Crystal Structure and Molecular Conformation of 2',3'-*O*-Methoxymethyleneuridine: *X*-Ray and Nuclear Magnetic Resonance Investigation

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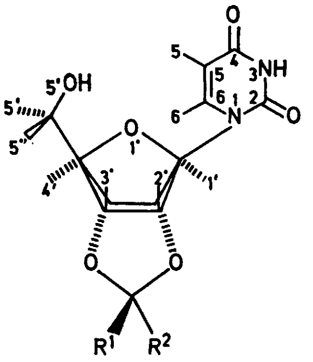
Crystals of the title compound are orthorhombic, space group $P2_12_12_1$, with $Z = 4$ in a unit cell of dimensions: $a = 16.48(1)$, $b = 4.752(4)$, and $c = 15.94(1)$ Å. The structure was determined from three-dimensional diffractometer data by direct methods, and refined by least-squares methods to R 4.04%. The dioxolan and ribofuranose rings are *cis*-connected about the C(2')–C(3') bond. The relatively flattened ribose ring ($\phi_{\text{max.}}$ 23°) has the C(2')-*endo* S-conformation, the glycosidic torsion angle lies in the normal *anti* range (χ_{CN} 56.5°) and the orientation of the C(5')–O(5') side-chain is *gauche–gauche*. The crystal packing is governed by O(2) ··· N(3) (2.896 Å) and O(4) ··· O(5') (2.907 Å) hydrogen bonds.

^{1}H n.m.r. spectroscopy reveals the existence of various conformational equilibria in solution. Vicinal coupling constants between ribose protons are consistent with an N [C(3')-*endo*] $\rightleftharpoons$ S [C(2')-*endo*] equilibrium with a slightly more puckered ribose ring than occurs in the crystal structure. The C(5')–O(5') side-chain shows almost equal populations of *gauche–gauche*- and *gauche–trans*-rotamers with a minor contribution of the *trans–gauche*-conformation. The c.d. spectrum and the chemical shifts of the base protons H(5) and H(6) indicate a preference for the *anti*-conformation about the glycosidic bond.

KNOWLEDGE of conformation and geometry of fragments of nucleic acids or their analogues may be helpful for the interpretation of physical data (n.m.r., c.d.), for correlation with tRNA structures and, more generally, helical structures of nucleic acids.



endo R^1=H, R^2=OMe *(S)*
exo R^1=OMe, R^2=H *(R)*

The title compound, 2',3'-*O*-methoxymethyleneuridine [hereafter MMU shown in (I)] was synthesized according to the method described by Griffin *et al.*[1] In principle, two diastereoisomers may be formed in the reaction of nucleosides with orthoesters such as trimethyl orthoformate. Usually, a pure diastereoisomer crystallizes from the reaction mixture in the reaction of uridine with trimethyl orthoformate at 20 °C, whereas the two possible diastereoisomers are found in the case of methoxymethyleneadenosine (MMA), formed at 130° C;[1] one can be obtained in an optically pure state by repeated crystallizations from ethanol.

It has been argued that the absolute configuration can be assigned by comparing the chemical shifts of formyl proton in both isomers: deshielding was assumed to occur if a proton is located *endo* [2] to the ribose ring. In fact we observed two isomers in the ^{1}H n.m.r. spectrum of MMA, one of them showing similar shifts for formyl and OMe protons as observed for the pure diastereoisomer of MMU, whereas the formyl proton of the other diastereoisomer is deshielded by 0.11 p.p.m. and the OMe signal is shielded by 0.12 p.p.m.

The present *X*-ray study was undertaken in order to allow an unambiguous stereochemical assignment of this class of compounds and to assess the effect on the conformation of the ribofuranoside ring of orthoester bridging of the *cis*-diol function. It turns out that the isolated diastereoisomer of MMU has the *S*-configuration around C(7) (formyl proton *endo* to the ribose ring) in accordance with earlier chemical-shift considerations.

Since a detailed conformational analysis of 2',3'-*O*-methoxymethylene derivatives of nucleosides in solution has not been carried out, we also undertook a ^{1}H n.m.r. study of MMU. The conformational properties in the crystal structure and in solution are compared.

X-*Ray Analysis*

MMU was recrystallized from ethanol as irregular orthorhombic crystals. Lattice dimensions were determined manually with a three-circle diffractometer. The crystal was mounted about the direction perpendicular to ($\bar{1}02$) and Mo-K_α radiation was used.

Crystal Data.—$C_{11}H_{14}N_2O_7$. Orthorhombic, $M = 286.2$, $a = 16.48(1)$, $b = 4.752(4)$, $c = 15.94(1)$ Å, $U = 1\,248$ Å^3, $D_m = 1.50$, $Z = 4$, $D_c = 1.523$ g cm^{-3}, $F(000) = 600$. Space group $P2_12_12_1$ from systematic absences (and in agreement with optical activity). Mo-K_α radiation, $\lambda = 0.710\,7$ Å; μ(Mo-K_α) = 1.4 cm^{-1}.

The reflection intensities of a crystal with dimensions $0.5 \times 0.4 \times 0.2$ mm were recorded with a three-circle diffractometer employing the θ—2θ scanning mode in the range θ 3.5—28° and by use of graphite-monochromatized Mo-K_α-radiation. Altogether 1 781 symmetry-independent reflections were scanned of which 206 had intensities $< 2\sigma$ from counting statistics and 159 were considered unobserved. The remaining 1 416 reflection intensities were reduced to structure factors, but correction for absorption

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[1] B. E. Griffin, M. Jarman, C. B. Reese, and J. E. Sulston. *Tetrahedron*, 1967, **23**, 2301.

[2] N. Baggett, A. B. Foster, J. M. Webber, D. Lipkin, and B. E. Phillips, *Chem. and Ind.*, 1965, 136.

was not applied since the value of μ is very small. A preliminary scale and temperature factor (B *ca.* 3.4 Å^2) was calculated from a Wilson plot.

