/3	−0.3384/3	0.0018/1	0.0059/3	0.0161/5	−0.0003/1	0.0001/2	−0.0007/3
O(1′)	−0.0846/1	0.1003/3	−0.3721/3	0.0014/0	0.0086/3	0.0114/4	0.0006/1	0.0006/1	0.0020/3
H(3)	−0.310/2	−0.063/7	−0.207/8	0.002	0.010	0.015	0.000	0.000	0.000
H(5)	−0.305/2	0.429/7	−0.217/7	0.001	0.008	0.012	0.000	0.000	0.000
H(7)	−0.148/2	0.482/7	−0.107/7	0.002	0.010	0.014	0.000	0.000	0.000
H(7)	−0.137/2	0.475/7	−0.333/7	0.002	0.010	0.014	0.000	0.000	0.000
H(7)	−0.206/2	0.567/7	−0.251/7	0.002	0.010	0.014	0.000	0.000	0.000
H(1′)	−0.094/2	0.261/6	−0.160/6	0.001	0.006	0.009	0.000	0.000	0.000
H(2′)	−0.105/2	−0.054/6	−0.024/6	0.001	0.006	0.009	0.000	0.000	0.000
H(C2′)	−0.112/2	0.104/7	0.193/7	0.002	0.009	0.014	0.000	0.000	0.000
H(3′)	0.005/2	−0.091/6	−0.058/6	0.001	0.006	0.009	0.000	0.000	0.000
H(C3′)	0.035/2	0.166/7	0.076/7	0.001	0.008	0.012	0.000	0.000	0.000
H(4′)	0.014/2	0.079/6	−0.374/7	0.001	0.007	0.010	0.000	0.000	0.000
H(5′)	0.020/3	−0.199/7	−0.424/7	0.002	0.009	0.013	0.000	0.000	0.000
H(5′)	−0.036/2	−0.137/7	−0.578/7	0.002	0.009	0.013	0.000	0.000	0.000
H(O5′)	−0.106/3	−0.188/7	−0.340/8	0.002	0.009	0.013	0.000	0.000	0.000
				Molecule B					
N(1)	−0.3035/1	−0.4598/3	0.2553/3	0.0011/1	0.0074/3	0.0136/5	−0.0001/1	0.0007/1	−0.0000/4
C(2)	−0.3425/1	−0.6009/4	0.2660/4	0.0012/1	0.0074/4	0.0144/6	−0.0002/1	0.0012/2	0.0014/4
O(2)	−0.4030/1	−0.5986/3	0.2712/4	0.0013/1	0.0082/3	0.0292/7	−0.0002/1	0.0021/1	0.0021/4
N(3)	−0.3076/1	−0.7449/3	0.2678/4	0.0015/1	0.0066/3	0.0158/6	−0.0003/1	0.0012/1	0.0003/4
C(4)	−0.2386/1	−0.7640/4	0.2661/4	0.0014/1	0.0091/4	0.0116/6	0.0008/1	0.0007/2	0.0007/4
O(4)	−0.2143/1	−0.9021/3	0.2625/4	0.0018/1	0.0089/4	0.0254/7	0.0008/1	0.0012/2	−0.0001/4
C(5)	−0.2021/1	−0.6139/4	0.2699/4	0.0011/1	0.0107/5	0.0127/6	0.0004/1	0.0004/1	−0.0007/4
C(6)	−0.2330/1	−0.4684/4	0.2629/4	0.0010/1	0.0090/4	0.0099/5	−0.0002/1	0.0004/1	−0.0019/4
C(7)	−0.1938/1	−0.3127/4	0.2621/5	0.0011/1	0.0096/5	0.0255/9	−0.0004/2	0.0000/2	−0.0009/5
C(1′)	−0.3379/1	−0.3004/3	0.2465/4	0.0011/1	0.0064/4	0.0113/5	−0.0002/1	0.0008/1	−0.0005/4
C(2′)	−0.3945/1	−0.2814/4	0.0892/4	0.0012/1	0.0079/4	0.0112/5	−0.0005/1	0.0005/1	−0.0001/4
O(2′)	−0.3664/1	−0.2440/3	−0.0936/3	0.0017/1	0.0121/4	0.0106/4	−0.0015/1	0.0004/1	0.0004/3
C(3′)	−0.4352/1	−0.1439/4	0.1776/4	0.0012/1	0.0080/4	0.0140/6	−0.0001/1	−0.0002/2	0.0011/4
O(3′)	−0.4060/1	0.0124/3	0.1466/3	0.0024/1	0.0081/3	0.0181/5	−0.0004/1	0.0011/1	0.0015/4
C(4′)	−0.4285/1	−0.1801/4	0.3990/4	0.0011/1	0.0079/4	0.0124/6	0.0001/1	0.0002/1	−0.0018/4
C(5′)	−0.4863/1	−0.2801/4	0.4697/4	0.0011/1	0.0134/5	0.0145/6	−0.0006/2	0.0004/2	−0.0003/5
O(5′)	−0.4769/1	−0.3088/4	0.6751/3	0.0015/1	0.0253/6	0.0133/5	−0.0013/2	0.0008/1	0.0023/4
O(1′)	−0.3658/1	−0.2685/3	0.4304/3	0.0011/1	0.0016/5	0.0114/5	0.0006/1	0.0004/1	0.0009/4
H(3)	−0.332/2	−0.836/7	0.255/7	0.001	0.008/	0.012	0.000	0.000	0.000
H(5)	−0.155/2	−0.608/7	0.284/7	0.001	0.008/	0.012	0.000	0.000	0.000
H(7)	−0.201/3	−0.256/7	0.136/8	0.002	0.011/	0.016	0.000	0.000	0.000
H(7)	−0.149/3	−0.330/8	0.286/8	0.002	0.011	0.016	0.000	0.000	0.000
H(7)	−0.205/3	−0.233/7	0.381/8	0.002	0.011	0.016	0.000	0.000	0.000
H(1′)	−0.304/2	−0.223/6	0.222/6	0.001	0.006	0.009	0.000	0.000	0.000
H(2′)	−0.425/2	−0.382/7	0.074/7	0.001	0.008	0.011	0.000	0.000	0.000
H(C2′)	−0.396/2	−0.279/7	−0.178/7	0.002	0.009	0.013	0.000	0.000	0.000
H(3′)	−0.478/2	−0.144/7	0.127/7	0.001	0.008	0.012	0.000	0.000	0.000
H(C3′)	−0.406/2	0.023/8	0.020/8	0.002	0.012	0.017	0.000	0.000	0.000
H(4′)	−0.425/2	−0.089/7	0.477/7	0.001	0.007	0.011	0.000	0.000	0.000
H(5′)	−0.527/2	−0.215/7	0.437/7	0.002	0.009	0.013	0.000	0.000	0.000
H(5′)	−0.490/2	−0.382/7	0.394/7	0.002	0.009	0.013	0.000	0.000	0.000
H(O5′)	−0.519/3	−0.337/8	0.657/8	0.002	0.013	0.018	0.000	0.000	0.000

