Intramolecular Hydrogen Bonding and Molecular Conformations of Nucleosides: Uridine Derivatives

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The effect of intramolecular hydrogen bonding on the molecular conformations of uridine derivatives in solution was investigated by i.r., c.d., and n.m.r. spectroscopy. The 220 MHz ^{1}H n.m.r. spectra of three uridine derivatives in $CDCl_3$ solutions [3-methyl-5′-*O*-methyl-2′,3′-*O*-isopropylideneuridine (1), 3-methyl-2′,3′-*O*-isopropylideneuridine (2), 3,6-dimethyl-2′,3′-*O*-isopropylideneuridine (3)] were analysed in terms of the conformational properties of the O(5′)–C(5′) and C(5′)–C(4′) bonds, and the sugar ring conformation. The presence of the hydrogen bond in compounds (2) and (3) was confirmed directly by i.r. measurements whereas 5′-*O*-methylation precludes such hydrogen bond formation between the exocyclic CH_2OH group and the base ring 2-carbonyl group in compound (1). The presence of the hydrogen bond was confirmed indirectly by c.d. measurements which showed that the base ring adopts the *syn*-conformation which is necessary for such hydrogen bond formation whereas the base ring of the 5′-*O*-methyl compound adopts a predominant *anti*-conformation. Changes in conformational properties of compound (2) in different solvents monitored by c.d. and n.m.r. measurements support the conformational model that hydrogen bond formation between the exocyclic CH_2OH group and the pyrimidine ring 2-carbonyl group necessitates the base ring being in the *syn*-conformation and the O(5′)–C(5′) and C(5′)–C(4′) bonds adopting specific *gauche*-conformers. Hydrogen bond formation also promotes the sugar ring S [*ca.* C(2′)-*endo*] conformation though not as markedly as for an analogous purine derivative.

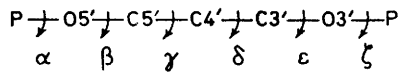
INTRAMOLECULAR hydrogen bonding has recently been shown to exist for a purine nucleoside derivative (6,6-dimethyl-2′,3′-*O*-isopropylideneadenosine) in non-polar solvents by i.r. and n.m.r. studies.[1] The hydrogen bond exists between the exocyclic CH_2OH group and the N(3) atom of the base ring. In order to facilitate such hydrogen bond formation the base ring adopts the *syn*-conformation, the C(5′)–C(4′) bond exists exclusively in the γ_+ (*ca.* 60°) conformation and the O(5′)–C(5′) bond exists exclusively in the β_+ (*ca.* 60°) conformation.† These conformational features are also observed in *X*-ray crystal structures of purine derivatives which exhibit this intramolecular hydrogen bonding.[3] For such molecules in solution the presence of the hydrogen bond is sufficient to tilt the balance between conformations separated by relatively low energy barriers and to promote the sugar ring **S**-type [*ca.* C(2′)-*endo*] conformation (80—90%) compared with cases where the hydrogen bond is unlikely to be present (40—50%).[1]

An analogous intramolecular hydrogen bond has been observed for a pyrimidine derivative in the solid state by *X*-ray crystallographic studies of 6-methyluridine [4] where the hydrogen bond between O(5′) of the sugar and O(2) of the base ring was accompanied by the base ring being in the *syn*-conformation, the sugar ring in the C(2′)-*endo* (**S**-type) conformation, and the exocyclic C(5′)–C(4′) bond in the *gauche-gauche* (γ *ca.* 51°) conformation. It should be noted that the second molecule in the asymmetric unit of 6-methyluridine [4] does not exhibit an intramolecular hydrogen bridge between the base ring O(2) and exocyclic O(5′) because the C(5′)–C(4′) bond has the *trans-gauche* (γ *ca.* 180°) conformation in contrast to the *gauche-gauche* (γ *ca.* 51°) observed for the first molecule even though both molecules are found with similar base ring (*syn*) and sugar ring [C(2′)-*endo*] conformations. Conformational properties observed for 6-methyluridine in aqueous solution by n.m.r. spectroscopy,[5] indicated that the presence of the base ring in the *syn*-conformation was accompanied by the exocyclic group existing predominantly in either the *trans-gauche* (γ *ca.* 180°) or *gauche-trans* (γ *ca.* 300°) conformation. Differentiation between these two latter conformations is only possible by n.m.r. spectroscopy if the two 5′-methylene protons are assigned unambiguously.[6-8] Evidence for intramolecular hydrogen bonding being involved in stabilising particular conformations of nucleosides in solution has been presented utilising i.r.[9] and other spectroscopic techniques [10,11] though no complete studies of pyrimidine derivatives have been made in which all the various conformational features necessary for such hydrogen bond formation have been elucidated.

The present work reports the observation of hydrogen bonding between the exocyclic CH_2OH group and the base ring 2-carbonyl group of uridine derivatives in solution by i.r. spectroscopy and the concomitant conformational features necessary for such hydrogen bonding have been investigated by n.m.r. and c.d. spectroscopy. It is found that the hydrogen bond for the pyrimidine derivative is not as strong as for an analogous purine derivative [1] and the resulting effect on the conformational properties of the base and sugar rings is not as marked.

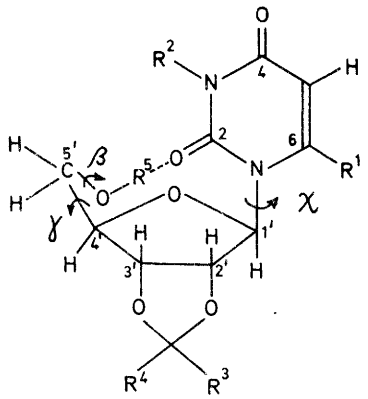
The uridine derivatives measured in this work are shown in Figure 1 together with the atom numbering

† The new α—ζ notation recommended to I.U.P.A.C.–I.U.B. for description of conformations of polynucleotide chains is used in this work.[2] The notation labels bonds α—ζ along the polynucleotide chain starting at the phosphorus atoms as in (A).