Structure Determination and Refinement.—A straightforward solution of the structure was obtained by application of direct methods by use of the program MULTAN.[3] With the exception of C(4), O(4), and C(8) and hydrogen atoms, all atoms were located in the first trial E map. The missing heavy atoms were found in the first Fourier map using F structure factors as coefficients, while the hydrogen atoms were located in difference-Fourier maps. The structure was refined by least-squares techniques, the function minimized being $Ew(F_o - F_c)^2$. The weighting scheme was of the form $w = (0.004F^2 + \sigma^2)^{-1}$ (σ from counting statistics) and only significant reflections were used. Scattering factors for non-hydrogen atoms were taken from ref. 4 and for hydrogen from ref. 5.

All heavy atoms were refined anisotropically using as temperature factors the function $\exp[-2\pi^2\Sigma_{i,j}h_ih_ja_i{}^*a_j{}^*U_{ij}]$. The hydrogen atoms of the methyl group C(8) were kept at calculated staggered positions with fixed values B 7 Å^2. Remaining hydrogen atoms were constrained to isotropic refinement with individual Debye–Waller temperature factors.

During the final two cycles the full-matrix version of the program was used resulting in R 4.04% and R' 3.35% $\{R' = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_c|^2]^{1/2}\}$. Observed and calculated structure factors and anisotropic thermal parameters are listed in Supplementary Publication No. SUP 21917 (6 pp., 1 microfiche).* Positional parameters of the heavy atoms are listed in Table 1 and positional and thermal parameters of hydrogen atoms in Table 2. Estimated positional standard errors are H 0.02, C 0.002 5, N 0.001 9, and O 0.001 8 Å. The corresponding standard deviations of C(7), C(8), O(2′), and O(7) are, however, 0.003 6 and 0.002 2. Å No allowance for systematic errors was made, but the estimated errors in lattice dimensions were taken into account in the calculation of geometric entities (see Discussion of the structure).

TABLE 1

Fractional co-ordinates of the heavy atoms of MMU, with estimated standard deviations in parentheses

Atom	x	y	z
C(1′)	0.211 7(2)	−0.224 9(2)	0.096 4(2)
C(2′)	0.141 6(2)	−0.016 3(2)	0.092 9(3)
C(3′)	0.088 9(2)	−0.127 4(2)	0.023 2(2)
C(4′)	0.142 1(2)	−0.309 3(2)	−0.030 5(2)
C(5′)	0.164 4(2)	−0.185 2(2)	−0.113 8(2)
C(6)	0.324 8(2)	0.084 9(3)	0.056 2(2)
C(5)	0.396 5(2)	0.202 2(3)	0.068 2(2)
C(4)	0.443 6(2)	0.134 2(3)	0.140 7(2)
C(2)	0.333 6(2)	−0.184 3(3)	0.183 1(2)
C(7)	0.020 5(3)	−0.170 1(4)	0.146 8(3)
C(8)	−0.118 0(3)	−0.072 3(5)	0.150 9(3)
O(1′)	0.215 4(2)	−0.361 3(2)	0.017 7(2)
O(2′)	0.093 7(2)	−0.027 3(3)	0.166 2(2)
O(3′)	0.032 1(2)	−0.295 1(2)	0.068 5(2)
O(5′)	0.197 9(2)	0.087 0(2)	−0.100 4(2)
O(4)	0.509 4(2)	0.236 8(2)	0.159 1(2)
O(2)	0.306 3(2)	−0.346 8(2)	0.234 6(2)
O(7)	−0.038 7(2)	0.035 6(3)	0.147 0(2)
N(1)	0.291 3(2)	−0.101 6(2)	0.112 7(2)
N(3)	0.408 7(2)	−0.064 8(2)	0.191 3(2)

DISCUSSION OF THE STRUCTURE

Bond lengths and angles are given in Figure 1. Standard errors in bond angles are *ca.* 0.2°. Taking into consideration the σ values the most striking features of the observed pattern of distances are as follows. (i) The rather small single-bond C–C distances, 1.507 Å. The generally accepted[6] C(sp^3)–C(sp^3) distance is 1.533 Å. Since corrections for thermal motion (not applied) might add amounts up to 0.01—0.015 Å to the observed distances, however, this low value is not significant.

TABLE 2

Fractional co-ordinates, and isotropic B values (Å^2) of hydrogen atoms, with estimated standard deviations in parentheses

Atom	x	y	z	B
H(1′)	0.205(2)	−0.359(2)	0.142(2)	2.6(0.4)
H(2′)	0.159(2)	0.157(2)	0.086(2)	2.7(0.5)
H(3′)	0.065(2)	0.021(2)	−0.003(2)	2.5(0.5)
H(4′)	0.116(2)	−0.489(2)	−0.041(2)	2.5(0.5)
H(5′)	0.113(2)	−0.178(2)	−0.146(2)	2.5(0.4)
H(5″)	0.205(2)	−0.296(2)	−0.140(2)	3.6(0.5)
H(6)	0.293(2)	0.123(2)	0.008(2)	3.3(0.5)
H(5)	0.413(2)	0.337(2)	0.034(2)	3.7(0.5)
H(7)	0.009(2)	−0.334(2)	0.188(2)	5.5(0.7)
H(O5′)	0.201(2)	0.180(3)	−0.147(3)	6.0(0.7)
H(N3)	0.436(2)	−0.126(2)	0.231(2)	5.2(0.6)
H(81) *	−0.130	−0.199	0.103	7.0
H(82) *	−0.160	0.077	0.152	7.0
H(83) *	−0.126	−0.188	0.203	7.0

* Fixed positions and B values (see text).

