Assignment and Conformational Properties of the Exocyclic 5′-Hydroxymethyl Group of Nucleosides by Nuclear Magnetic Resonance Spectroscopy

By **David B. Davies,*** Dept. of Chemistry, Birkbeck College, Malet Street, London WC1E 7HX
Andrzej Rabczenko, Institute of Biochemistry and Biophysics, Polish Academy of Science 02–532, Warsaw, Poland

The assignment of the methylene protons of the exocyclic group of nucleosides has been confirmed by comparison of the H(5′) chemical shifts of corresponding 2′- and 3′-nucleotides and by direct observation of hydrogen bonding between the 5′-hydroxy- and 2-keto-groups of *N*(3)-methyl-2′,3′-*O*-isopropylideneuridine in $CDCl_3$ and CCl_4 solutions. The assignment of the methylene protons permits calculation of the relative populations of the three C(4′)–C(5′) bond rotamers. The results are consistent with crystal structure analyses of β-nucleosides, *i.e.* *gg* > *gt* > *tg*. Analysis of the contributions to vicinal proton spin coupling constants and chemical shifts of the exocyclic hydroxymethyl group together with the correct signal assignment confirms the approximate correlation between the sum of proton spin coupling constants and the chemical shift differences between the methylene protons of pyrimidine nucleosides. The method is extended to purine nucleosides.

The polynucleotide backbone of 3′,5′-phosphodiester bonds is described by six torsional angles for the bonding sequence C(4′)–C(5′)–O(5′)–P–O(3′)–C(3′)–C(4′) as shown in Figure 1. N.m.r. spectroscopy provides detailed

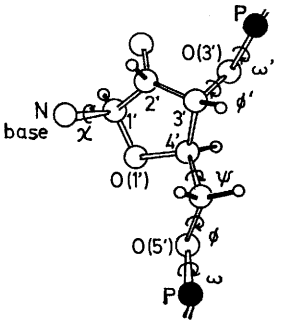


FIGURE 1 Nomenclature for torsional angles of the sugar phosphate backbone of nucleic acids [5]

conformational properties for the C(4′)–C(5′), C(5′)–O(5′), O(3′)–C(3′), and C(3′)–C(4′) bonds. The C(4′)–C(5′) bond rotamer populations are determined from analysis of the vicinal proton–proton spin coupling constants between the C(4′) and two C(5′) protons in the n.m.r. spectrum of the nucleotide in solution. At present such detailed information cannot be determined for the ribose–phosphate backbone of polynucleotides in solution though considerable information is available for mononucleotides and nucleosides. Previous analyses of the magnitudes of the observed coupling constants of the exocyclic groups of nucleosides [1,2] and 5′-nucleotides [3] has shown that the exocyclic group interconverts rapidly on the n.m.r. time-scale between the three staggered conformers shown in Figure 2 and that one conformer (*gauche-gauche*) is preferred compared to the other two (*gauche-trans* and *trans-gauche*). In order to determine the relative populations of the *gt* and *tg* conformers it is necessary to assign the observed signals to the individual methylene protons. The correct assignment is particularly important for determining the conformational properties of the ribose–phosphate chain of dinucleoside phosphates, dinucleotides, and oligonucleotides.

From an analysis of published n.m.r. spectra of uridine, β-pseudouridine, and their 3′-monophosphates it was suggested [4] that specific assignment of the signals of the two C(5′) protons is possible by taking account of the deshielding effect of the phosphate group. A number of objections to this analysis are possible as no account was taken of either the charged state of the molecules in solution or the possible effect of different conformational properties of nucleosides compared to 3′-nucleotides [*i.e.* *syn* ⇌ *anti* equilibrium of the base-ring, N ⇌ S equilibrium of the ribose ring, and C(5′)–O(5′) bond rotation]. Most of these objections are overcome by comparing results from 3′-nucleotides with those for the corresponding 2′-nucleotides; these results substantiate the assignment of the two methylene protons suggested by Remin and Shugar.[4] Further independent evidence for the same assignment is given by observation of hydrogen bonding between the 5′-hydroxy-group [with

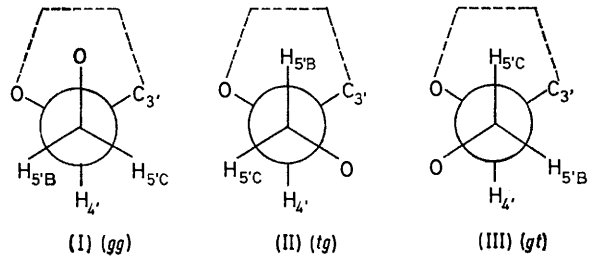


FIGURE 2 The three staggered conformations for rotation about the C(4′)–C(5′) bond

the C(4′)–C(5′) bond predominantly in the *gauche-gauche* conformation] and the 2-keto-oxygen (with the pyrimidine ring predominantly in the *syn*-conformation) of *N*(3)-methyl-2′,3′-*O*-isopropylideneuridine (1) in $CDCl_3$ and CCl_4 solutions. The markedly different coupling of

[1] B. J. Blackburn, A. A. Grey, I. C. P. Smith, and F. E. Hruska, *Canad. J. Chem.*, 1970, **48**, 2866.
[2] F. E. Hruska, in ' Conformation of Biological Molecules and Polymers,' Proceedings of 5th Jerusalem Symposium, eds. F. D. Bergmann and B. Pullman, Academic Press, New York, 1973, and references therein.
[3] D. B. Davies and S. S. Danyluk, *Biochemistry*, 1974, **13**, 4417.
[4] M. Remin and D. Shugar, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 636.

the 5′-OH group to the C(5′) protons serves to assign the latter.