(A)

scheme, bond torsional angles (β γ, χ), and a diagrammatic representation of the features necessary for $CH_2OH \cdots O{=}C$ intramolecular hydrogen bond formation. The compounds are 3-methyl-5′-*O*-methyl-2′,3′-*O*-isopropylideneuridine (1), 3-methyl-2′,3′,-*O*-isopropylideneuridine (2), and 3,6-dimethyl-2′,3′-*O*-isopropylideneuridine (3). The compounds were designed to promote intramolecular hydrogen bonding between O(5′) and O(2) by substitution at O(2′) and O(3′) with the isopropylidene group and to minimise intermolecular hydrogen bonding by methylation of N(3). Gelation of uridine derivatives in non-polar solvents had previously been observed by Hart and Davis [12] but no such gelation occurred for the present set of molecules because no concentration dependence of hydrogen bonding was observed by i.r.



	R^1	R^2	R^3	R^4	R^5
(1)	H	CH_3	CH_3	CH_3	CH_3
(2)	H	CH_3	CH_3	CH_3	H
(3)	CH_3	CH_3	CH_3	CH_3	H
(4)	H	D*	H	OCH_3	D*

FIGURE 1 Structure of the uridine derivatives (1)—(4) showing the atom numbering scheme, bond torsional angles β, γ, and χ, and a representation of the conformational features necessary for $CH_2OH \cdots O{=}C$ intramolecular hydrogen bond formation

spectroscopy. The three compounds enabled the effect of promoting the base ring *syn*-conformation [comparison of (3) with (2)] and precluding hydrogen bond formation [comparison of (2) with (1)] to be assessed and the results were compared with those of a similar derivative, 2′,3′-*O*-methoxymethyleneuridine (4), measured in D_2O solution [13] where intramolecular hydrogen bonding is not expected to be significant. It was found that the same conformational features needed to promote hydrogen bond formation in a purine nucleoside were observed for the pyrimidine nucleosides [base ring in the *syn*-conformation and the exocyclic CH_2OH group existing predominantly with the C(5′)–C(4′) bond in the γ *ca.* 60° conformation and the O(5′)–C(5′) bond in the β *ca.* 60° conformation], though the effect of such hydrogen bond formation on sugar ring conformation is smaller for pyrimidine than for purine nucleosides.

EXPERIMENTAL

(i) *Materials.*—The uridine derivatives were synthesised according to established procedures. 5′-*O*-Methyl-2′,3′-*O*-isopropylideneuridine (1) was synthesised by methylation of 2′,3′-*O*-isopropylideneuridine with methyl iodide in dimethylformamide in the presence of sodium hydride.[14] 3-Methyl-2′,3′-*O*-isopropylideneuridine (2) was synthesised by methylation of 2′,3′-*O*-isopropylideneuridine using diazomethane in methanol according to the method of Szer and Shugar.[15] 3,6-Dimethyl-2′,3′-*O*-isopropylideneuridine (3) was synthesised by isopropylidenation of 6-methyluridine (a kind gift from Dr. H. Vorbrüggen, Schering A.G.) using acetone and 2,2-dimethoxypropane in solution with toluene-*p*-sulphonic acid as a catalyst and then by methylation with diazomethane in methanol [15] as for compound (2).

(ii) *C.d. Measurements.*—The c.d. spectra of compounds (1)—(3) were measured in CCl_4 solution and compound (2) was also measured in a range of different solvents (cyclohexane, carbon tetrachloride, chloroform, and 1,3-dioxan). Three independent samples were measured in each case and the concentrations of solutions were all *ca.* 10^{-3}M. The experiments were performed on a Russel Jouan III spectrophotometer using 1 mm cells.

(iii) *I.r. Measurements.*—The i.r. absorption spectra of compound (2) for CCl_4, CH_2Cl_2, and $CHCl_3$ solutions were run on a UR 20 Zeiss Jena spectrophotometer within the spectral regions 1 500—1 800 and 2 800—3 700 cm^{-1}. The concentrations of solutions were 10^{-4}M in the case of

TABLE 1

Observed ^1H n.m.r. parameters and conformational properties of compounds (1)—(3) in $CDCl_3$ solution at 294 ± 1 K [a]

	(1) [a]	(2) [a]	(3) [a]	(4) [b]
(i) Chemical shifts (δ)				
6-H	7.55	7.427	(2.318) [c]	7.791
5-H	5.763	5.764	5.664	5.873
1′-H	5.863	5.614	5.693	5.871
2′-H	4.776	4.995	5.257	5.193
3′-H	4.796	4.963	5.166	5.005
4′-H	4.409	4.311	4.221	4.346
5′-H	3.654	3.917	3.875	3.872
5″-H	3.589	3.806	3.802	3.807
5′-OH	(3.37) [c]	2.92		
NCH_3	3.43	3.218	3.52	
CH_3	1.62	1.591	1.55	3.558
CH_3	1.38	1.373	1.33	(6.181) [e]
(ii) Spin coupling constants (*J*/Hz)				
1′,2′	~1.8	2.8	2.2	2.5
2′,3′	6.0	6.5	6.5	6.5
3′,4′	~0.5	2.5	4.5	4.0
4′,5′	3.0	2.5	2.9	3.6
4′,5″	3.8	3.1	4.5	5.8
5′,5″	−10.7	−11.8	−12.1	−12.3
5′,OH		3.0 [d]		
5″,OH		6.0 [f]		
(iii) Conformational properties				
%S	78	53	33	38
$\%\gamma_+$	62	74	56	36
$\%\beta_+$		40		