(ii) The large scatter in individual single-bond C–O and C–C distances. Excluding the bonds O(1′)–C(4′) and C(1′)–C(2′), the scatter is much smaller. The difference in bond lengths (0.039 Å) between values for the bonds O(1′)–C(4′) and O(1′)–C(1′) is consistent with literature values. Mean O(1′)–C(4′) and O(1′)–C(1′) distances calculated from 21 observations quoted in ref. 7 are 1.451(1) and 1.414(1) Å: our observed values are 1.452(3) and 1.414(3) Å. Similar differences between C–O bonds occurring in sugars and halogen-substituted dioxan compounds were discussed at some length by Romers *et al.*[8] (anomeric effect).

Relevant torsion angles are listed in Table 3. The ribose ring displays an S-type [C(2′)-*endo*] conformation [C(2′) pointing to the same side as the C(5′) side-chain]; its calculated phase angle (P) of pseudorotation[9,10] (162.8°) is close to the C_s(2′)-envelope form, (162°), and occupies the centre of the observed range of values for

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* See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1976, Index issue.

[3] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

[4] 'International Tables for *X*-Ray Crystallography,' vol. III, Kynoch Press, Birmingham, 1962, p. 202.

[5] R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

[6] L. E. Sutton, in *Chem. Soc. Special Publ.*, No. 18, 1965.

[7] M. Sundaralingam, in 'Conformation of Biological Molecules and Polymers,' Proc. 5th Jerusalem Symposium, eds. E. D. Bergmann and B. Pullman, Academic Press, New York, 1973, **5**, 417.

[8] C. Romers, C. Altona, H. R. Buys, and E. Havinga, *Topics in Stereochem.*, E. L. Eliel and N. L. Allinger, 1967, **4**, 39.

[9] C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, 1968, **24**, 13.

[10] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1972, **94**, 8205.

S-type ribonucleoside derivatives[10] (we calculated a mean value of 161.5° from 43 independent observations). The calculated puckering amplitude of MMU ($\phi_{\text{max.}}$ 23.1°) is significantly lower than the mean (39.3°) obtained from the same 43 observations. Apparently, the *cis*-connection of the dioxolan and ribose rings [see Figure 2(c)] may cause some flattening of the ribose ring.

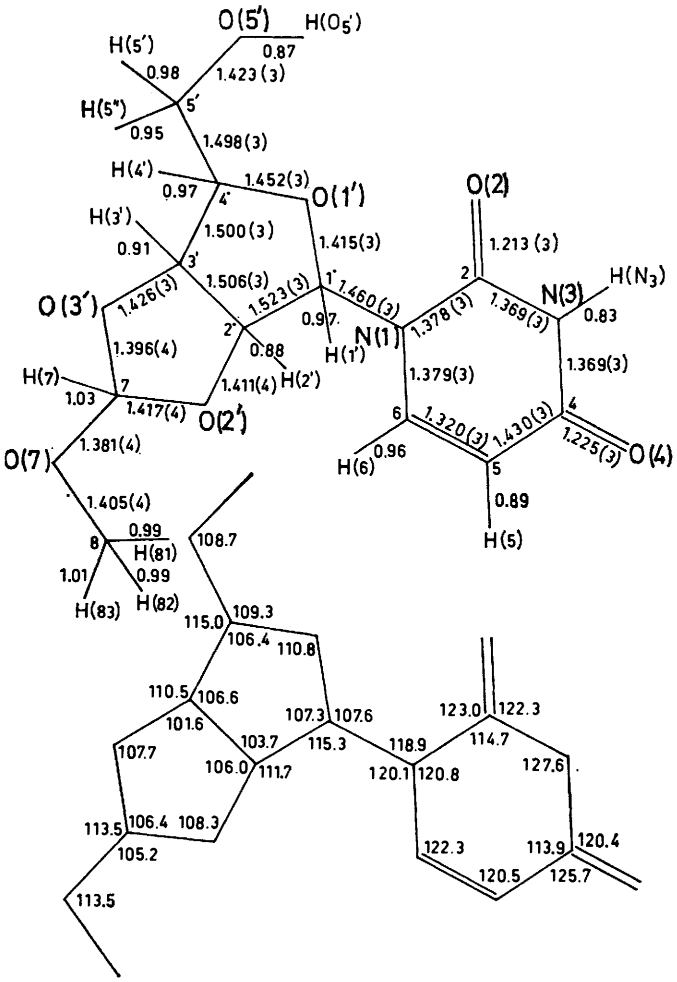

FIGURE 1 Bond lengths (Å), bond angles (°), and atom numbering system in 2′,3′-*O*-methoxymethyleneuridine

In only one instance, a comparable ribose ring conformation has been observed in crystal structures of nucleosides, modified with a 2′,3′-*cis*-diol bridging function. For the bis(pyridine)osmate(IV) ester of adenosine[11] an S-type ribose ring was observed with phase angle *P* 166° and puckering amplitude $\phi_{\text{max.}}$ 32°, whereas the ribose ring in crystal structures of nucleoside 2′,3′-*cyclic* phosphates is either flat[12] or puckered with the furanose oxygen out of the ring plane.[12,13] The observed value of 32.3° for the maximum puckering angle of the dioxolan ring in MMU agrees with the mean (31.5°) observed for six dioxolan rings quoted by Braun *et al.*;[14] no influence of the *cis*-connection is apparent in the puckering of the dioxolan ring. The calculated phase angle of pseudorotation of the latter ring [taking the symmetrical C(2′)*endo*–C(3′)*exo* form as the reference conformation, defining *P* 0°], amounts to *P* 138.6°, close to the C(3′)*endo*–O(3′)*exo* half-chair conformation.