The consequences of the assignment on the conformational properties of the C(4′)–C(5′) bond of nucleosides are also discussed. For example, the observation [4] that the exocyclic methylene group of nucleosides exhibits regular behaviour such that the downfield methylene signal [δH(5′) > δH(5″)] has the smaller coupling to the C(4′) proton (*i.e.* $J_{4'5'} < J_{4'5''}$) can be interpreted in terms of a greater preference for the *gauche-trans* compared to the *trans-gauche* conformer for rotation about the C(4′)–C(5′) bond. This conclusion bears out the conformational behaviour of the exocyclic group of β-nucleosides in the solid state [5] where the results of 56 crystal structures show *gg* > *gt* > *tg* conformations in the ratio 36 : 16 : 4. One important consequence of the correct assignment resulting from a detailed analysis of the contributions to $J_{obs.}$ and $\delta_{obs.}$ is that the correlation observed by Hruska *et al.*[6] between the sum of the vicinal proton spin coupling constants and the chemical shift difference between the exocyclic methylene group of pyrimidine nucleosides can be rationalised. The analysis is extended to purine nucleosides, a number of which are also measured in this work.

EXPERIMENTAL

Commercial samples of adenosine, deoxycytidine, deoxyguanosine, thymidine, and deoxycytidine (Sigma Chemical Co.) were lyophilised from D_2O to minimise the residual HDO peak. The 220 MHz proton magnetic resonance spectra of the nucleosides were measured in 100% D_2O (Diaprep) using the sodium salt of [2,2,3,3-$^2H_4$]-3-trimethylsilylpropionic acid (Merck, Sharp and Dohme) as internal reference standard. Owing to the low solubility of adenosine and deoxyguanosine in D_2O solutions 220 MHz Fourier transform n.m.r. measurements were made by kind permission of Dr. S. S. Danyluk, Argonne National Laboratory, Chicago. The relevant chemical shift and coupling constant data of the exocyclic group of these nucleosides are summarised in Table 4.

N(3)-Methyl-2′,3′-*O*-isopropylideneuridine (1) was synthesised from 2′,3′-*O*-isopropylideneuridine by methylation with diazamethane in methanol according to the method of Szer and Shugar [7] and 5′,2-*O*-cyclo-2′,3′-*O*-isopropylideneuridine (2) was synthesised according to the method of West.[8]

The 220 MHz proton magnetic resonance spectra of (1) in $CDCl_3$ and CCl_4 were measured at a probe temperature of 22° using tetramethylsilane as internal reference standard. In order to determine all the C(5′) proton spin coupling constants measurements were made on both a deuteriated sample [OD(5′), enabling $J_{4'5'}$ and $J_{4'5''}$ to be observed] and a solution prepared using distilled solvents and dried sample and finally dried over molecular sieve for a few minutes prior to measurement. In the latter case a separate OH(5′) signal was observed together with the coupling to the C(5′) protons. The complete assignments of the spectra were checked by computer simulation and the relevant chemical shifts and spin-coupling constants are given in Table 1.

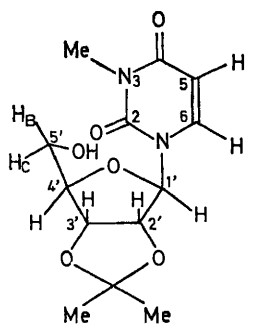
TABLE 1

Observed proton chemical shifts and spin coupling constants

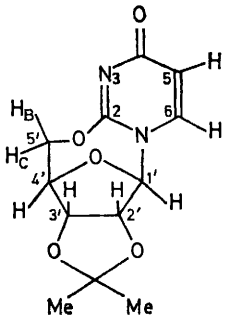
Compound	(1) [a]		(2) [b]
Solvent	$CDCl_3$	CCl_4	$CDCl_3$
Temp.(*T*/K)	293	295	303
Chemical shifts (δ)			
H(6)	7.427	7.282	7.40
H(5)	5.772	5.636	6.17
H(1′)	5.614	5.350	5.36
H(2′)	5.008	4.985	5.00
H(3′)	4.973	4.924	4.91
H(4′)	4.318	4.168	4.68
H(5′)	3.918	3.800	4.44
H(5″)	3.808	3.673	4.18
OH(5′)	2.92	3.03	
Coupling constants (*J*/Hz)			
1′2′	2.6	2.8	0.4
2′3′	6.3	6.3	5.6
3′4′	3.0	3.0	0.2
4′5′	2.5	2.2	1.6
4′5″	3.0	2.6	1.0
5′5″	−11.9	−12.1	−12.5
5′OH	3.0 [c]	2.5 [c]	
5″OH	6.0 [c]	7.0 [c]	

[a] 220 MHz ^1H N.m.r. measurements; error limits in *J*(±0.1 Hz) and δ(±0.002) p.p.m. [b] 100 MHz ^1H n.m.r. measurements taken from ref. 17. [c] Error limits on *J*(±0.5 Hz).

Owing to the low solubility of (1) in CCl_4 it was necessary to accumulate spectra for both solutions using a c.a.t. (16 scans). Nuclear Overhauser experiments were performed on degassed and sealed samples of (1) and (2) in $CDCl_3$ using a JEOL MH100 spectrometer operating in the internal lock mode. Ten successive measurements were performed by irradiation of H(6) and observing H(1′) or *vice versa*, and the integrated intensities compared with decoupling experiments in which the irradiation frequency was applied off resonance.[9] An N.O.E. was observed for both (1) (12 ± 2%) and (2) (18 ± 2%) using the same experimental conditions.



(1)



(2)

DISCUSSION

A distinction is made in the nomenclature of the chemical shifts and spin coupling constants of the

[5] M. Sundaralingam, in ref. 2.

[6] F. E. Hruska, D. J. Wood, T. N. McCaig, A. A. Smith, and A. Holy, *Canad. J. Chem.*, 1974, **52**, 497.

[7] W. Szer and D. Shugar, ' Synthetic Procedures in Nucleic Acid Chemistry,' eds. W. W. Zorbach and R. S. Tipson, Wiley, London, 1968, vol. 1, p. 433.

[8] B. West, ref. 7, p. 313.