[a] Chemical shifts δ (±0.002) p.p.m. and coupling constants *J* (±0.1 Hz) were determined by computer simulation of the spectra. [b] Data taken from ref. 3 for (4) in D_2O solution (0.05M; pD 7.3; 298 K) observed at 360 MHz [chemical shifts relative to internal 3-(trimethylsilyl)propanesulphonic acid, accuracy 0.001 p.p.m.; coupling constants expressed in Hz, accuracy 0.1 Hz]. [c] CH_3 group. [d] Error limits on *J* (±0.5 Hz). [e] CH group.

CCl_4 and *ca.* 10^{-2}M in the case of other solvents The solutes were checked for intermolecular self-associaton and no dependence of absorption was detected over an order of magnitude change in concentration. All samples were prepared in a dry box to minimise contamination from traces of water. The solutions were prepared in glass ampoules sealed after weighing to avoid both evaporation of solvent and absorption of atmospheric water during the prolonged solubilisation process, particularly for compounds of low solubility, *e.g.* (2) in CCl_4. Before i.r. measurements were made, small traces of water were removed by activated molecular sieve for 10—15 min. It was found that drying for this period of time did not alter the species concentration but adsorption of the solute on to the sieves occurred after a few hours.

(iv) *N.m.r. Measurements.*—220 MHz ^{1}H N.m.r. spectra of (1)—(3) in $CDCl_3$ solutions were measured at a probe temperature of 294 (±1) K and spectra of compound (2) obtained in different solvents [$CDCl_3$, CD_3CN, $(CD_3)_2CO$, CD_3OD, $(CD_3)_2SO$, and D_2O]. Chemical shifts were recorded with respect to tetramethylsilane (TMS) used as internal reference standard and spin coupling constants were checked by computer simulation of spectra and the relevant parameters are given in Tables 1 [compounds (1)—(3) in $CDCl_3$] and 2 [compound (2) in different solvents].

A feature of the spectra of compound (2) is observation of the 5′-OH signal as a doublet of doublets resulting from the different magnitudes of coupling to the two 5′-protons. In order to determine all 5′-proton spin coupling constants of (2), measurements were made on both a deuteriated sample (5′-OD, enabling $J_{4',5'}$ and $J_{4',5''}$ to be observed) and a solution prepared using distilled solvents and dried sample with the final solution being dried over molecular sieve for a few minutes prior to measurement. In the latter case the separate 5′-OH signal was observed together with the coupling to the 5′-protons.

RESULTS AND DISCUSSION

(1) *Molecular Conformations.*—(i) *Exocyclic* C(5′)–C(4′) *bond,* γ. The conformational properties of the C(5′)–C(4′) bond are determined by analysis of the vicinal spin coupling constants between the C(4′) and two C(5′) protons in terms of the fractional populations (p_+, p_a, p_-) of the three classical staggered rotamers (γ_+, γ_a, γ_- of Figure 2) according to established procedures.[16-18] Absolute values of bond conformer populations were calculated using the parameters of Hruska and Sarma [17,18] (J_t 11.5, J_g 1.5 Hz) and the results, which are summarised in Table 1, indicate a strong preference for the γ_+ conformer for each compound with increasing proportions of this conformation found for compounds (3) (56%), (1) (62%), and (2) (75%) in $CDCl_3$ solutions at 294 ± 1 K whereas compound (4) in D_2O solution exhibits only 36% of the γ_+ conformer for C(4′)–C(5′) bond rotation. Differences between the calculated populations of γ_+ conformers for compounds (1)—(4) are significant because the observed errors in J (±0.1 Hz) lead to variations in p_+ of *ca.* ±2%. The differences in relative proportions of C(4′)–C(5′) bond conformations of compounds (1)—(4) are subsequently correlated with other conformational features of the molecules and the hydrogen bond properties of these compounds determined by i.r. measurements.

(ii) *Exocyclic* O(5′)–C(5′) *bond,* β. The conformational properties of the O(5′)–C(5′) bond were determined by analysis of ^3J(HCOH) magnitudes in terms of the relative proportions of staggered conformers (β_+, β_a, and β_- in Figure 2) using the method adopted for the adenosine derivative.[1] The parameters of the Karplus relation for the HCOH molecular fragment determined by Fraser

TABLE 2

Observed ^{1}H n.m.r. parameters and conformational properties of compound (2) in different solvents