TABLE 3

Selected torsion angles (°) of MMU *

Notation	Designation	Angle	Conformation
τ_0	C(4′)–O(1′)–C(1′)–C(2′)	−13.7	
τ_1	O(1′)–C(1′)–C(2′)–C(3′)	21.8	Type S [*C*(2′) *endo*]
τ_2	C(1′)–C(2′)–C(3′)–C(4′)	−21.7	*P* 162.8°
τ_3	C(2′)–C(3′)–C(4′)–O(1′)	14.3	$\phi_{\text{max.}}$ 23.1°
τ_4	C(3′)–C(4′)–O(1′)–C(1′)	−0.3	
τ_0'	C(3′)–O(3′)–C(7)–O(2′)	−28.6	
τ_1'	C(2′)–C(3′)–O(3′)–C(7)	31.9	C(3′)*endo*–O(3′) *exo*
τ_2'	O(2′)–C(2′)–C(3′)–O(3′)	−23.6	*P* 138.6°
τ_3'	C(7)–O(2′)–C(2′)–C(3′)	7.4	$\phi_{\text{max.}}$ 32.3°
τ_4'	O(3′)–C(7)–O(2′)–C(2′)	12.4	
χ_{CN}	O(1′)–C(1′)–N(1)–C(6)	56.5	*anti*
ψ	O(3′)–C(4′)–C(5′)–O(5′)	52.9	
	O(1′)–C(4′)–C(5′)–O(5′)	−66.7	*gauche–gauche*
ϕ'	C(4′)–C(3′)–O(3′)–C(7)	144.8	

* Standard deviations 0.3°.

Having established the relatively low degree of puckering of the ribose ring compared with the average value for unconstrained ribonucleosides, a side-step towards the observed endocyclic valency angles proved to be interesting. It has been argued,[15] that the valency angles of cyclopentane vibrate between the values $\tau - b$ and $\tau + b$ during pseudorotational motion of the molecule according to equation (1) where ν is an

$$\nu_j = \tau + b \sin\left\{2\left(P + \frac{4\pi j}{5}\right) + \delta\right\},\ j = 1, 2 \ldots 5 \quad (1)$$

endocyclic valency angle, δ is a phase for which a value of $7\pi/10$ (or $-3\pi/10$) was calculated, and τ and b represent a set of constants (in degrees), which are dependent upon the degree of puckering $\phi_{\text{max.}}$. This dependence is plotted in Figure 2 of ref. 15 for the case of cyclopentane. It can be estimated from this Figure and equation (1), that the sum of endocyclic valency angles $\Sigma\nu_j$ for a cyclopentane ring with a given phase-angle P is enhanced by 10°, if the degree of puckering $\phi_{\text{max.}}$ is allowed to decrease from 39 to 23°. This is indeed found to be the case: transferring this situation to the case of MMU *versus* normal type S ribonucleosides, we find $\Sigma\nu_j$ 534.8° for MMU and mean $\Sigma\nu_j$ 524.8° for the 43 type S ribonucleosides already discussed, all other conditions being fulfilled.

In Figure 2, important torsion angles of MMU are depicted by means of Newman projections. The

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[11] J. F. Conn, J. J. Kim, F. L. Suddath, P. Blatmann, and A. Rich, *J. Amer. Chem. Soc.*, 1974, **96**, 7152.

[12] C. L. Coulter, *J. Amer. Chem. Soc.*, 1973, **95**, 570.

[13] W. Saenger and F. Eckstein, *J. Amer. Chem. Soc.*, 1970, **92**, 4712.

[14] P. B. Braun, J. Hornstra, C. Knobler, E. W. M. Rutten, and C. Romers, *Acta Cryst.*, 1973, **B29**, 463.

[15] H. J. Geise, C. Altona, and C. Romers, *Tetrahedron Letters*, 1967, **15**, 1383.

glycosidic torsion angle, χ_{CN} 56.5° [Figure 2(b)], lies in the normal *anti* range for type S ribonucleosides (38—73°),[10] and the orientation of the exocyclic 5′-CH_2OH group [Figure 2(a)] is seen to be *gauche–gauche* with respect to O(1′) and C(3′) (ϕ −66.7° is approximately in the centre of the range observed for ribonucleosides). Finally, the orientation of C(8) [Figure 2(d)] is *gauche* with respect to O(3′) (79.8°) and *trans* with respect to O(2′) (−164.2°).

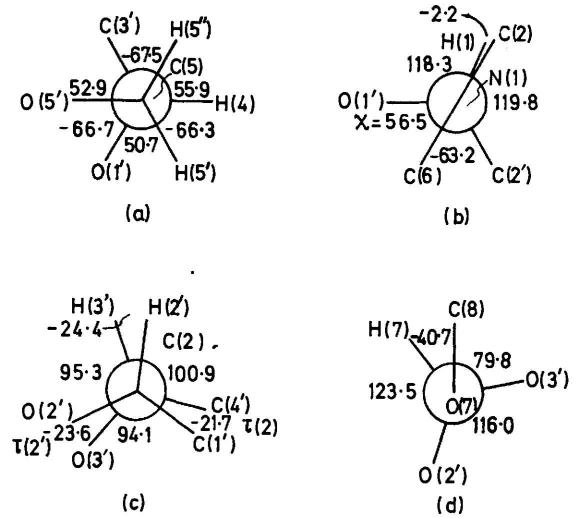

FIGURE 2 Newman projections of relevant torsion angles in MMU

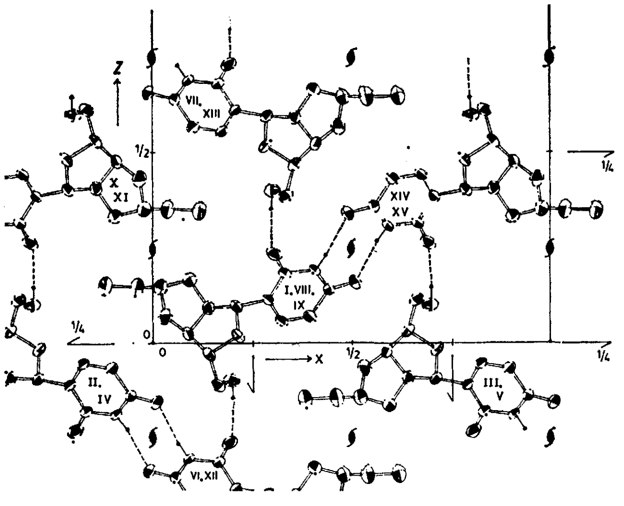

FIGURE 3 Projection of the structure viewed along [010] direction; hydrogen bonds are indicated by dashed lines