[9] J. H. Noggle and R. E. Schirmer, ' The Nuclear Overhauser Effect: Chemical Applications,' Academic Press, New York, 1971.

observed signals and expected values used in the subsequent analysis. The observed methylene signals are labelled H(5′) for the downfield signal and H(5″) for the upfield signal in accordance with previous nomenclature.[1,4] Theoretical signals of the exocyclic methylene group are labelled according to particular protons as in Figure 2, *i.e.* H(5′B) and H(5′C). There are two ways of assigning the theoretical signals to the observed signals:

	Assignment A	Assignment B
H(5′)	H(5′B)	H(5′C)
H(5″)	H(5′C)	H(5′B)
$J_{4'5'}$	$J_{4'5'B}$	$J_{4'5'C}$
$J_{4'5''}$	$J_{4'5'C}$	$J_{4'5'B}$

It is shown in this work that A is the correct assignment which agrees with the previous analysis by Remin and Shugar.[4] The consequences of the correct assignment on the conformational properties of the exocyclic methylene group of nucleosides is also discussed. The analysis depends on the interpretation of observed coupling constants ($J_{4'5'}$ and $J_{4'5''}$) in terms of the relative proportions of the three staggered conformers for rotation about the exocyclic C(4′)–C(5′) bond. A simplifying assumption is made that a single torsional angle represents the range of angles which lie within the accessible region of the potential energy well for each staggered conformer.[10] For rotation about the C(4′)–C(5′) bond that is rapid on the n.m.r. time-scale expected values of spin coupling constants for the C(5′) protons are weighted averages of the value in each conformer and the relative proportion that conformer makes to the equilibrium, *i.e.* p_{I}, p_{II}, and p_{III}. The consequences of the previous assumption is that observed coupling constants are given by equations (1)—(3) where the

$$J_{4'5'} = J_0^{\mathrm{B}} = p_{\mathrm{I}}^{\mathrm{B}}J + p_{\mathrm{II}}J_{\mathrm{II}}^{\mathrm{B}} + p_{\mathrm{III}}^{\mathrm{B}}J_{\mathrm{III}}^{\mathrm{B}} \quad (1)$$

$$J_{4'5'} = J_0^{\mathrm{C}} = p_{\mathrm{I}}J_{\mathrm{I}}^{\mathrm{C}} + p_{\mathrm{II}}J_{\mathrm{II}}^{\mathrm{C}} + p_{\mathrm{III}}J_{\mathrm{III}}^{\mathrm{C}} \quad (2)$$

$$1 = p_{\mathrm{I}} + p_{\mathrm{II}} + p_{\mathrm{III}} \quad (3)$$

subscript of J refers to particular conformers and the superscript of J refers to particular methylene protons. Equations (1) and (2) are simplified by assuming that protons in either *gauche* or *trans* relationships for each conformer exhibit the same coupling constants *i.e.* $J_g = J_{\mathrm{I}}^{\mathrm{B}} = J_{\mathrm{I}}^{\mathrm{C}} = J_{\mathrm{II}}^{\mathrm{C}} = J_{\mathrm{III}}^{\mathrm{B}}$ and $J_t = J_{\mathrm{II}}^{\mathrm{B}} = J_{\mathrm{III}}^{\mathrm{C}}$. Such an approximation is not strictly correct as the orientation of an attached electronegative substituent is known to affect vicinal coupling.[11-13] However, possible modifications of J_g and J_t for different conformers which in turn affects the absolute values of p_{I}, p_{II}, and p_{III} will be discussed after the main analysis and conclusions. By manipulation of equations (1)—(3), equations (4)—(6) are derived that enable the populations (p_{I}, p_{II}, and p_{III}) to be determined. It is seen from equation (4) that p_{I} can be determined from the sum of observed coupling constants ($J_0^{\mathrm{B}} + J_0^{\mathrm{C}}$), but from equations (5) and (6) determination of p_{II} and p_{III} depends on the specific assignment of observed coupling constants to particular methylene protons.

$$p_{\mathrm{I}} = [(J_t + J_g) - (J_0^{\mathrm{B}} + J_0^{\mathrm{C}})]/(J_t - J_g) \quad (4)$$

$$p_{\mathrm{II}} = (J_0^{\mathrm{B}} - J_g)/(J_t - J_g) \quad (5)$$

$$p_{\mathrm{III}} = (J_0^{\mathrm{C}} - J_g)/(J_t - J_g) \quad (6)$$

A *Assignment of Methylene Protons.*—(i) *2′- and 3′-nucleotides.* It was suggested by Remin and Shugar [4] that specific assignment of the signals of the two C(5′) protons is possible by taking account of the deshielding effect of the phosphate group of a number of pyrimidine-3′-mononucleotides compared to the corresponding nucleosides. It is expected that chemical shifts of nucleoside-2′- and -3′-monophosphates in aqueous solution provide data for comparison of the effect of the charged phosphate group on C(5′) proton signals that is more realistic than comparison of chemical shifts of a nucleoside with its 3′-monophosphate made by Remin and Shugar; [4] first, the properties of the two solutions being compared are the same and, secondly, the nucleoside-2′- and -3′-monophosphate molecules are in the same charged state with the phosphate group at different distances from the two C(5′) protons in these two series of molecules.

A comparison was made of the chemical shift differences of the exocyclic methylene protons of purine and pyrimidine nucleoside-2′- and -3′-monophosphates as shown in Table 2. It is observed that the chemical shift differences of both H(5′) and H(5″) signals exhibit a

TABLE 2

Chemical shift differences of H(5′) and H(5″) for nucleoside-2′- and -3′-monophosphates [a]

	Chemical shift differences (p.p.m.)	
	H(5′)	H(5″)
(AMP-3′ – AMP-2′)	0.021	0.094
(GMP-3′ – GMP-2′)	0.045	0.084
(UMP-3′ – UMP-2′)	0.032	0.059
(CMP-3′ – CMP-2′)	0.066	0.093

[a] Original data given in ref. 14.

consistent pattern with the effect for the upfield signal [H(5″), 0.082 (±0.012) p.p.m.] greater than for the downfield signal [H(5′), 0.041 (±0.014) p.p.m.]. It has been shown that conformational properties of pyrimidine-2′- and -3′-mononucleotides are similar in aqueous solution; [14] the same conclusion was made for purine-2′- and -3′-mononucleotides.[14] Thus it is feasible to ascribe the chemical shift differences summarised in Table 2 to the effect of the phosphate group. In the pH range 5—6 an average charge of −1.5 resides on the mononucleotide phosphate group. The electric field of the charged group is responsible for deshielding the C(5′) protons. Magnitudes of δH(5′C) and δH(5′B) were

[10] T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, 1972, **11**, 137.

[11] D. H. Williams and N. S. Bhacca, *J. Amer. Chem. Soc.*, 1964, **86**, 2742.