[a] Estimated standard deviations are separated by a slash.

E's greater than 1.7. The reflection 13 3 5 simultaneously served to define the enantiomorph. Four out of the 64 phase sets resulting from the above combinations of starting sets showed approximately equally satisfactory figures of merit and R indices and were significantly more self-consistent that the other 60 phase sets. An attempt was then made to determine the phases of the 245 E's of magnitude greater than 1.6 by application of the tangent formula, based on the starting phases of these best four phase sets with $E > 1.7$. However, as was only later discovered, the sequence of reflections of the first set was accidentally interchanged and the starting phase

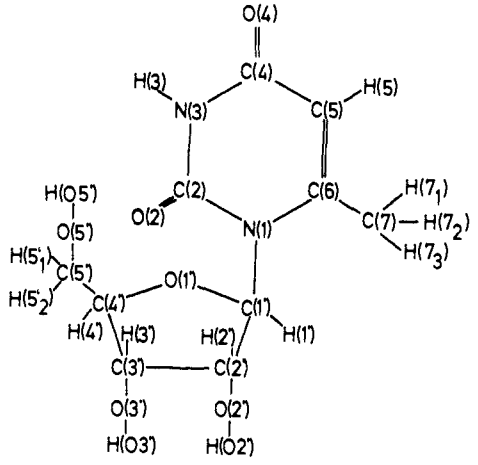

Figure 1. Chemical formula and numbering scheme of 6-methyluridine.

Chart II

h	k	l	E	Starting phase, deg	Phase angle after tangent refinement
3	3	0	1.79	360	318
22	1	2	1.96	315	331
23	1	3	1.74	180	180
5	3	1	1.82	315	190
0	7	1	4.46	180	180
5	9	2	1.69	45	320
15	2	4	2.36	45	93
18	0	3	2.34	360	180
7	5	4	1.79	180	180

assignments (Chart II) were in fact essentially "random." Nevertheless, from an "E map" based on the phases of the 245 E's greater than 1.6 derived from the above random starting set, the positions of 20 atoms (the two pyrimidine rings plus substituents) could be correctly located. These 20 maxima were among the strongest maxima found in the map. From a subsequent Fourier synthesis phased with the information from these 20 atoms, the whole (nonhydrogen) structure could be deduced.