Packing and Hydrogen Bonds.—Figure 3 shows a part of the structure projected along [010]. The reference molecule (I) at *x,y,z* has 98 contacts <3.2 Å (see Table 6) and is surrounded by 14 neighbouring molecules. There are two types of hydrogen bonds: (i) bonds O(5′) · · · O(2) established by the screw operation $\frac{1}{2} - x, -y, \pm\frac{1}{2} + z$, connecting the molecules in the direction [001] [since strong O · · · O type hydrogen bonds[16] have a length of *ca.* 2.7 Å, the bond O(5′) · · · O(2) must be considered as rather weak] and (ii) the helical arrangement of bonds N(3) · · · O(4) brought about by the screw operation $-x, \pm\frac{1}{2} + y, \frac{1}{2} - z$. In view of quoted N · · · O distances[16] in the range 2.8—3.1 Å, the latter hydrogen bond (2.896 Å) may be classed as moderately strong.

TABLE 4

A survey of 98 contacts <3.2 Å

Molecule	Operation	No.	Mean dist.	Min. dist.
(*a*) 36 H · · · H interactions				
II, III	$\mp\frac{1}{2} + x, -\frac{1}{2} - y, -z$	8	2.83	2.61
IV, V	$\mp\frac{1}{2} + x, \frac{1}{2} - y, -z$	8	2.77	2.57
VIII, IX	$x, \mp 1 + y, z$	10	2.61	2.49
X, XI	$-x, \mp\frac{1}{2} + y, \frac{1}{2} - z$	6	3.14	3 09
XII, XIII	$\frac{1}{2} - x, -y, \pm\frac{1}{2} + z$	4	2.78	2.57
(*b*) 54 C · · · H, N · · · H, and O · · · H interactions				
II, III	$\mp\frac{1}{2} + x, -\frac{1}{2} - y, -z$	6	3.00	2.83
IV, V	$\mp\frac{1}{2} + x, \frac{1}{2} - y, -z$	12	3.00	2.72
VI, VII	$\frac{1}{2} - x, -1 - y, \mp\frac{1}{2} + z$	2	2.63	2.63
VIII, IX	$x, \mp 1 + y, z$	16	2.95	2.60
X, XI	$-x, \mp\frac{1}{2} + y, \frac{1}{2} - z$	10	2.92	2.68
XII, XIII	$\frac{1}{2} - x, -y, \mp\frac{1}{2} + z$	6	2.94	2.76
XIV, XV	$1 - x, \pm\frac{1}{2} + y, \frac{1}{2} - z$	2	3.07	3.07
(*c*) 4 Hydrophilic O · · · H interactions				
XII, XIII	$\frac{1}{2} - x, -y, \mp\frac{1}{2} + z$	2	2.05	2.05
XIV, XV	$1 - x, \mp\frac{1}{2} + y, \frac{1}{2} - z$	2	2.07	2.07
(*d*) 4 Hydrophilic O · · · O and O · · · N distances				
XII, XIII	$\frac{1}{2} - x, -y, \mp\frac{1}{2} + z$	2	2.907	2.907 O · · · O
XIV, XV	$1 - x, \mp\frac{1}{2} + y, \frac{1}{2} - z$	2	2.896	2.896 O · · · N

Nuclear Magnetic Resonance Spectroscopy

Experimental.—Preliminary investigations of the ^1H n.m.r. spectrum of MMU at 100 MHz showed considerable overlap of resonances. More accurate chemical shifts and coupling constants could be obtained by means of n.m.r. spectroscopy at 360 MHz.

The ^1H n.m.r. spectrum of MMU (0.05M in D_2O, pD = 7.3, 25 °C, internal references 0.001M-TMA and DSS) was recorded on a Bruker HX-360 spectrometer operating in the Fourier transform mode at 360 MHz. The free induction decay signals (100 accumulations) were stored in a Bruker BNC-12 computer using a spectral window of 3 311 Hz with 16 K data points. Use was made of a WEFT (π–τ–$\pi/2$) pulse sequence[17] (τ = 1.62 s) to eliminate the HDO signal. The lock signal was provided by the deuterium signal of D_2O. Spectral parameters were obtained by careful simulation of the expanded ribose proton region with the aid of the computer program LAME. The resulting chemical shifts and coupling constants are estimated to be reproducible to ±0.001 p.p.m. and ±0.1 Hz respectively (Table 5). The accuracy of the determination of the coupling constants is rather better than the resolution per data point (0.4 Hz) because each line is determined by *ca.* 10 data points, in addition all couplings occur at least twice. A considerable decrease of the relative intensities of the signals of H(1′), H(2′), H(3′), and H(4′) as a result of the pulse sequence technique was noted.

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[16] W. C. Hamilton and J. A. Ibers, 'Hydrogen Bonding in Solids,' Benjamin, New York, 1968.

[17] F. W. Benz, J. Feeney, and G. C. K. Roberts, *J. Magnetic Resonance*, 1972, 8, 114.