[12] S. Sternhell, *Quart. Rev.*, 1969, **23**. 236.

[13] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669, and references therein.

[14] D. B. Davies and S. S. Danyluk, *Biochemistry*, 1975, **14**, 543

calculated * assuming a planar ribose ring and free rotation about the C(3')–O(3') and C(2')–O(2') bonds. As the calculation assumes a point charge, the charge on the phosphate group was located along the O–P bond at the intersection of the plane containing the three, unsubstituted phosphate oxygen atoms. Calculated values of δH(5'C) — δH(5'B) between 2'- and 3'-mononucleotides for different C(4')–C(5') bond rotamers are *gg*(0.15), *gt*(—0.20), and *tg* (—0.02 p.p.m.). It can be seen that a predominance of conformer (I) (*ca.* 70% *gg*) leads to a greater deshielding on H(5'C) compared to H(5'B).† It is concluded that H(5' C) corresponds to the upfield signal [H(5'')], and H(5' B) the downfield signal [H(5'')]; this, in turn, corresponds to assignment A.

(ii) N(3)-*Methyl*-2',3'-*O*-*isopropylideneuridine* (1).—It is found that in non-polar solvents ($CHCl_3$ and CCl_4) compound (1) preferentially exists in the *syn*-conformation, that the C(4')–C(5') bond substantially prefers the *gauche-gauche* conformation and that hydrogen-bonding occurs between the 5'-OH group and 2-keto-oxygen of the base-ring. The concomitant observation of different proton coupling between the 5'-OH and two C(5') protons confirms the assignment of the methylene protons.

C.d. measurements of (1) have shown that the pyrimidine base-ring preferentially exists in the *anti*-conformation in polar solvents (water and acetonitrile) and in the *syn*-conformation in non-polar solvents (carbon tetrachloride and cyclohexane).[15] By comparison of c.d. spectra of substituted uridine derivatives, it was concluded that formation of an intramolecular hydrogen bond involving the 5'-OH group and 2-keto-oxygen of the base is mainly responsible for promotion of the *syn*-conformer of (1) in non-polar solvents.[15] Recently the thermodynamic properties of this hydrogen bond formation of (1) in CCl_4, $CHCl_3$, and CH_2Cl_2 have been measured by i.r. spectroscopy and the results are consistent with changes in the ^1H n.m.r. spectra of (1) in $CDCl_3$ and CCl_4 solutions as a function of temperature.[16] In this work an N.O.E. of 12 (±2)% was observed between H(6) and H(1') of (1) in degassed $CDCl_3$ solution which confirms that the *syn*-conformer exists in solution. Although a larger theoretical N.O.E. might be predicted for protons *ca.* 2.6 Å apart (from Dreiding models), measurements were made for comparison on 5',2-*O*-cyclo-2',3'-*O*-isopropylideneuridine (2) under the same solution conditions. It was shown[17] that the base ring of (2) exists in the same *syn*-conformation in $CDCl_3$ solution as in the solid state such that the C(6)–H(6) bond is approximately *cis*-coplanar with the C(1')–H(1') bond (torsional angle 14°) with a distance of 2.2 Å between the H(6) and H(1') atoms. The observed N.O.E. of 18(±4)% between H(6) and H(1') of (2) is considerably lower than that predicted[9,18] for such a molecular configuration(>40%). Although direct comparison of N.O.E. for (1) and (2) cannot be made because of the likelihood of different relaxation mechanisms as discussed for β-pseudouridine,[19] the measurements suggest that the *syn*-conformation exists for a significant fraction of the total glycosidic bond conformers.

^1H N.m.r. also leads to determination of the conformational properties of the exocyclic C(4')–C(5') bond. It has been shown that the *gg* rotamer population can be determined from the sum of $(J_{4'5'} + J_{4'5''})$ and that for nucleosides in aqueous solution the *gg*-conformer (I in Figure 2) is preferred.[1,2,4] Using the same analysis the observed $(J_{4'5'} + J_{4'5''})$ for compound (1) (5.6 in $CDCl_3$; 4.8 Hz in CCl_4 at 20°) corresponds to an overwhelming predominance of the *gg*-conformer (84% in $CDCl_3$ and 92% in CCl_4 solutions). In this conformation the C(5')–O(5') bond projects over the ribose ring toward the pyrimidine base-ring on C(1') similar to that found

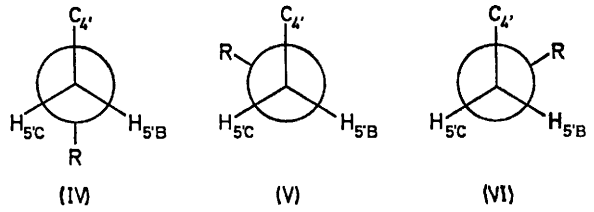


FIGURE 3 The three staggered conformations for rotation about the C(5')–O(5') bond; R = H

for the 5',2-*O*-cyclouridine derivative. For the base-ring in the *syn*-conformation it is possible to form a hydrogen bond between 5'-OH and the 2-keto-oxygen. The presence of the hydrogen bond is confirmed by observation of the vicinal coupling between the C(5') methylene protons and 5'-OH of compound (1) in $CDCl_3$ and CCl_4 solutions. It is found that ^3J(H,OH) is markedly different for the two C(5') protons, *i.e.* in Table 1 $J(5'', OH) = 7$ is much greater than $J(5', OH) = 2.5$ Hz, which indicates different relative populations for rotation about the C(5')–O(5') bond. The three classical staggered C(5')–O(5') bond rotamers are shown in Figure 3 with R = H. Analysis of the relative rotamer populations is made similar to that for the C(4')–C(5') bond; the *gg*-rotamer population (IV) is determined from the sum of $(J_{5',OH} + J_{5'',OH})$ by an equation analogous to equation (4) whereas *tg* (V) and *gt* (VI) rotamer populations can only be calculated from equations (5) and (6) if the assignment of the individual methylene protons is known. Using the Karplus relation determined by Fraser *et al.*[20] for coupling in the H–C–O–H molecular

* Calculations were made according to the equation $\Delta\delta = 12.5 \times 10^{-6}(\Sigma q_i \cos\theta_i/R_i^2) - 17.0 \times 10^{-6}(\Sigma q_i^2/R_i^2)$ with $q_i = -1.5$ and the distances (R_i) and orientation (θ_i) of the charge from the C–H bond determined from Dreiding molecular models. We thank a referee for pointing out the importance of θ_i as well as R· for the electric field effect.