The parameters of the 36 atoms were varied isotropically by least-squares full-matrix refinement[12] to yield a disagreement index $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ of 10.3% for the significant 2670 reflections. The data were assigned weights based on counting statistics[13] with 2% allowance for machine error and data with $F_o < 3\sigma F_o$ were treated as unobserved and not included in the refinement process. Fifteen hydrogen atoms could be located in a difference Fourier synthesis at this stage, and the remaining hydrogen atoms, namely those of the methyl groups and of the O(2'), O(3'), and O(5') hydroxyl groups, were found later. In the following four cycles of anisotropic refinement the hydrogen atoms were assigned the isotropic temperature factors of the atoms to which they were covalently bound. The positional parameters of the hydrogen atoms were refined in the last two cycles. The average parameter changes after the fourth least-squares refinement cycle were less than $^1/_3$ the estimated standard deviations. The final R factor is 4.9% for the 2670 significant data and 5.4% for all the 2723 measured data. Atomic scattering factors from the International Tables for X-Ray Crystallography[14] were utilized throughout, and the function minimized was $\Sigma 1/\sigma^2(|F_o| - |F_c|)^2$.

Results and Discussion

In Tables I–VII are gathered the crystallographic data, the final atomic parameters with standard deviations estimated from the least-squares variance–covariance matrix elements, the deviations of atoms from least-squares "best" planes through base and ribose units, dihedral angles within the riboses, bond distances and angles involving hydrogen atoms, geometrical data of the hydrogen bonds, and short intermolecular distances, respectively. The observed and calculated structure factors are listed in the microfilm

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Table III. Least-Squares Planes through the Bases and Ribose Units of Molecules A and B[a]

Atoms	Displacements, Å: Molecule A	Molecule B
	Bases[b]	
N(1)$^+$	−0.055	−0.030
C(2)$^+$	0.047	0.024
N(3)$^+$	0.003	0.003
C(4)$^+$	−0.046	−0.025
C(5)$^+$	0.042	0.021
C(6)$^+$	0.009	0.007
O(2)	0.137	0.072
O(4)	−0.156	−0.080
C(7)	0.042	0.022
C(1')	−0.204	−0.054
C(2')	−1.312	−1.107
H(3)	−0.07	−0.10
H(5)	−0.04	0.11
H(7$_1$)	−0.80	−0.82
H(7$_2$)	0.73	0.17
H(7$_3$)	0.17	0.85
	Ribose Units[c]	
C(1')$^+$	0.012	0.018
C(3')$^+$	−0.011	−0.017
C(4')$^+$	0.019	0.027
O(1')$^+$	−0.020	−0.029
C(2')	−0.570	0.571
O(2')	−0.122	0.133
O(3')	1.323	−1.385
C(5')	−1.111	1.275
O(5')	−2.397	1.250

[a] The plane equations are of the form $lX + mY + nZ + p = 0$ where X, Y, and Z are along a, b, and c^*, respectively. The plane defining atoms are marked by $^+$. [b] Molecule A, $l = -0.0059$, $m = -0.0085$, $n = -0.9999$, $p = -1.5149$. Molecule B, $l = -0.0097$, $m = 0.0224$, $n = 0.9997$, $p = -1.7372$. [c] Molecule A, $l = 0.5140$, $m = 0.8548$, $n = -0.0718$, $p = -0.1002$. Molecule B, $l = -0.5207$, m −0.8458, $n = -0.1163$, $p = -5.4128$.

Table IV. Endocyclic and Exocyclic Dihedral Angles within the Ribose Units

	Molecule A	Molecule B
C(1')-N(1)-C(6)-C(7)	+5.2	+0.0
C(6)-N(1)-C(1')-C(2')	+130.1	+131.3
C(6)-N(1)-C(1')-O(1')	−109.1	−107.3
C(2)-N(1)-C(1')-C(2')	−51.2	−51.9
C(2)-N(1)-C(1')-O(1')	+69.6	+69.4
N(1)-C(1')-C(2')-O(2')	−82.5	−81.9
N(1)-C(1')-C(2')-C(3')	+158.6	+156.8
O(1')-C(1')-C(2')-O(2')	+155.8	+155.7
O(1')-C(1')-C(2')-C(3')	+36.9	+34.3
C(1')-C(2')-C(3')-C(4')	−33.0	−35.8
O(2')-C(2')-C(3')-O(3')	−37.0	−37.8
C(1')-C(2')-C(3')-O(3')	+82.4	+79.4
O(2')-C(2')-C(3')-C(4')	−152.4	−153.1
C(2')-C(3')-C(4')-O(1')	+19.7	+26.4
C(2')-C(3')-C(4')-C(5')	−101.2	−94.1
O(3')-C(3')-C(4')-C(5')	+140.3	+147.4
O(3')-C(3')-C(4')-O(1')	−98.7	−92.2
O(1')-C(4')-C(5')-O(5')	−69.0	+61.9
C(3')-C(4')-C(5')-O(5')	+51.4	−179.9
C(3')-C(4')-O(1')-C(1')	−3.6	−5.2
C(5')-C(4')-O(1')-C(1')	+129.2	+117.0
C(4')-O(1')-C(1')-C(2')	−25.8	−18.6
C(4')-O(1')-C(1')-N(1)	−152.5	−145.5