The Conformations of the Ribose Ring in Solution.—Vicinal coupling constants between ribose protons in nucleosides and nucleotides have been used to yield quantitative information about the equilibrating type N [C(3′)-*endo*] and type S [C(2′)-*endo*] ribose ring conformations and their relative populations in solution.[18] It was shown that time-average values of $J_{2'3'}$ and $J_{1'2'} + J_{3'4'}$ are to a good approximation independent of the equilibrium constant K. Although it is hard to disentangle changes in maximum degree of pucker from pseudorotation towards P-values not normally observed in β-ribonucleosides, it was argued that a decrease in $\phi_{\text{max.}}$ (flattening of the ribose ring) would result in an increase in $J_{2'3'}$ and a decrease in $J_{1'2'} + J_{3'4'}$. The individual coupling constants $J_{1'2'}$ and $J_{3'4'}$ reflect the equilibrium composition and can be used to calculate the equilibrium constant.

TABLE 5

Chemical shifts [a] and coupling constants [b] for MMU in D_2O solution

Proton	δ	$^3J_{HH}$	J
H(6)	7.791	5 6	8.0
H(7)	6.181	1′2′	2.5
H(5)	5.873	2′3′	6.5
H(1′)	5.871	3′4′	4.0
H(2′)	5.193	4′5′	3.6
H(3′)	5.005	4′5″	5.8
H(4′)	4.346	5′5″	−12.3
H(5′)	3.872		
H(5″)	3.807		
(OCH_3)	3.558		

[a] Chemical shifts are expressed in p.p.m. relative to internal DSS [3-(trimethylsilyl)propanesulphonic acid], shifts relative to TMA (tetramethylammonium chloride) can be obtained by subtracting 3.197 p.p.m., accuracy 0.001 p.p.m. [b] Coupling constants are expressed in Hz, accuracy 0.1 Hz.

The observed vicinal coupling constants for MMU in D_2O will be discussed along these guidelines. One of the proposed 'probes for ribose ring geometry,[18]' $J_{2'3'}$, amounts to 6.5 Hz in MMU, 1.5 Hz higher than the typical value of ~5.0 Hz observed in ribonucleoside derivatives not constrained by a *cis*-diol bridging methoxymethylene group. The second probe for ribose ring geometry, *viz.* $J_{1'2'} + J_{3'4'}$ also indicates relative flattening of the ribose ring on a time-average basis; $J_{1'2'} + J_{3'4'} = 6.5$ Hz compared with normal values of ~9.5 Hz observed for an N⇌S equilibrium between ribose conformers with puckering amplitudes around 39°. This is consistent with the solid state geometry of MMU, a puckering of 23° was observed, substantially lower than the normal values.

To test if the solid-state geometry alone can account for the observed vicinal coupling constants in MMU, we calculated the vicinal coupling constants by means of the modified Karplus equation: $^3J_{HH} = A\cos^2\phi_{HH} + B\cos\phi_{HH} + C$ using two sets of parameters applicable to the ribofuranoside ring: $A = 10.5$, $B = -1.2$, and $C = 0$ (ref. 18) and $A = 9.8$, $B = -0.9$, and $C = 0$ (ref. 19).* Proton–proton torsion angles (ϕ_{HH}) were derived from the crystal structure of MMU following method (b) of Altona and Sundaralingam[18] and the results are collected in Table 6.

TABLE 6

Calculated coupling constants [a] for individual ribose ring conformers and for an equilibrium composition of 60% type N and 40% type S

	$\phi_{1'2'}$	$J_{1'2'}$	$\phi_{2'3'}$	$J_{2'3'}$	$\phi_{3'4'}$	$J_{3'4'}$	$\Sigma J_{1'2'} + J_{3'4'}$
Type S							
Solid-state	138°	6.2	−23°	7.5	−101°	0.5	6.7
geometry		6.8		7.7		0.6	7.4
$P = 162.8°$,							
$\phi_{\text{max.}} - 23.1°$							
Type N	85°	0.1	33°	6.2	−138°	6.2	6.3
$P = -18°$,		0.1		6.4		6.8	6.9
$\phi_{\text{max.}} = 34°$							
N ⇌ S		2.5		6.7		3.9	6.5
equilibrium,		2.8		7.1		4.4	7.2
60% N, 40% S.							
Experimental		2.5		6.5		4.0	6.5

[a] Coupling constants were calculated utilizing the Karplus relation $J_{HH} = A\cos^2\phi_{HH} + B\cos\phi + C$. Upper values for coupling constants in the Table: parameters $A = 9.8$, $B = 0.9$, and $C = 0$ were employed. Lower values: $A = 10.5$, $B = -1.2$, $C = 0$.

Agreement between experimental (6.5 Hz) and calculated (6.7 Hz) values for $J_{1'2'} + J_{3'4'}$ is rather good, but the calculated value $J_{2'3'} = 7.5$ Hz is 1 Hz higher than the experimental value. Moreover, we note the interesting fact that the calculated 'equilibrium-sensitive' coupling constants, $J_{1'2'} = 6.2$ Hz and $J_{3'4'} = 0.5$ Hz from the solid-state geometry do not match the experimental values at all (observed $J_{1'2'} = 2.5$ and $J_{3'4'} = 4.0$ Hz).

In other words, the experimental set of vicinal coupling constants between ribose protons cannot be explained by assuming a single S-type ribose conformer for MMU in solution. However, the data can be rationalized by assuming a fast conformational exchange process, resulting in dynamic averaging of the coupling constants. A possible candidate for such a dynamic process is a two-state equilibrium between an S-type ribose like the one found in the *X*-ray structure determination and an N-type ribose conformer. Assuming that the geometrical properties of the S-form can be transferred from the solid into the solution state, an approximate model geometry for the hypothetical N-conformer can be deduced on the basis of the following two conditions: (i) $(J_{2'3'})_N$ should be somewhat smaller than 6.5 Hz and (ii) $(J_{1'2'} + J_{3'4'})_N$ should be about equal

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* The set of Karplus parameters used in ref. 18 was obtained using a limited number of observations. A great number of accurate coupling constants have since become available and a slight downward revision of the original Karplus parameters for the ribose ring towards those used by Davies and Danyluk (this reference) seems appropriate. The *cis*-bridging group is not expected to affect the Karplus parameters to a significant extent because both electronegativity and geometrical changes, relative to the 'free' 2′,3′-diols, are small.