† The conclusion is also the same for the ribose ring in equilibrium between *N*[C(3')-*endo*] and *S*[C(2')-*endo*] conformations.

[15] A. Rabczenko, K. Jankowski, and K. Zakrewska, *Biochim. Biophys. Acta*, 1974, **353**, 1.
[16] D. B. Davies, D. Plochocka, and A. Rabczenko, to be published.
[17] P. C. Manor, W. Saenger, D. B. Davies, K. Jankowski, and A. Rabczenko, *Biochim. Biophys. Acta*, 1974, **340**, 472.
[18] R. A. Bell and J. K. Saunders in ' Topics in Stereochemistry,' eds. N. L. Allinger and E. L. Eliel, Wiley, New York, 1973, vol. 7.
[19] R. K. Nanda, R. Tewari, G. Govil, and I. C. P. Smith, *Canad. J. Chem.*, 1974, **52**, 371.

fragment, *i.e.* J_t 12.1 and J_g 2.0 Hz, it is calculated that that the *gg*-conformer is populated *ca* 50% of the time and that either *gt* or *tg* is populated *ca.* 50%. Hydrogen-bond formation between 5'-OH and the 2-keto-group of the base-ring in the *syn*-conformation is only possible for the C(5')–O(5') bond in the *gt*-conformation (VI). A significant population of *gt* (VI) rather than *tg* (V) is consistent with such hydrogen-bond formation. In conformer (VI) the 5'-OH group is *trans* to H(5'C) and *gauche* to H(5'B). Consequently the upfield signal, H(5''), which exhibits the larger 5'-OH coupling (*ca.* 7 Hz) corresponds to H(5'C). This assignment is the same as that determined in the previous section. The agreement between two independent methods of assigning the C(5') methylene protons is strong evidence that assignment A is correct.

B *Consequences of Correct Assignment.*—(i) *Relative values of* p_{II} *and* p_{III}. It has been observed [4] that the proton spin coupling constants of the two C(5') protons with the C(4') proton exhibit regular behaviour such that the downfield signal [now assigned to H(5'B)] has a smaller coupling constant than the upfield methylene signal, *i.e.* $J_{4'5'}$ $(= J_0^B) < J_{4'5''}$ $(= J_0^C)$. From equations (5) and (6) it is calculated that $p_{III} > p_{II}$ for $J_0^C > J_0^B$. In conformer (II) C(5')–O(5') is *antiperiplanar* to C(4')–O(1') whereas in conformer (III) C(5')–O(5') is *antiperiplanar* to C(4')–C(3') which brings the two oxygen atoms, O(5') and O(1'), into a *gauche*-relationship. It is observed that both preferred rotamers (I) and (III) have the two oxygen atoms, O(1') and O(5'), in a *gauche*-relationship and the least likely conformer (II) has the oxygen atoms in a *trans*-relationship. This distribution of conformers ($p_I > p_{III} > p_{II}$) is similar to that found for β-nucleosides in the solid state determined by *X*-ray crystallography.[5] The results show that for a total of 56 crystal structures of β-nucleosides the C(4')–C(5') exocyclic group is found predominantly in the *gg*-conformation [(I), 64%], significantly in the *gt* conformation [(III), 29%], and seldom in the *tg* conformation [(II), 7%]. The distribution found in the solid state is similar to the relative populations of each conformer for nucleosides in aqueous solution and provides supporting evidence for the correct assignment of the C(5') protons. Theoretical calculations of the conformational energies of pyrimidine nucleosides substantiates [21-25] n.m.r. results that the *gg* conformer is significantly more stable than *gt* and *tg* though calculations have not predicted that *gt* is more stable than *tg* as observed in solution.

(ii) *Absolute values of conformer populations.* Unequivocal assignment of the methylene protons permits calculation of each conformer population for the C(4')–C(5') bond rotamers. Absolute values of calculated conformer populations depend on magnitudes of J_t and J_g used for proton coupling in conformers (I)—(III). Previous determinations for nucleosides [1,2,4] and nucleotides [3,14] assumed that protons in *gauche* and *trans* relationships exhibit the same coupling constants in conformers (I)—(III). In this work approximate values of J_t 12 Hz (close to that suggested by Lemieux in ref. 1) and J_g 2 Hz (close to that suggested by many workers [1,26]) are used. Calculations of rotamer populations using this procedure have been made for some representative examples of J_0^B and J_0^C as summarised in Table 3. Modifications of the Karplus relation [27,28] should also take into account the orientation of attached electronegative substituents as their maximum effect is exerted on vicinal proton coupling when antiperiplanar to protons involved in the coupling path.[11,12,29] For nucleosides the substituent effect on coupling between protons in a *gauche*-relationship depends on the relative orientation of the two electronegative oxygen atoms.

An analysis has been made of a similar situation for the rotamer populations of the glycerol fragment of dipalmitoyl-lecithin.[30] The method does not rely on a Karplus relation but utilises observed coupling constants in model compounds for the individual conformers (I)—(III) (Figure 2). Coupling constants in conformers

TABLE 3

Comparison of calculated p_I—p_{III} values

	Coupling constants (*J*/Hz)			Conformer populations					
				Equations (4)—(6)			Equations (3), (7), (8)		
Assignment	J^C	J_0^B	$J_0^B + J^C$	p_I	p_{II}	p_{III}	p_I	p_{II}	p_{III}
A,B	3	3	6	0.80	0.10	0.10	0.77	0.03	0.20
A	4	3	7	0.70	0.10	0.20	0.67	0.03	0.30
B	3	4	7	0.70	0.20	0.10	0.71	0.14	0.15
A,B	4	4	8	0.60	0.20	0.20	0.62	0.14	0.24
A	5	3	8	0.60	0.10	0.30	0.58	0.03	0.39
B	3	5	8	0.60	0.30	0.10	0.64	0.26	0.10
A	5	4	9	0.50	0.20	0.30	0.53	0.14	0.33
B	4	5	9	0.50	0.30	0.20	0.55	0.26	0.19

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[30] N. J. M. Birdsall, J. Feeney, A. G. Lee, Y. K. Levine, and J. C. Metcalfe, *J.C.S. Perkin II*, 1972, 1441.