Dielectric constant	$CDCl_3$ 4.8	CD_3CN	$(CD_3)_2CO$ 21	CD_3OD 33	$(CD_3)_2SO$ 40	D_2O 78
(i) Chemical shifts (δ)						
6-H	7.427	7.627	7.850	7.91	7.850	7.773
5-H	5.764	5.677	5.668	5.81	5.764	5.950
1′-H	5.614	5.836	5.909	5.97	5.855	5.909
2′-H	4.995	4.814	4.895	4.86	4.891	5.091
3′-H	4.963	4.786	4.864	4.78	4.750	4.936
4′-H	4.311	4.177	4.120	4.28	4.093	4.414
5′-H	3.917	3.718	3.820	3.837	3.551	3.869
5″-H	3.806	3.660	3.770	3.777	3.508	3.777
5′-OH	2.92	2.236	4.364		5.114	
NCH_3	3.318	3.173	3.191	3.34	3.114	3.727
CH_3	1.591	1.518	1.509	1.62	1.477	1.477
CH_3	1.373	1.305	1.309	1.42	1.273	1.409
(ii) Spin-coupling constants (J/Hz)						
1′,2′	2.8	2.4	2.4	2.2	2.4	2.5
2′,3′	6.5	6.2	6.3	6.5	6.2	6.4
3′,4′	2.5	3.0	3.1	3.1	3.4	3.7
4′,5′	2.5	3.3	2.8	3.1	4.6	3.9
4′,5″	3.1	4.4	3.8	4.8	5.1	5.7
5′,5″	−11.8	−12.1	−12.0	−12.0	−12.1	−12.2
(1′,2′ + 3′,4′)	5.3	5.4	5.5	5.3	5.8	6.2
(4′,5′ + 4′,5″)	5.6	7.7	6.6	7.9	9.7	9.6
(iii) Conformational properties						
%S	53	44	44	42	41	40
%γ_+	74	53	64	51	33	34

et al.,[19] *i.e.* J_t 12.1, J_g 2.1 Hz, were used in this work to calculate p_+, p_a, and p_- though previous work has shown [1] that the values might be modified to accommodate smaller observed J_g (1.8—1.9 Hz). However small changes in J_g and J_t produce only small changes in absolute magnitudes of p_+, p_a, and p_- and have little effect on their relative values so the values suggested by Fraser *et al.*[19] will be used until the projected modifications are confirmed. By analogy with the analysis for

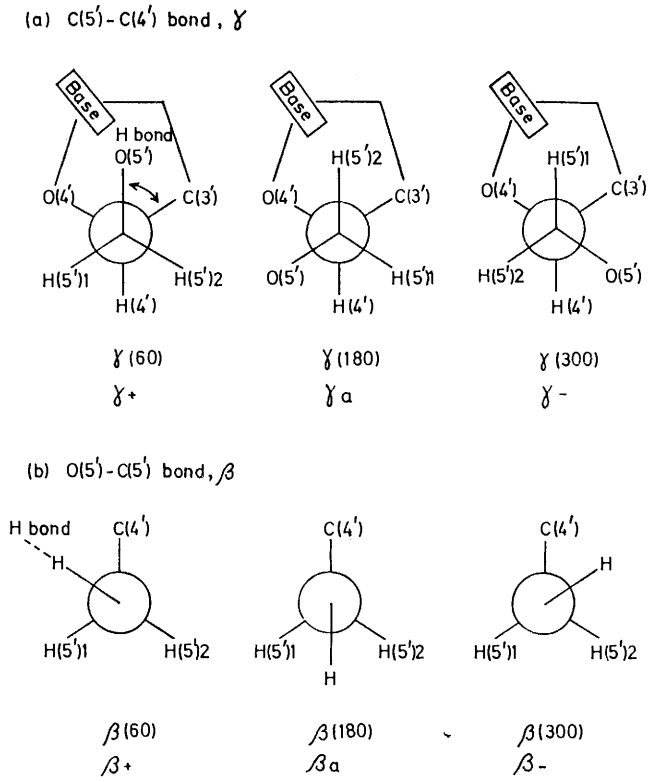


FIGURE 2 Classical staggered conformers for (a) C(5')–C(4'), γ, and (b) O(5')–C(5') bonds, β, showing the conformations needed for intramolecular hydrogen bonding between exocyclic CH_2OH and base-ring 2-oxo-group, *i.e.* $\beta_{60°}$ and $\gamma_{60°}$. The (+,a,−) notation used for different rotamers (60, 180, 300°, respectively) [16] is shown in terms of the new torsion angle nomenclature recommended to I.U.P.A.C.–I.U.B.[2]

the C(5')–C(4') bond rotamers it was shown that the relative proportion of the β_a conformer could be determined from the sum of observed coupling constants ($J_{5',OH5'} + J_{5'',OH5'}$), but that p_+ and p_- can only be determined by unequivocal analysis of the two C(5') protons. The observed ^3J(HCOH) values for compound (2) listed in Table 1 were analysed in terms of the relative proportions of O(5')–C(5') bond conformers. Using the C(5') proton assignment of Remin and Shugar,[7] it was found that significant populations of two conformers (β_+ *ca.* 0.4 and β_a *ca.* 0.5) exists for compound (2) whereas there is only a small contribution of the β_- conformer (*ca.* 0.1). The relatively large proportion of the β_+ conformer is consistent with $CH_2OH \cdots O{=}C$ intramolecular hydrogen bonding as shown in Figure 2.

(iii) *Sugar ring conformation.* Criteria have been established for determining the conformations of ribose rings of nucleosides and nucleotides in solution and the pseudorotational analysis of Altona and Sundaralingam [20,21] has been shown to be applicable to nucleoside-2'-, -3'-, and -5'-monophosphates.[22,23] The analysis relies on observed sugar ring coupling constant values of $J_{2',3'}$ (*ca.* 5.1 Hz) and ($J_{1',2'} + J_{3',4'}$) (*ca.* 9.9—10.1 Hz). The data for compounds (2) and (3) in Table 1 indicate that substantial differences from these values can occur for ribose rings constrained by the isopropylidene ring, *i.e.* $J_{2',3'}$ 6.2—6.5 and ($J_{1',2'} + J_{3',4'}$) 4.5—6.7 Hz. Similar trends were observed previously [24] for isopropylidene derivatives of adenosine and guanosine in ND_3 solutions [$J_{2',3'}$ 6.2—6.4 and ($J_{1',2'} + J_{3',4'}$) 4.9—5.6 Hz] over the temperature range 213—331 K, for 2',3'-*O*-methoxymethylene uridine in D_2O solution, [$J_{2',3'}$ 6.5 and ($J_{1',2'} + J_{3',4'}$) 6.5 Hz [13]], and for 6,6-dimethyl-2',3'-*O*-isopropylideneadenosine in $CDCl_3$ solutions over the temperature range 245—317 K [$J_{2',3'}$ 5.8—5.9 and ($J_{1',2'} + J_{3',4'}$) 5.7—5.9 Hz].[1]