[18] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1973, **95**, 2333.

[19] D. B. Davies and S. S. Danyluk, *Biochemistry*, 1974, **13**, 4417.

to 6.5 Hz with $J_{1'2'}$ 2.5 Hz and $J_{3'4'}$ 4.0 Hz. Condition (i) determines the lower limit of $(\phi_{\text{max.}})_{\text{N}}(30°)$. A search of possible P-values revealed that acceptable solutions occur in the range characterized by $P = 0°$ ($\phi_{\text{max.}} = 30°$) to $P = -27°$ ($\phi_{\text{max.}} = 36°$). Outside this narrow range the calculated coupling constants rapidly diverge from experimental values. For the sake of convenience we adopt a parameter combination well within the range, *viz.* $P = -18°$, $\phi_{\text{max.}} = 34°$ (Table 6).

Then the relative populations of N- and S-conformers in solution were calculated from the established equations:

$$\overline{J_{1'2'}} = X_{\text{S}}J_{1'2'}(\text{S}) + (1 - X_{\text{S}})J_{1'2'}(\text{N})$$
$$\overline{J_{3'4'}} = X_{\text{S}}J_{3'4'}(\text{S}) + (1 - X_{\text{S}})J_{3'4'}(\text{N})$$

A slight bias in favour of the N-type ribose conformer is apparent for MMU in D_2O solution; 60% N-conformer in the $\text{N} \rightleftharpoons \text{S}$ equilibrium follows from both equations. As a final check, one calculates the time-average values corresponding to this equilibrium composition; $J_{2'3'} =$ 6.7 Hz and $J_{1'2'} + J_{3'4'} = 6.5$ Hz which are in good agreement with the experimental probes for ribose ring geometry, 6.5 Hz both (Table 6).

To our knowledge, no N-type ribose rings have as yet been encountered in X-ray structure determinations of 2′,3′-*O*-methoxymethylene nucleosides or closely related compounds, but we feel safe to predict their existence from the n.m.r. data in this paper. However, in the absence of X-ray evidence concerning the exact geometry of the N-type conformer and in view of the additional constraint of fixing the S-type partner in the equilibrium at its exact solid state geometry, the pseudorotational parameters and coupling constants of the pure conformers participating in the equilibrium should, for the time being, be regarded with some caution. In particular, an equilibrium between N and S conformers with approximately the same phase angles of pseudorotation but puckering amplitudes intermediate between the values of 23 and 34° would also show reasonable agreement with the experimental set of vicinal coupling constants, but the equilibrium composition would hardly differ from the above calculated values.

Unfortunately, no information regarding the conformations of the dioxolan ring can be obtained by the ^{1}H n.m.r. method because only the time-average value of $J_{2'3'}$ is known.

Conformation of the Exocyclic 5′-CH$_2$OH *Group.*—The conformation of the exocyclic 5′-CH$_2$OH group relative to the ribose ring may be studied by an analysis of the vicinal proton–proton coupling constants $J_{4'5'}$ and $J_{4'5''}$ [20] if a rapid interconversion between three staggered rotamers about the C(4′)–C(5′) bond is assumed (see Figure 4). Wood *et al.*[21,22] have argued that the relative population of the *gauche–gauche* rotamer can be estimated from the approximate equation: $P_{gg} \simeq (13 - \Sigma)/10$ where P_{gg} is the fractional population of the *gauche, gauche* rotamer and Σ denotes the experimental sum $J_{4'5'} + J_{4'5''}$. For MMU one calculates a *gauche,-gauche* population of 36% ($\Sigma = 9.4$).

The observed chemical shift difference between H(5′) and H(5″), $\Delta\delta = 0.065$ p.p.m., combined with the experimental value $J_{4'5'} + J_{4'5''} = 9.4$ Hz form a pair that comes close to the correlation line $\Delta\delta$ against Σ presented by Hruska[23] for a series of pyrimidine nucleosides. This observation, together with the fact that $\delta\text{H}(5') > \delta\text{H}(5'')$ and $J_{4'5'} < J_{4'5''}$ strongly suggests that the Remin and Shugar assignment of the methylene hydrogens in β-nucleosides[24] also holds for the constrained nucleoside MMU. (This configurational assignment is already indicated in Figure 4.) Additional support for this assignment comes from a recent reinvestigation of the conformational properties of the 5′-CH$_2$OH group in nucleosides and nucleotides performed by Davies and Rabczenko.[25] The populations of all three staggered rotamers can be calculated from the following equations:

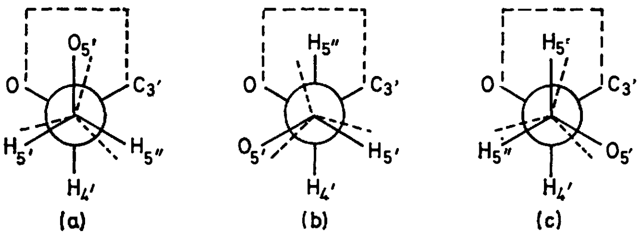

FIGURE 4 Newman projections of the exocyclic CH$_2$OH group showing classical staggered rotamers and rotamers with 15° oxygen–oxygen repulsion: (*a*) *gauche,gauche*; (*b*) *gauche,trans*; (c) *trans, gauche*

$$\overline{J_{\text{HH}}} = P_{gg}J_{gg} + P_{gt}J_{gt} + P_{tg}J_{tg}$$

and

$$P_{gg} + P_{gt} + P_{tg} = 1$$

$J_{4'5'}$ and $J_{4'5''}$ values for the three rotamers participating in the equilibrium were calculated for a classical staggered rotamer model and for a 15° oxygen–oxygen repulsion model[20] employing the Karplus relation with constants $A = 10.5$, $B = -1.2$, and $C = 0$.[18] In addition, the vicinal coupling constants for pure rotamers adopted by Davies and Rabczenko[25] were utilized to calculate the populations [equations (3), (7), and (8) in ref. 25].