(I) and (III) were determined to be $J_{\mathrm{I}}^{\mathrm{B}}$ 2.7, $J_{\mathrm{I}}^{\mathrm{G}}$ 0.6, $J_{\mathrm{III}}^{\mathrm{G}}$ 11.5, and $J_{\mathrm{III}}^{\mathrm{B}}$ 2.7 Hz using *trans*-2,3-dimethyl-1,4-dioxan as a model compound.[31] Assuming a negligible electronegativity difference between H and C, an estimate was made of $J_{\mathrm{II}}^{\mathrm{B}}$ 11.7 and $J_{\mathrm{II}}^{\mathrm{G}}$ 5.8 Hz from the work by Abraham and Gatti on 1,2-disubstituted ethanes.[32] Substituting these values in equations (1) and (2) leads to relationships (7) and (8) from which p_{I}—p_{III} can be determined. Calculations of rotamer populations

$$J_0^{\mathrm{B}} = 2.7p_{\mathrm{I}} + 11.7p_{\mathrm{II}} + 2.7p_{\mathrm{III}} \quad (7)$$

$$J_0^{\mathrm{G}} = 0.6p_{\mathrm{I}} + 5.8p_{\mathrm{II}} + 11.5p_{\mathrm{III}} \quad (8)$$

using equations (3), (7), and (8) have been made for the same examples of J_0^{B} and J_0^{G} calculated previously and the results are summarised in Table 3. Both methods of calculation lead to similar values of p_{I} which is the dominant conformer but different values of p_{II} and p_{III}. It can be seen that using the unmodified method [equations (4)—(6)] differences in magnitudes between p_{II} and p_{III} do not depend on the assignment of individual methylene protons but differences occur in these values using the modified method [equations (3) (7) and (8)]. Using the correct assignment (A) and modified method of calculation it is found that the predominance of the *gt* over the *tg* conformation is particularly marked.*

(iii) *Correlation between* $(J_{4'5'} + J_{4'5''})$ *and* $\delta H(5') - \delta H(5'')$. A correlation has been observed between the sum of proton spin coupling constants and the chemical shift difference of C(5′) methylene protons of many pyrimidine nucleosides in aqueous solution.[6] A linear correlation was observed between $(J_{4'5'} + J_{4'5''})$ and $\delta H(5') - \delta H(5'')$ with a negative slope and positive intercepts on both axes. By an analysis of the C(5′) proton chemical shifts and spin coupling constants the basis for such a correlation is shown and another correlation is predicted which leads to further understanding of the conformational properties of nucleosides in solution.

The C(4′)–C(5′) bond rotamers shown in Figure 2 make contributions to the observed chemical shifts of individual methylene protons in proportion to their relative populations according to equations (9) and (10).

$$\delta H(5'B)_{\mathrm{obs}} = \delta_0^{\mathrm{B}} = p_{\mathrm{I}}\,\delta_{\mathrm{I}}^{\mathrm{B}} + p_{\mathrm{II}}\delta_{\mathrm{II}}^{\mathrm{B}} + p_{\mathrm{III}}\delta_{\mathrm{III}}^{\mathrm{B}} \quad (9)$$

$$\delta H(5'C)_{\mathrm{obs}} = \delta_0^{\mathrm{G}} = p_{\mathrm{I}}\,\delta_{\mathrm{I}}^{\mathrm{G}} + p_{\mathrm{II}}\delta_{\mathrm{II}}^{\mathrm{G}} + p_{\mathrm{III}}\delta_{\mathrm{III}}^{\mathrm{G}} \quad (10)$$

Subscripts and superscripts of δ mean the same as for J in equations (1) and (2). It is likely that absolute values of observed chemical shifts of the two methylene protons vary considerably for different nucleosides and different solution conditions though the observed chemical shift difference is less dependent on different environments. The difference in chemical shifts of the two C(5′) protons determined from equations (9) and (10) is given by equation (11). In order to calculate the sign and

$$(\delta_0^{\mathrm{B}} - \delta_0^{\mathrm{G}}) = p_{\mathrm{I}}\,(\delta_{\mathrm{I}}^{\mathrm{B}} - \delta_{\mathrm{I}}^{\mathrm{G}}) + p_{\mathrm{II}}\,(\delta_{\mathrm{II}}^{\mathrm{B}} - \delta_{\mathrm{II}}^{\mathrm{G}}) + p_{\mathrm{III}}\,(\delta_{\mathrm{III}}^{\mathrm{B}} - \delta_{\mathrm{III}}^{\mathrm{G}}) \quad (11)$$

magnitude of $(\delta_0^{\mathrm{B}} - \delta_0^{\mathrm{G}})$ chemical shift differences of methylene protons for each individual conformer are needed together with the relative population of each conformer. It was shown [33] that the chemical shift difference between geminal groups may be partitioned into a ' conformational ' term (depending on appropriate conformer populations) and an ' intrinsic ' term that is independent of these conformer populations. It was also shown for some substituted ethane molecules [34] (*i.e.* compounds of the type CX_2Y–CABC where X = F) that intrinsic diastereiosmerism in individual conformers may be appreciable even though increases in temperature tend to equalise rotamer populations and the magnetic non-equivalence tends toward zero. At present such detailed information for the methylene protons in each individual conformer in Figure 2 is not known and, therefore, in order to analyse the observed chemical shifts it is assumed that the oxygen atoms attached to C(4′) and C(5′) predominantly determine the chemical shifts of the neighbouring protons and that protons in similar symmetrical situations with respect to the oxygen atoms have the same chemical shifts, *i.e.* $\delta_{\mathrm{I}}^{\mathrm{B}} = \delta_{\mathrm{III}}^{\mathrm{G}}$, $\delta_{\mathrm{II}}^{\mathrm{B}} = \delta_{\mathrm{II}}^{\mathrm{G}}$, and $\delta_{\mathrm{I}}^{\mathrm{G}} = \delta_{\mathrm{III}}^{\mathrm{B}}$. The consequences of these assumptions are that $(\delta_{\mathrm{II}}^{\mathrm{B}} - \delta_{\mathrm{II}}^{\mathrm{G}}) = 0$ and $(\delta_{\mathrm{I}}^{\mathrm{B}} - \delta_{\mathrm{I}}^{\mathrm{G}}) = -(\delta_{\mathrm{II}}^{\mathrm{B}} - \delta_{\mathrm{III}}^{\mathrm{G}})$.