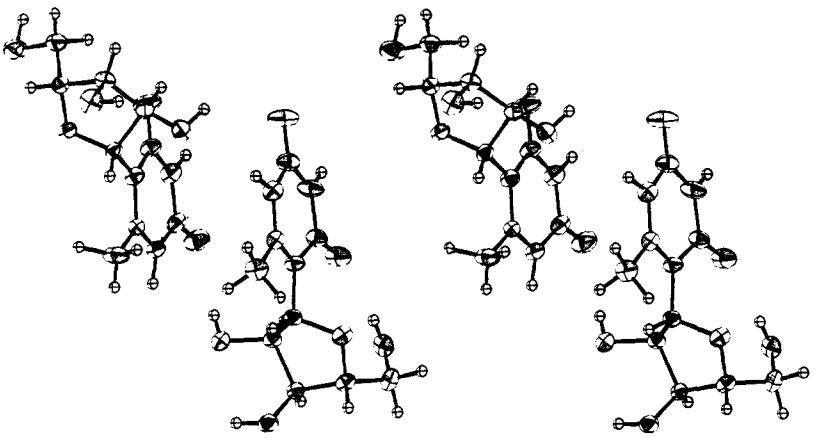


Figure 2. Stereoscopic view of the asymmetric unit. The atoms are represented by their 50% probability thermal ellipsoids.

Table V. Bond Distances and Angles Involving Hydrogen Atoms[a]

Atoms	Molecule A	Molecule B
Bond Distances, Å		
H(3)-N(3)	0.93	0.90
H(5)-C(5)	0.95	0.94
$H(7_1)$-C(7)	0.98	0.98
$H(7_2)$-C(7)	1.05	0.90
$H(7_3)$-C(7)	1.00	1.08
H(1′)-C(1′)	0.92	0.95
H(2′)-C(2′)	0.97	1.03
H(O2′)-O(2′)	0.78	0.85
H(3′)-C(3′)	0.97	0.91
H(O3′)-O(3′)	0.90	0.85
H(4′)-C(4′)	0.93	0.92
$H(5'_1)$-C(5′)	0.98	0.98
$H(5'_2)$-C(5′)	1.05	0.98
H(O5′)-O(5′)	0.88	0.86
Bond Angles, Deg		
H(3)-N(3)-C(2)	113	116
H(3)-N(3)-C(4)	120	116
H(5)-C(5)-C(4)	119	124
H(5)-C(5)-C(6)	119	114
$H(7_1)$-C(7)-C(6)	109	110
$H(7_2)$-C(7)-C(6)	117	112
$H(7_3)$-C(7)-C(6)	110	113
$H(7_1)$-C(7)-$H(7_2)$	101	110
$H(7_1)$-C(7)-$H(7_3)$	110	110
$H(7_2)$-C(7)-$H(7_3)$	110	102
H(1′)-C(1′)-N(1)	107	105
H(1′)-C(1′)-O(1′)	113	110
H(1′)-C(1′)-C(2′)	105	109
H(2′)-C(2′)-C(1′)	110	114
H(2′)-C(2′)-O(2′)	110	110
H(2′)-C(3′)-C(3′)	111	108
H(O2′)-O(2′)-C(2′)	102	103
H(3′)-C(3′)-C(2′)	107	111
H(3′)-C(3′)-O(3′)	115	109
H(3′)-C(3′)-C(4′)	111	114
H(O3′)-O(3′)-C(3′)	109	105
H(4′)-C(4′)-C(3′)	104	114
H(4′)-C(4′)-O(1′)	110	106
H(4′)-C(4′)-C(5′)	111	107
$H(5'_1)$-C(5′)-C(4′)	111	105
$H(5'_2)$-C(5′)-C(4′)	111	110
$H(5'_1)$-C(5′)-O(5′)	109	112
$H(5'_2)$-C(5′)-O(5′)	111	112
$H(5'_1)$-C(5′)-$H(5'_2)$	102	108
H(O5′)-O(5′)-C(5′)	97	80

[a] The averaged estimated standard deviations are 0.05 Å and 2° for distances and angles, respectively.