It is not yet clear if the pseudorotational analysis of ribose rings may be applied to those constrained by the 2',3'-*O*-isopropylidene group as all the criteria for such an analysis are not met. Indeed a recent analysis of *X*-ray crystal structures indicates that a second pseudorotational pathway [O(1')-*endo* ⟷ planar ⟷ O(1')-*exo*] is possible for such constrained rings.[25] Although this conformational pathway has also been used to describe n.m.r. results of some isopropylidene derivatives,[24] it was shown that its use is rather restricted because the model predicts equal magnitudes of $J_{1',2'}$ and $J_{3',4'}$ for each conformational equilibrium whereas other cases have been observed.[1] In this work the pseudorotational analysis of ribose rings is applied to those constrained by isopropylidene groups and, even though this is a dubious procedure, it allows the results to be compared with those previously obtained by this method.[1]

First, it is assumed that the modified Karplus relation determined for ribose rings [22] holds for ribose rings constrained by the isopropylidene group. Secondly, it is assumed that the curves computed by Guschlbauer and Son [26] can be used to determine the pseudorotational parameters (P, τ_m) of the sugar ring from observed $J_{2',3'}$ and ($J_{1',2'} + J_{3',4'}$) values and can also be used to calculate the puckering equilibrium from individual values of $J_{1',2'}$ and $J_{3',4'}$. It is found that the sugar rings of compounds (3) and (4) are somewhat flattened (τ_m *ca.* 32) and the sugar ring of compound (2) (τ_m *ca.* 42) is more puckered than for normal ribose rings (τ_m *ca.* 38). For all compounds the calculated pseudorotational angles (^NP 310—350, ^SP 190—230°) correspond to approximate C(2')-*exo* and C(3')-*exo* conformations, respectively, whereas unrestrained ribose rings (^NP *ca.* 18 and ^SP, *ca.* 162°) correspond to C(3')-*endo* and C(2')-*endo* conformations, respectively. Similar behaviour is found for other isopropylidenenucleosides in different solvents.[1] Calculation of the puckering equilibrium indicates that

compounds (3) and (4) have 30—40% of the S conformer whereas compound (2) has 40—50% of the S conformer. These results are similar to those observed for other isopropylidenenucleosides except for 6,6-dimethyl-2′,3′,-*O*-isopropylideneadenosine which exhibited an increase in the S conformer (to *ca.* 80%) resulting from stabilisation by $CH_2OH \cdots N(3)$ hydrogen bonding.[1] Following this line of thought it can be seen that compound (2), where $CH_2OH \cdots O{=}C$ intramolecular hydrogen bonding is most likely to occur, has a greater proportion of the S conformer than compounds (3) and (4) and at the same time we found that compound (2) has a greater proportion of the C(5′)–C(4′) bond γ_+ conformer which is also needed for hydrogen bond formation.

(iv) *Glycosidic bond conformation*, χ (*Figure* 3). A number of physical methods have been used to determine the glycosidic bond conformation of nucleosides in

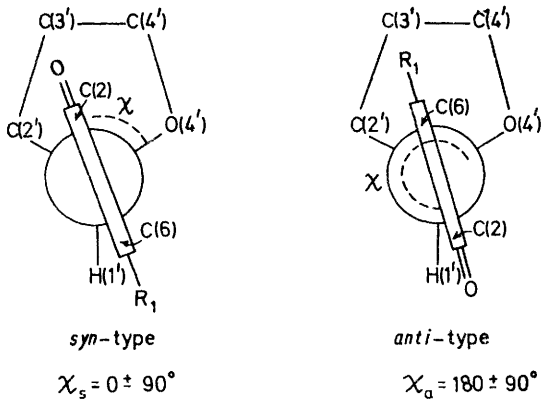


FIGURE 3 Glycosidic bond conformational ranges for *syn*- and *anti*-conformations of pyrimidine nucleosides looking down the N(1)–C(1′) bond

solution though none are able to specify the conformation as accurately as in the solid state because the low energy barrier to rotation about the glycosidic bond results in a *syn* ⇌ *anti* conformational equilibrium.[27] Reasonably reliable methods have been devised to determine the glycosidic bond conformational preference by c.d.,[10,28,29] nuclear Overhauser enhancements,[30,31] and vicinal carbon–proton coupling of base to sugar ring [32-34] though discrepancies between these methods have been noted.[16] The most convenient method for the present work is c.d. though a quantitative estimate of the *syn* ⇌ *anti* equilibrium is only feasible by n.m.r. measurements at present.[16]

The c.d. spectra of compounds (1)—(3) in CCl_4 solution are shown in Figure 4. A positive Cotton effect is observed for compound (1), the 5′-*O*-methyl derivative ($\Delta\varepsilon = +1.4$), which indicates a predominant *anti*-conformation whereas a negative Cotton effect is observed for compounds (2) and (3) which indicates a predominant *syn*-conformation for the base ring.[10] The magnitude of the effect for the 6-methyl derivative (3) ($\Delta\varepsilon$ −0.9) is a little larger than that for compound (2) ($\Delta\varepsilon$ −0.8) indicating predominant *syn*-conformations for both compounds in CCl_4 solution. C.d. spectra of compound