There is some justification for assuming the term $p_{\mathrm{II}}\,(\delta_{\mathrm{II}}^{\mathrm{B}} - \delta_{\mathrm{II}}^{\mathrm{G}})$ in equation (11) can be neglected. It was shown for *erythro*-(1,2-dibromo[2-^{2}H]ethyl)benzene (*erythro*-PhCHBrCHDBr) that the dominant conformer can be unequivocally determined as the one with the hydrogen atoms antiperiplanar.[35] In this conformer the bromine atoms are also antiperiplanar similar to conformer (II) of Figure 2 assuming that the oxygen atoms are replaced by bromine atoms. The chemical shifts of the methylene group of the undeuteriated molecule $PhCHBrCH_2Br$ are similar; [36] differences in chemical shift depend on solvent and vary from 0.06 (CCl_4) to −0.035 p.p.m. (CH_3COCH_3). These chemical shift differences can be compared with those found for conformer (III) in similar molecules. It was shown for *threo*-1,2-dibromo-3,3-dimethyl[1-^{2}H]butane that the dominant conformer can be unequivocally determined as the one in which the hydrogen atoms are antiperiplanar.[35] In this conformation the bromine atoms are *gauche* to one another and the t-butyl residue is antiperiplanar to one of the bromine atoms similar to conformer (III) in Figure 2 assuming that the oxygen atoms are replaced by bromine atoms. Although the

*At present the use of the constants in equations (7) and (8) is limited to aqueous solutions of nucleosides. From equations (3) and (7) it is calculated that $p_{\mathrm{II}} = (J_0^{\mathrm{B}} - 2.7)/9.0$ so that the analysis needs $J_{4'5}$ (or $J_{4'5''}$) $\geqslant$ 2.7 Hz whereas it has been found [16] for a number of compounds [*N*(3)-methyl-2′,3′-dimethyl- and *N*(3)-methyl-2′,3′,5′-trimethyl-uridine in $CDCl_3$ or CCl_4 solutions that $J_{4'5'}$ (and $J_{4'5''}$) $\leqslant$ 2.3 Hz.

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chemical shift differences of the methylene group of the undeuteriated molecule $(CH_3)_3CCHBrCH_2Br$ depend on solvent, *i.e.* 0.28 (benzene), 0.38 (CCl_4), 0.47 (CH_3CN), and 0.51 p.p.m. (acetone),[36] these values for conformer (III) are much greater than those for conformer (II). As a result of the correct assignment of the methylene protons it is also found for nucleosides that $p_{III} > p_{II}$. These two observations indicate that the term involving p_{II} $(\delta^B_{II} - \delta^C_{II})$ in equation (11) can be neglected.

$(\delta^B_I - \delta^C_I)$ is constant for a set of nucleosides, it can be seen that equation (13) describes a straight line in which a plot of $(J^B_0 + 2J^C_0)$ against $(\delta^B_0 - \delta^C_0)$ gives a negative slope of value $(J_t - J_g)/(\delta^B_I - \delta^C_I)$ and the intercept on the $(J^B_0 + 2J^C_0)$ axis is given by a constant $(J_t + 2J_g)$. The expected magnitude of the intercept is *ca.* 16 Hz using values of J_t 12 and J_g 2 Hz.

Equation (13) provides the basis for the empirical correlation observed by Hruska *et al.*[6] where it was shown

TABLE 4

Relative chemical shifts and spin coupling constants of nucleoside C(5′) protons in D_2O solution [a]

Compound	Spin coupling constants (J/Hz) $J_{4'5'}$	$J_{4'5''}$	$J^B_0 + J^C$	$J^B_0 + 2J^C_0$	δH(5′) − δH(5″) (p.p.m.)	Ref.
Pyrimidine nucleosides						
1. Uridine (23°)	2.9	4.4	7.3	11.7	0.104	10
2. Uridine (80°)	3.3	4.6	7.9	12.5	0.092	10
3. Cytidine (23°)	2.8	4.3	7.1	11.4	0.114	37
4. Cytidine (65°)	3.1	4.6	7.7	12.3	0.107	37
5. 2′-*O*-Methyluridine (28°)	2.9	4.3	7.2	11.5	0.109	37
6. 2′-*O*-Methyluridine (60°)	3.0	4.4	7.4	11.8	0.105	37
7. 2′-*O*-Methylcytidine (28°)	2.8	4.4	7.2	11.6	0.117	37
8. 2′-*O*-Methylcytidine (60°)	3.0	4.6	7.6	12.2	0.109	37
9. 2′,3′,5′-Trimethyluridine	2.8	4.8	7.6	12.4	0.101	38
10. 2′,3′,5′-Trimethylcytidine	2.8	4.9	7.7	12.6	0.096	38
11. *N*(3)-Methyl-2′,3′-dimethyluridine	2.8	4.0	6.8	10.8	0.141	*b*
12. 2′-Deoxyuridine (23°)	3.4	5.1	8.5	13.6	0.075	39
13. 2′-Deoxyuridine (80°)	3.8	5.5	9.3	14.8	0.073	46
14. 2′-Deoxycytidine (20°)	3.3	5.4	8.7	14.1	0.079	*b*
15. Thymidine (43°)	3.3	5.2	8.5	13.7	0.066	*b*
16. Thymidine	3.6	4.9	8.5	13.4	0.07	39
17. Dihydrouridine	3.6	4.8	8.4	13.2	0.077	46
Purine nucleosides						
1. Adenosine (amb.)	*c*	*c*	6.1		0.084	6
2. Adenosine (55°)	*c*	*c*	6.8		0.077	6
3. 2′-Deoxyadenosine (amb.)	*c*	*c*	7.3		0.063	6
4. 2′-Deoxyadenosine (60°)	*c*	*c*	8.3		0.063	6
5. 2′-*O*-Methyladenosine (amb.)	*c*	*c*	5.7		0.066	6
6. Inosine (amb.)	*c*	*c*	7.1		0.074	6
7. Adenosine (20°)	2.6	3.0	5.6	8.6	0.081	*b*
8. 2′-Deoxyadenosine (20°)	3.1	4.3	7.4	11.7	0.060	*b*
9. 2′-Deoxyguanosine (20°)	3.5 [d]	4.5 [d]	8.0	12.5	0.047	*b*
10. 2′-Deoxyadenosine	3.46	4.72	8.2	12.9	0.06	40

[a] Only the most accurate results checked by calculation are included, *i.e.* error in $J(\pm 0.1)$ Hz and $\delta(\pm 0.002)$. [b] This work. [c] Individual values not quoted. [d] Error in $J(\pm 0.2)$ Hz.