Table VI. Geometric Data of the Hydrogen Bonds and Short Nonbonded Interactions

Donor (D)	Acceptor (A)	Distances D···A, Å	Distances H···A, Å	Angle H-A-D, deg
N(3)A	O(2′)B	2.930	2.04	14
O(2′)A	O(4)B	2.870	2.11	9
O(3′)A	O(5′)A	2.849	1.99	16
O(5′)A	O(2)A	2.817	2.01	20
N(3)B	O(3′)B	2.881	2.03	15
O(2′)B	O(5′)B	2.692	1.87	12
O(3′)B	O(4)A	2.731	1.99	26
O(5′)B	O(3′)B	3.048	2.39	35
O(5′)B	O(2)B	2.979	2.56	53
C(2′)A	O(2)A	2.924	2.29	41
C(2′)B	O(2)B	2.888	2.26	44

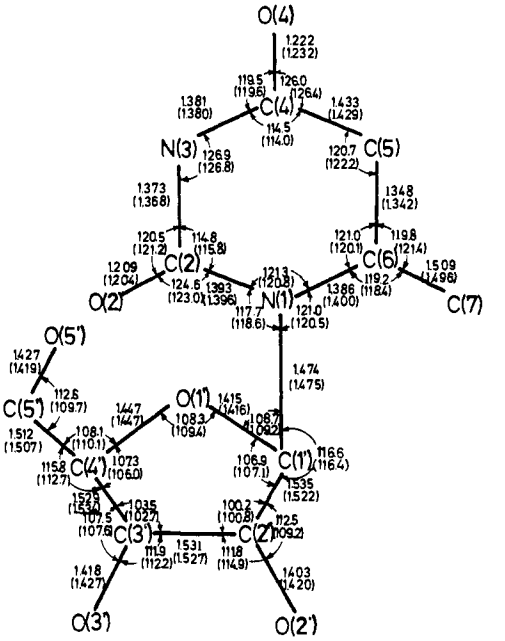


Figure 3. Bond angles and distances in molecules A and B. (Data for molecule B in parentheses.) The average estimated standard deviations are 0.005 Å and 0.3°, respectively.

edition of this journal.[15] Figures 2–5 illustrate a stereoscopic view of the asymmetric crystallographic unit, bond distances and angles, and projections of the

(15) A table of structure factors will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N. W., Washington, D. C. 20036, by referring to code number JACS-72-6520. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

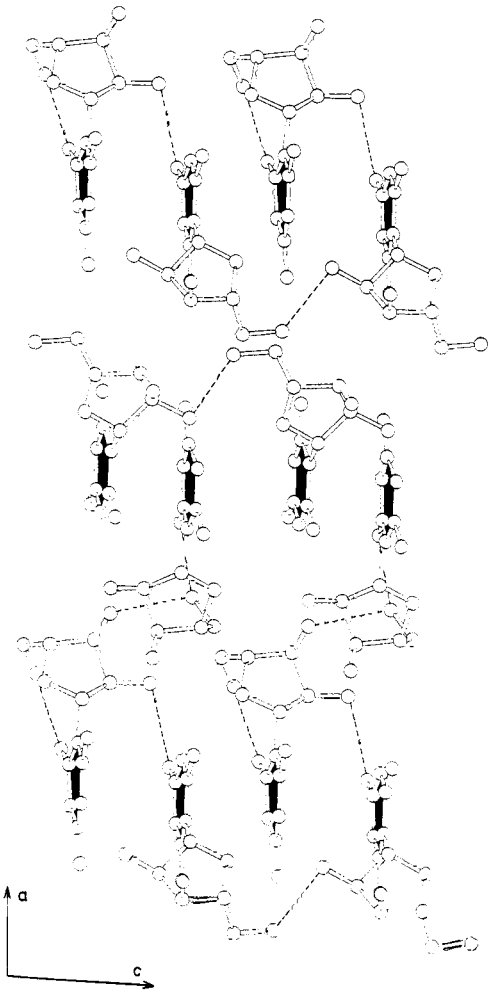


Figure 4. Projection of the crystal structure along the *b* axis (rotated by 10° about the *a* axis). Hydrogen bonds are indicated by dotted lines.

crystal structure along the *b* axis and the *c** axis, respectively.

Conformation of 6-Methyluridine. Ribose Units. Both ribose units exhibit a C(2′)-endo puckering conformation with atom C(2′) being 0.57 Å from the least-squares mean plane through the other four atoms of the ribose ring and on the same side as atom C(5′) (Table III). This ribose conformation is also indicated by the values of the five endocyclic ribose torsion angles and evident from the small dihedral angles C(3′)–C(4′)–O(1′)–C(1′), −3.6° in molecule A and −5.2° in molecule B, respectively (Table IV). The most prominent difference between the two crystallographically independent 6-methyluridine molecules of the asymmetric unit is found in the orientation of the C(5′)–O(5′) bond with respect to ribose moieties. In molecule A the conformation about the C(4′)–C(5′) bond is gauche,-gauche[16,17] (φ_{OO} = −69.0°, φ_{OC} = 51.4°) with O(5′) located "above" the ribose and in intramolecular hydrogen bonding contact with the base oxygen atom O(2), Table VI. In molecule B however, this conformation is gauche,trans (φ_{OO} = 61.9°, φ_{OC} = −179.9°) and O(5′) cannot participate in an intramolecular hydrogen bonding interaction with the heterocycle O(2)

(16) M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 1067 (1965).
(17) E. Shefter and K. N. Trueblood, *Acta Crystallogr.*, **18**, 1067 (1965).