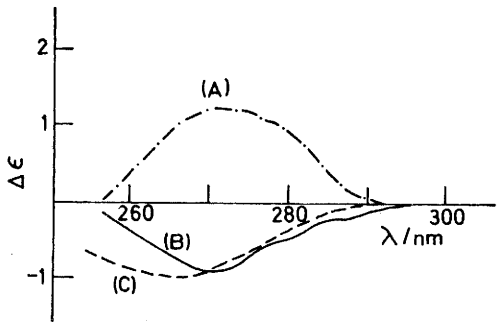


FIGURE 4 C.d. spectra of compounds (1) (A, $\Delta\epsilon$ +1.4); (2) (B, $\Delta\epsilon$ −0.8); and (3) (C, $\Delta\epsilon$ −0.9) in CCl_4 solution

(2) in different solvents (Figure 5) exhibits a positive Cotton effect in the most polar solvent (1,3-dioxan) which indicates a predominant *anti*-conformation whereas a negative Cotton effect is observed for (2) in non-polar solvents ($CHCl_3$, CCl_4, C_6H_{12}) which indicates an increasing proportion of the *syn*-conformer in solvents with smaller relative permittivities.

Chemical shifts of compound (2) in different solvents (Table 2) indicate a downfield trend with increasing polarity of solvent; the downfield trend is most marked for the 6- and 1′-H signals which suggests that they might be used to monitor the glycosidic bond equilibrium. However similar trends in chemical shifts are observed in some analogous compounds which are rigid (cyclonucleosides) and which mimic *syn*-type (5′,2-*O*-cyclo-2′,3′-*O*-isopropylideneuridine) and *anti*-type (5′,6-*O*-cyclo-2′,3′-*O*-isopropylideneuridine) pyrimidine nucleosides.[35] Hence changes in chemical shifts with solvent may not be used to determine the glycosidic bond equilibrium. On the other hand, it is found that the glycosidic bond conformations of the uridine derivatives in the same solvent ($CDCl_3$) and at the same temperature (299 ± 1 K) are reflected in the proton chemical shifts of the sugar ring protons. The results in Table 1 show that the 2′- and 3′-H signals of the sugar ring move downfield with progressively more *syn*-conformation of the base ring [(3) > (2) > (1)] and that the opposite trend is observed for the 1′- and 4′-H signals. If it is assumed that compound (3) exists with a *syn*-type glycosidic bond conformation in solution and compound (1) exists with an *anti*-type conformation, then results

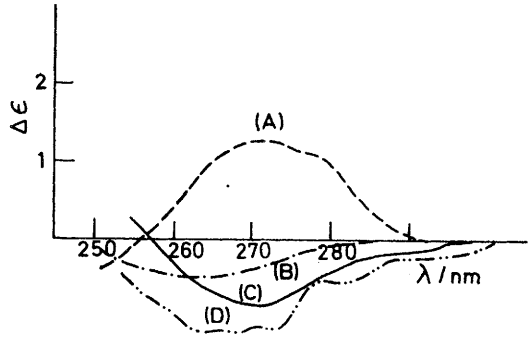


FIGURE 5 C.d. spectra of 3-methyl-2′,3′-*O*-isopropylideneuridine (2) in 1,3-dioxan (A), $CHCl_3$ (B), CCl_4 (C), and cyclohexane (D) solutions

for 2′-, 3′-, and 4′-H signals of compound (2) indicate a *ca.* 1 : 1 *syn–anti* equilibrium. Results for δ(1′-H) of compound (3) do not follow this trend because of the effect on 1′-H of the magnetic anisotropy of the CH_3 group compared to the normal 6-H group for the uracil ring in the *syn*-conformation. The chemical shifts of sugar ring protons of these pyrimidine derivatives may be used in a qualitative manner to determine glycosidic bond conformations of closely related nucleosides measured in the same solvent and at the same temperature.

(2) *Hydrogen Bond Formation* (*I.r.*).—The i.r. absorption spectrum of compound (2) in $CHCl_3$ solution at 303 K exhibits the expected carbonyl (1 500—1 800

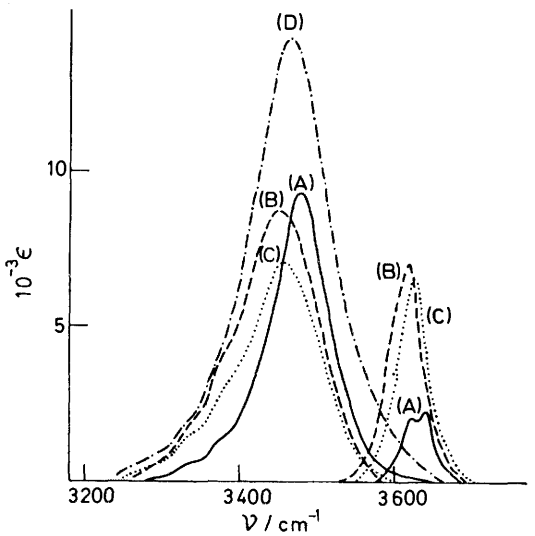


FIGURE 6 High frequency part of i.r. spectra of 3-methyl-2′,3′-*O*-isopropylideneuridine (2) in CCl_4 (A), CH_2Cl_2 (B), $CHCl_3$ (C), and 1,3-dioxan (D) solutions at 293 K. Maxima of the absorption bands: in CCl_4, 3 480, 3 622, 3 640 cm^{-1}, in CH_2Cl_2, 3 456, 3 605(shoulder), 3 622 cm^{-1}; in $CHCl_3$, 3 450, 3 602(shoulder), 3 618 cm^{-1}; in 1,3-dioxan 3 466 cm^{-1}