The results, collected in Table 7, show considerable differences (>20%) in the fractional populations obtained by the three methods though the general preferences $P_{gg} > P_{gt} > P_{tg}$ for MMU in D_2O solution are clear. At present, no clear-cut choice of method can be

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[21] D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, *Canad. J. Chem.*, 1973, **51**, 2571.

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made.* A slight preference for the *gauche, gauche* rotamer for MMU in D_2O solution is observed, but the energy differences between the three rotamers are small. In the crystal structure the *gauche–gauche* rotamer receives additional stabilization by means of a hydrogen bond between 5′-OH and the O(2) atom of a neighbouring residue.

TABLE 7

Calculated coupling constants $J_{4'5'}$ and $J_{4'5''}$, relative populations of rotamers around the C(4′)–C(5′) bond of MMU

		Classical staggered rotamers		Rotamers with 15° O–O repulsion		Direct model [a]
		ϕ_{HH}	J_{HH}	ϕ_{HH}	J_{HH}	J_{HH}
$J_{4'5'}$	*gg*	−60°	2.0	−75°	0.7	2.7
	gt	60°	2.0	75°	0.7	2.7
	tg	180°	11.7	165°	11.1	11.7
$J_{4'5''}$	*gg*	60°	2.0	45°	4.5	0.6
	gt	180°	11.7	−165°	11.1	11.5
	tg	−60°	2.0	−75°	0.7	5.8
P_{gg}		0.44		0.36		0.58
P_{gt}		0.39		0.36		0.32
P_{tg}		0.17		0.28		0.10

[a] Calculated using equations (3), (7), and (8) in ref. 26.

Conformation around the Glycosidic Linkage.—Information concerning the conformation about the glycosidic bond was obtained from the c.d. spectrum of MMU in aqueous solution (see Table 8 for experimental details).

TABLE 8

C.d. spectra of MMU [a] and 2′,3′-*O*-isopropyleneuridine [b] (Urd > CMe_2) in aqueous solution

	B_{2u} transition	B_{1u} transition	E_{1ua} transition	E_{1ub} transition
MMU	1.6 (267 nm)	−0.5 (246 nm)	−0.6 (222 nm)	1.2 (194 nm)
Urd > CMe_2	2.0 (268 nm)	−1.1 (238 nm)	−1.6 (213 nm)	

[a] Experimental: a solution of 0.87×10^{-4}M-MMU in H_2O, pH = 7 was measured in a 1-cm cuvette on a Roussel-Jouan Mark III dichrograph calibrated with 0.1% (+)-camphorsulphonic acid. The optical density was 0.902 at the long-wavelength absorption maximum (262 nm). [b] Ref. 26.

The sign and magnitude of the B_{2u} transition (267 nm) in MMU is comparable to that of the closely related 2′,3′-*O*-isopropyleneuridine in aqueous solution. For the latter compound an *anti*-conformation around the glycosidic bond was inferred from c.d. spectra in polar solvents.[26] The qualitative agreement between the c.d. spectra of MMU and isopropyleneuridine (Table 8) suggests that MMU in aqueous solution also prefers the *anti*-conformation of the uracil base relative to the ribose ring, in accordance with the solid-state geometry.

In addition the chemical shifts of the base protons H(6) (7.791 p.p.m.) and H(5) (5.973 p.p.m.) for MMU in D_2O solution are close to the values observed for uridine [20] (7.862 and 5.887 p.p.m. respectively), also suggesting that the uracil base exists in the *anti*-conformation about the *N*-glycosidic bond. From the observation of a long-range spin–spin coupling $^5J_{H(5),H(1')} = 0.8$ Hz,[27] for uridine in basic solution the *anti*-conformation was inferred, but unfortunately neither uridine nor MMU show such a long range coupling in neutral solutions.

Conclusions.—The combination of *X*-ray and n.m.r. methods in this investigation of the conformational properties of 2′,3′-*O*-methoxymethyleneuridine proved to be useful. The *X*-ray investigation provided detailed geometrical information about the solid-state conformation of MMU. This conformation is characterized by a relatively flattened ribose ring ($\phi_{max.} = 23.1°$) with a phase angle of pseudorotation $P = 162.8°$ in the normal range observed for type S [C(2′)-*endo*] ribonucleosides. The base ring exists in the *anti*-conformation relative to the ribose ring and the exocyclic CH_2OH-group occurs in the familiar *gauche, gauche* rotamer. The solid-state conformation was considered as one of the low-energy conformations (conformers) for MMU in aqueous solution. Vicinal coupling constants in the n.m.r. spectrum of MMU revealed the presence of conformational equilibria in solution. The time-average ribose ring conformation is best described as a rapid equilibrium between type S and type N conformers with a 60% preference for the latter. The relative preferences for orientation of the 5′-CH_2OH side-chain are in the order $gg > gt > tg$. The c.d. spectrum of MMU as well as the chemical shifts of the base protons suggested that the uracil base exists in an *anti*-conformation relative to the ribose ring.

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* The method proposed by Davies and Rabczenko [25] utilizes observed vicinal coupling constants in model compounds for the individual rotamers of the CH_2OH-group in nuclesides to account for the geometry-dependent electronegativity effect. Their assumption that the torsion angles in those model compounds can be carried over to the situation in nucleoside derivatives seems not *a priori* justified.

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