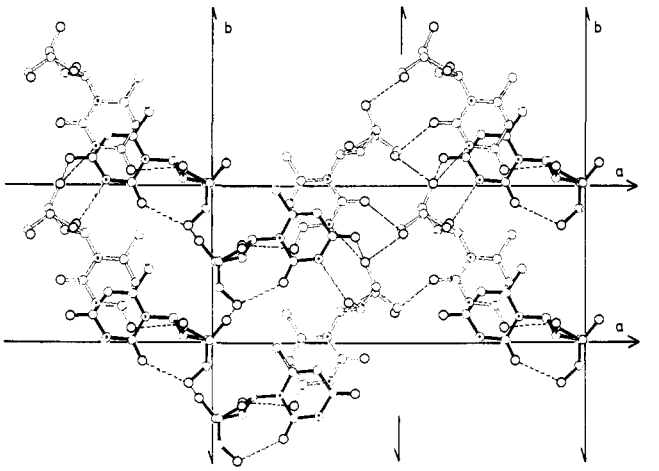


Figure 5. Projection of the crystal structure along the *c** axis. Oxygen and nitrogen atoms are marked by heavy lines and dots, respectively. Bonds in molecules A are filled and hydrogen bonds are indicated by dotted lines.

atom. The latter conformation seems to be preferred in 6-methyluridine since it was also found from nmr spectroscopic investigation that the conformation about the C(4′)–C(5′) bond in 6-methyluridine in aqueous solution is not gauche,gauche but trans,gauche or gauche,trans.[18]

Conformation about the Glycosidic Bond. The torsion angles C(2′)–C(1′)–N(1)–C(6) which define the conformation about the glycosidic C(1′)–N(1) bond[7] are 130.1° in molecule A and 131.3° in molecule B. Thus, both 6-methyluridine molecules are in syn conformation. Since 6-methyluridine is in the syn conformation even in solution[8] whereas 4-thiouridine assumes the anti conformation as soon as the crystals dissolve,[8,19,20] one could infer that 6-methyluridine is in the syn conformation due to the bulky methyl group. But then in 6-methyluridine the O(5′)–H···O(2) intramolecular hydrogen bond should be of only minor importance for the stabilization of the syn conformation as is indicated in its crystal structure. Only one of the two molecules in the asymmetric unit shows this intramolecular hydrogen bond.

Bond Distances and Angles. 6-Methyluracil Units. Bond angles and distances within the 6-methyluracil residues of molecules A and B are showing no significant differences and are in reasonable agreement with averaged data for other uracil derivatives.[21] The heterocycles are in the usual diketo form with double bonds between atoms C(2)–O(2), C(4)–O(4), and C(5)–C(6).

In Table III are collected the deviations of some atoms from the best planes through the pyrimidine rings. The deviation from planarity is greater for molecule A than for molecule B. The atom C(1′) deviates from the mean base plane by 0.204 Å in molecule A but by only 0.054 Å in molecule B; in both molecules the deviation is toward the same side of the base plane as atom C(2′).

(18) F. E. Hruska, "The Jerusalem Symposium on Quantum Chemistry and Biochemistry, Jerusalem, 1972," Vol. V, E. D. Bergmann and B. Pullman, Ed., Academic Press, New York, N. Y., in press.
(19) K. H. Scheit and W. Saenger, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **2**, 305 (1969).
(20) F. E. Hruska, K. K. Ogilvie, G. G. Smith, and H. Wayborn, *Can. J. Chem.*, **49**, 2449 (1971).
(21) D. Voet and A. Rich, *Progr. Nucleic Acid Res. Mol. Biol.*, 10, 183 (1970).

The carbon atoms of the methyl groups, C(7), are essentially coplanar with the pyrimidine heterocycles; the bonds N(1)–C(1′) and C(6)–C(7) are coplanar in molecule A but form an angle of 5.2° in molecule B.

The hydrogen atoms of the methyl groups in both molecules are orientated such that their conformation is "staggered" with respect to the C(1′)–H(1′) bond and "eclipsed" with respect to the C(5)–H(5) bond (Figure 2). The reversed orientation would result in an unfavorable close contact between the H(1′) proton and one of the methyl protons of about 1.6 Å.