cm^{-1}) and hydroxy (3 300—3 700 cm^{-1}) stretching vibration regions. Shoulders at the low frequency part of the OH absorption (3 300—3 400 cm^{-1}) are due to overtones of the C=O stretching vibration (1 600—1 720 cm^{-1}) [36] which was confirmed by observation of peaks in the 3 300—3 400 cm^{-1} region for the OD deuteriated compound as OH bands are not observed in this case. The OH stretching vibration region (3 200—3 700 cm^{-1}) of compound (2) in a number of different solvents at ambient temperatures (294 ± 1 K) is shown in Figure 6 (CCl_4, CH_2Cl_2, $CHCl_3$, and 1,3-dioxan); the lower frequency band (3 450—3 500 cm^{-1}) corresponds to OH groups involved in hydrogen bonding whereas the higher frequency band (max. 3 600—3 650 cm^{-1}) corresponds to the ' free ' OH group observed for CCl_4, CH_2Cl_2, and $CHCl_3$ solutions. There is no component for ' free ' OH groups of compound (2) in 1,3-dioxan because of the existence of additional solvent–solute interactions in this solvent.

Examination of the i.r. spectra of (2) in different solvents in more detail (Figure 6) shows that the maximum of the band corresponding to OH groups involved in hydrogen bonding occurs at 3 480 cm^{-1} for CCl_4 solution with shifts to lower frequencies for CH_2Cl_2 (3 456 cm^{-1}) and $CHCl_3$ (3 450 cm^{-1}) solutions. The low frequency shift is due to the interaction of the solvent (CH_2Cl_2 and $CHCl_3$) with the oxygen of the 5′-OH group which results in an increase of hydrogen bond strength of the type R–H • • • OH • • • O=C. At the same time the absorption band for ' free ' OH groups of compound (2) in CCl_4 solution consists of two components with maxima at 3 622 and 3 640 cm^{-1}. A similar situation occurs for CH_2Cl_2 and $CHCl_3$ solutions where one component appears as a shoulder to low frequencies; the absorption maxima for ' free ' OH groups in these solvents are also shifted toward lower frequencies compared to CCl_4 solutions which can also be explained in terms of the hydrogen bonding properties of the protonated solvents.

The equilibrium constants for internal hydrogen bond formation were determined from the integral band absorptions of the OH groups not involved in hydrogen bonding (D_f) and those involved in the internal hydrogen bridge (D_b) according to standard procedures.[37] The integral band intensity of the bound OH group, D_b, was obtained by two methods which lead to the same results (i, as the full area under the absorption curve; ii, as the area of the band after symmetrisation of the short wavelength part). The equilibrium constant for hydrogen bond formation is defined as $K = C_b/C_f$ (where C_b and C_f are the concentrations of the bound and free OH groups) and can be determined from equation (1) where D_f and D_b are the integral intensities of the bands corresponding to the vibrations of the free and bound OH group and $\varepsilon_f/\varepsilon_b$ is the ratio of the extinction coefficients of the two bands. Values of K at 293 K for compound

$$K = C_b/C_f = D_b\varepsilon_f/D_b\varepsilon_f \qquad (1)$$

(2) in different solvents have been calculated from equation (1) using the results in Figure 6 (CCl_4 1.6, CH_2Cl_2 0.4, and $CHCl_3$ 0.3). The degree of association (α%) for hydrogen bonding was calculated from the magnitudes of K, *i.e.* CCl_4 62%, CH_2Cl_2 30%, and $CHCl_3$ 24%. The results show that intramolecular hydrogen bonding between the base ring and the exocyclic CH_2OH group in (2) increases in non-polar compared to polar solvents. This observation is in line with conformational properties determined from n.m.r. and c.d. measurements of compound (2) in different solvents and combination of results from the different spectroscopic measurements leads to a conformational model for these molecules.

Conformational Model.—The trends in conformational properties of 3-methyl-2′,3′-*O*-isopropylideneuridine (2) with the polarity of the solvent are summarised in Table 3.

The inter-relation between all the conformational features was explored using Dreiding molecular models which show that the CH_2OH • • • O=C intramolecular hydrogen bond in pyrimidine nucleosides can be formed

TABLE 3

Trends in conformational properties of compound (2) in different solvents

	D_2O	$(CD_3)_2SO$	CD_3OD	$(CD_3)_2CO$	CD_3CN	$CDCl_3$	CCl_4	C_6H_{12}
K_r [a]	78.5		32.6	21.2		4.8	2.3	2.0
I.r.	Increase in intramolecular hydrogen bonding →							
C.d.	Increase in in *syn*-conformation →							
N.m.r.								
(i) $\%\gamma_+$ [b]	34	33	51	64	53	74		
	Increase in C(5′)–C(4′) bond γ_+ conformation →							
(ii) %S [c]	40	41	42	44	44	53		
	Increase in sugar ring S conformation →							

[a] Approximate relative permittivity (dielectric constant) at ambient temperature. [b] Calculated from $p(\gamma_+) = (13 - \Sigma)/10$; $\Sigma = (J_{4',5'} + J_{4',5''}$ Hz). [c] Calculated from $S_X = J_{1',2'}/(J_{1',2'} + J_{3',4'})$.