Ribose Units. Bond distances and angles within the two crystallographically independent ribose residues are essentially the same and agree well with data averaged for ribose units in the same C(2′)-endo conformation.[22] Small differences in the angles O(1′)–C(4′)–C(5′), C(3′)–C(4′)–C(5′), and C(4′)–C(5′)–O(5′) of the two 6-methyluridine molecules of 2–3° (Figure 3) might be due to the different conformations about the C(4′)–C(5′) bonds and the differences in hydrogen bonding involving the O(5′) hydroxyl groups. The difference in hydrogen bonding could also influence the angles at C(2′) (Figure 3) and the C(2′)–O(2′) bond distance.

Comparison of 6-Methyluridine with 4-Thiouridine. 4-Thiouridine which also occurs in syn conformation[7] exhibits some structural features which were not observed in the other pyrimidine nucleosides in anti conformation. (1) The ribose of 4-thiouridine is in the unusual C(3′)-endo–C(4′)-exo conformation. (2) The exocyclic angles at N(1) and C(1′) are strained by about 3 and 6°, respectively, so as to increase the intramolecular distance between the ribose and the base O(2) oxygen atom. (3) There is a short intramolecular distance between O(2) and the sugar protons attached to C(3′), 2.27 Å; the van der Waals distance would be 2.6 Å.

The corresponding parameters in 6-methyluridine, on the other hand, deviate only marginally from the values observed for pyrimidine nucleosides in the anti conformation. The ribose moiety is in a "normal" C(2′)-endo conformation, the conformation found in all syn purine nucleosides.[23] From studies on Dreiding wire models it is observed that only in the C(2′)-endo sugar conformation are the nucleobase and ribose as far apart as possible, irrespective of syn or anti conformation. One could argue that this particular sugar conformation is preferred when a nucleoside assumes the syn conformation since then the bulky pyrimidine O(2) or purine N(3) groups interfere least with the sugar residues. In 6-methyluridine the exocyclic angles at N(1) are not and the angle N(1)–C(1′)–C(2′) is only slightly (by 3°) distorted with respect to anti pyrimidine nucleosides. Oxygen atom O(2) is in short intramolecular contact with the hydrogen atom at C(2′) (2.29 Å in molecule A and 2.26 Å in molecule B), Table VI.

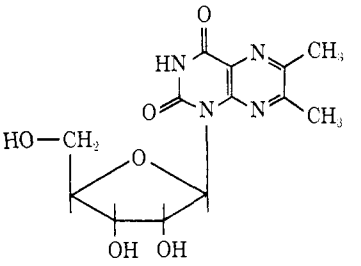
Packing and Hydrogen Bonding Scheme. Figure 4 shows a projection of the crystal structure of 6-methyluridine onto the *ac* plane. One readily recognizes a clear separation into regions of hydrophobic and hydrophilic character extending parallel to the *bc* plane at $a = 1/4$ and $3/4$ and $a = 0$ and $1/2$, respectively. Similar packing schemes had been observed for 4-thiouridine and 6-azauridine.[24]

Within the hydrophobic regions the 6-methyluracil residues, oriented almost parallel to the *ab* plane at $c = -0.22$ and 0.26, form a stack along the *c* axis with an interplanar spacing of 3.24 Å. The angle between the normals to the base planes is only 1.2°. The mutual overlap of neighboring bases is depicted in Figure 5, a projection onto the *ab* plane.

The ribose residues constitute the hydrophilic regions with hydrogen bonding interactions between the ribose hydroxyl groups and the functional groups of the pyrimidine heterocycles. These interactions are all intermolecular except the intramolecular O(5′)–H···O(2) bond in molecule A and the close contact between O(2) and the C(2′)–H(2′) group in both molecules. The geometrical details of these hydrogen bonds are presented in Table VI. The position of the proton attached to O(5′) in molecule B allows short intermolecular contacts to O(3′) (3.048 Å) as well as to O(2) (2.979 Å) of another molecule B related to the first by a screw operation.