with the sugar ring in either the N or S conformations but that specific conformations are required for exocyclic group C(5′)–C(4′) bond (γ_+) and O(5′)–C(5′) bond (β_+) conformations and the base ring must adopt the *syn*-conformation (χ_s) in line with the experimental findings. The hydrogen-bonded structure (shown in Figure 1) was assembled in Dreiding molecular models using the 2.8 Å hydrogen-bond spring which allowed the conformational flexibility to be manifested but conferred sufficient rigidity on the molecule for approximate conformational angles to be measured for the β, γ, and χ bonds with the sugar ring in either N-type, S-type, or planar (P) conformations. It was found that the molecule was extremely strained with the sugar ring in the planar conformation and that an O(4′)-*endo* conformation was preferred for the situation when no twist of the C(2)′–C(3′) bond occurred. In this conformation the sugar ring C(4′), C(3′), C(2′), and C(1′) atoms are in one plane so that C(4′)–H(4′) is eclipsed with C(3′)–O(3′) and C(1′)–H(1′) is eclipsed with C(2′)–O(2′) and, concomitantly, in the dioxolan ring the C(3′)–O(3′) and C(2′)–O(2′) bonds are eclipsed. The strain can be relieved by twisting the C(2′)–C(3′) bond to generate conformations of the N-type [*ca.* C(2′)-*exo*, C(3′)-*endo*] and S-type [*ca.* C(3′)-*exo*, C(2′)-*endo*] as determined from ^{1}H n.m.r. measurements. For each sugar ring conformation approximate angles were determined for the exocyclic O(5′)–C(5′) and C(5′)–C(4′) bonds, the glycosidic bond (χ), and, at the same time, the concomitant changes in the angle between O(5′)–H(5′) and C=O bond directions were used as a measure of the relative strength of the hydrogen bond for these three cases. The angles determined in this manner are shown in Figure 7. It seems that the strongest hydrogen bond (closest to colinearity) is observed with the sugar ring in the N conformation, a weaker bond for the ' planar ' [*i.e. ca.* O(4′-*endo*)] sugar ring, and the weakest hydrogen bond for the sugar ring in the S conformation. These results can be compared with the behaviour for the other bonds (β, γ, χ) which exhibit variations in angle with sugar ring conformations in that magnitudes of angles for N and S sugar ring conformations lie *ca.* 10° either side of that found for the ' planar ' sugar ring. Using molecular models and nearest neighbour repulsion as a criterion of stability it can be seen from the results in Figure 7 that the more stable conformation for the β and χ bonds might be given with the sugar ring in the S conformation whereas the more stable conformations for the γ bond *and* the hydrogen bond might occur for the sugar ring in the N conformation. Using this approach there is no clear cut case for the hydrogen bond being preferred for the sugar ring adopting either the N or S conformations though the present n.m.r. observations indicate a small

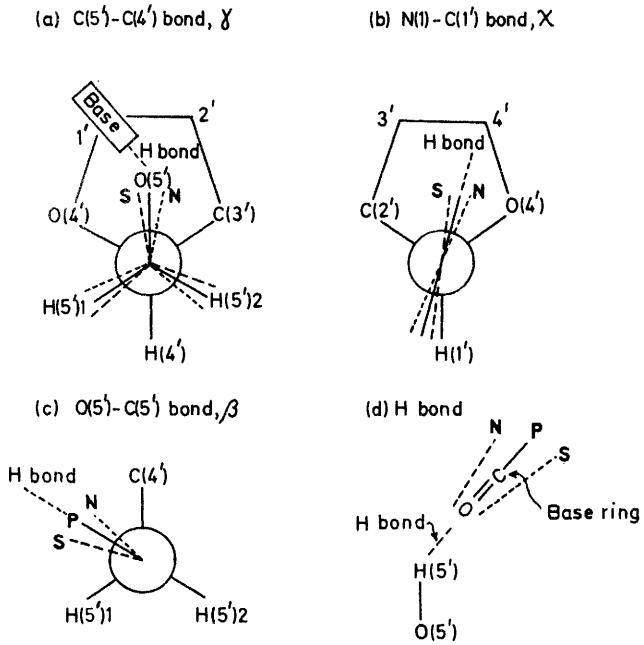


FIGURE 7 Representation of variations or conformational properties of (i), γ (ii) β, (iii) χ, (iv) hydrogen-bonds, with sugar ring conformations (N-type, S-type, and planar) of pyrimidine nucleoside which have $CH_2OH \cdots O{=}C$ intramolecular hydrogen bonding in Dreiding molecular models

increase in sugar ring S conformation with hydrogen bonding for these pyrimidine derivatives. A similar phenomenon previously observed for an analogous hydrogen bond in a purine derivative (6,6-dimethyl-2′,3′-*O*-isopropylideneadenosine in $CDCl_3$ solution), showed a marked effect on sugar ring conformation where hydrogen-bond formation resulted in 80—90% preference for the sugar ring S conformation compared to similar compounds with little or no intramolecular hydrogen bonding (40—50%).[1] It appears that the hydrogen bond in the purine derivative is stronger than that in the pyridine derivatives and so has a greater effect

on the other conformational features of the molecule, *viz.* an increase in exocyclic group O(5′)–C(5′) bond β_+ conformation, an increase in the C(5′)–C(4′) bond γ_+ conformation, an increase in sugar ring **S** conformation, and, probably, an increase in the glycosidic bond *syn*-conformation. It is hoped that such purine and pyrimidine derivatives, in which intramolecular hydrogen bonding can alter the equilibrium between the various conformational forms of nucleosides in solution, may be used to explore in a quantitative manner the relation between the thermodynamic properties of these bonds and their effect on the conformations of nucleosides. Such a study should lead to a greater understanding of the differences in conformational behaviour of purine and pyrimidine derivatives.

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