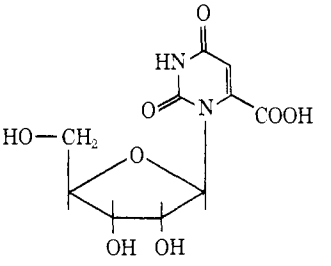
Conclusions

From the X-ray results on 4-thiouridine it appeared that pyrimidine nucleosides can exist in the syn conformation. However, in 4-thiouridine considerable strain is being imposed on the molecule as evidenced by the peculiar C(3′)-endo–C(4′)-exo ribose conformation and strained bond angles. From the results on 6-methyluridine and, very recently, on a 1-(β-D-ribofuranosyl)lumazine[25] which can be regarded as a uridine



derivative it was found that *pyrimidine nucleosides can occur in the syn conformation without strain and distortion of bond distances and angles, provided that the sugar conformation is C(2′)-endo.*

These considerations should also be applicable to the naturally occurring nucleoside orotidine which differs



from 6-methyluridine only by the substitution of the 6-methyl group by a carboxyl function. It was found by nmr evidence that orotidine is in the syn conformation in solution[26] and from the above mentioned one might

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(25) W. Saenger and W. Pfleiderer, *Angew. Chem.*, in press.
(26) F. Hruska, *J. Amer. Chem. Soc.*, **93**, 1795 (1971).

infer that orotidine is in an unstrained syn conformation with a C(2′)-endo ribose.

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Interaction of Metal Ions with Polynucleotides and Related Compounds. XXI. Metal Ions as Agents for the Stacking of Nucleotides. A Specific Interaction of Zinc(II) and Adenosine Monophosphate

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Abstract: Zinc(II) ions react with 5′-AMP at 1×10^{-3} *M* concentration in the presence of a polycation to produce a highly rotatory complex with conservative circular dichroism bands. This complex is characterized in the uv by hypochromicity and shoulders at 280 and 290 nm, and it exhibits a titration curve in which the phosphate p*K* is lowered and the p*K* for zinc hydrolysis is raised. Conservative CD bands are also produced by Zn(II) and 3′-AMP. These observations indicate that the zinc promotes parallel stacking of 3′- and 5′-AMP. The effect is remarkably specific. Other AMP isomers (2′, 2′,3′ cyclic, and 3′,5′ cyclic) and other nucleotides (5′-GMP, 3′-GMP, 5′-CMP, 5′-UMP, and 5′-IMP) do not produce a highly rotatory complex. Metal ions other than Zn(II), *e.g.*, Mn(II), Co(II), Ni(II), Cu(II), Cd(II), Hg(II), Ag(I), Fe(III), Al(III), and Ce(III), also do not produce such a complex. The failure to produce the effects that have been noted does not indicate a lack of complex formation, but only the inability of the metal to induce parallel stacking of the nucleotides. A metal that does not induce parallel stacking at low concentration may do so at high concentration, *e.g.*, Cu(II), but some metals apparently do not induce such stacking at any concentration. Highly rotatory complexes that do not display the conservative CD effect are formed between Pb(II) and 5′-AMP and Zn(II) and 3′- and 5′-dAMP. The absence of the 2′-OH group produces a much greater change in the CD effect of the 3′-AMP complex than of the 5′ complex, suggesting that the 2′-OH group plays a more significant role in the former than in the latter.

The participation of metal ions in the biochemical reactions of nucleotides and nucleic acids has provided a great deal of interest in the determination of the structures of the metal–nucleotide complexes.[1,2] Recently a nuclear magnetic resonance study has suggested that the reaction of Cu(II) with 3′-AMP and 5′-AMP results in a complex containing two atoms of Cu(II) and two molecules of AMP, with the AMP molecules located in such a way as to permit π interaction between the purines.[3] If metal ions are able to induce such stacking of nucleotide bases, this stacking should result in the drastic alterations of their ORD and CD spectra. The present study reveals that metal ions can indeed produce large increases in the rotatory strength of nucleotides which are consistent with an interaction between the bases. Perhaps the most striking aspect of the production of these Cotton effects is that, far from constituting a general phenomenon, they are produced only in certain very restricted cases. Copper ions unexpectedly do not produce the effects. Only zinc(II) and lead(II), of all common metal ions, and only AMP (either 3′ or 5′), of all the common nucleotides, will produce Cotton effects, and only the optical characteristics of zinc, and not lead, are indicative of parallel stacking. Such specificity in the reaction of metal and ligand is quite remarkable.

This paper describes the studies that demonstrate this specificity and provides some clues for its occurrence.

The study of the optical properties of metal–nucleotide complexes is complicated by the fact that many of these complexes precipitate at neutral pH. This problem has been overcome by carrying out the optical studies in the presence of poly-L-lysine and certain other polycations. It is then possible to probe the effect of metal ions on the uv, ORD, and CD spectra of various nucleotides and thus to determine whether metal ions can induce the stacking of nucleotide bases.

Experimental Section

The nucleosides and nucleotides were obtained from Sigma except for 3′-GMP which was obtained from P. L. Biochemicals. The basic polypeptides were obtained from Pilot Chemicals, polyethyleneimine from Pfaltz and Bauer, and DEAE-dextran from Pharmacia. All other chemicals were reagent grade.

The concentrations of nucleotides were determined from their uv spectra. The concentrations of basic polypeptides were deter-

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