Within the limitations imposed by the various assumptions equation (11) reduces to (12). Equation (12) shows

$$(\delta^B_0 - \delta^C_0) = (p_I - p_{III})(\delta^B_I - \delta^C_I) \qquad (12)$$

that the sign of $(\delta^B_0 - \delta^C_0)$ depends on the sign of $(\delta^B_I - \delta^C_I)$ and the relative magnitudes of p_I and p_{III}. In all cases [1,4] so far determined $p_I > p_{III}$, *i.e.* $(p_I - p_{III}) > 0$. It can be seen that relative chemical shifts of observed methylene signals is the same as that found in conformer (I), *i.e.* the sign of $(\delta^B_0 - \delta^C_0)$ is the same as that of $(\delta^B_I - \delta^C_I)$. Substitution of equations (4) and (6) in equation (12) leads to relation (13). As

$$(J^B_0 + 2J^C_0) = (J_t + 2J_g) - \frac{(J_t - J_g)(\delta^B_0 - \delta^C_0)}{(\delta^B_I - \delta^C_I)} \qquad (13)$$

$(J_t + 2J_g)$ and $(J_t - J_g)$ are constant and assuming that a plot of $(J_{4'5'} + J_{4'5''})$ against δH(5′) − δH(5″) is a straight line with a negative slope. As $(J_t - J_g) > 0$ the correlation observed for a number of pyrimidine nucleosides confirms that the sign of $(\delta^B{}_0 - \delta^C{}_0)$ is the same sign as $(\delta^B{}_I - \delta^C{}_I)$ as predicted from equation (12).

The available data were plotted according to equation (13). Only the most reliable observations for pyrimidine nucleosides have been summarised in Table 4 in which individual δ and J values have been quoted, the analysis checked by computer simulation and δ values quoted to an accuracy of ±0.002 p.p.m. Using this limited data and plotting equation (13) according to assignment A as in Figure 4, an approximate linear correlation is observed between $(J^B_0 + 2J^C_0)$ and $(\delta^B_0 - \delta)$, *i.e.* curve A. From the slope it is found that $(\delta^B_I - \delta^C_I) =$ 0.17 p.p.m. assuming J_t 12 and J_g 2 Hz. The intercept of 17.4 Hz is slightly greater than the $(J_t + 2J_g)$ *ca.*

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[39] D. J. Wood, F. E. Hruska, and K. K. Olgilvie, *Canad. J. Chem.*, 1974, **52**, 3353.
[40] K. N. Slessor and A. S. Tracey, *Carbohydrate Res.*, 1973, **27**, 407.

16 Hz predicted by equation (13) * but the values are sufficiently close to indicate that the assumptions used in deriving equation (13) are valid and that the relation can be used to investigate the exocyclic group conformational properties of pyrimidine nucleosides. A number of factors might account for the discrepancy between these two latter values: first, the n.m.r. observations show a considerable range of $\delta H(5') - \delta H(5'')$ for similar coupling constants as the nucleosides have been measured under various solution conditions; secondly, there are insufficient data available over a wide range of values to determine accurately the relation between $(J_0^B + 2J_0^C)$ and $(\delta_0^B - \delta_0^C)$; and thirdly, it is likely that assumptions made in the analysis are not strictly valid. For

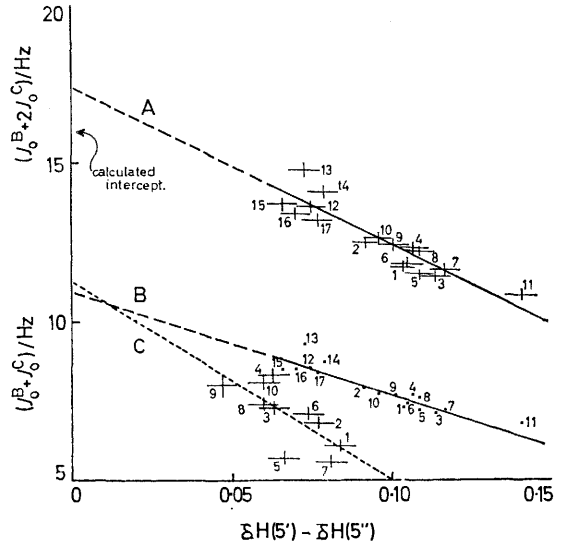

FIGURE 4 Correlation of nucleoside C(5') exocyclic group J and δ summarised in Table 4: A, pyrimidine nucleosides plotted according to equation (13) with error limits on data, intercept 17.4 Hz, slope −50 Hz (p.p.m.)$^{-1}$; B, pyrimidine nucleosides plotted according to Hruska correlation,[6] intercept 10.9 Hz, slope −31 Hz (p.p.m.)$^{-1}$; C, purine nucleosides plotted according to Hruska correlation [6] with error limits on data, intercept 11.3 Hz, slope −62 Hz (p.p.m.)$^{-1}$

example, it has been tacitly assumed that other conformational features such as *syn* ⇌ *anti* equilibrium of the base-ring or C(5')–O(5') bond rotation either have no effect on the C(5') proton chemical shifts or, at least, have the same effect on both protons. It has been shown [6] that a correlation exists between the C(4')–C(5') bond rotamer population (as expressed by the sum of $J_{4'5'}$ and $J_{4'5''}$) and the furanose ring conformer population (as expressed by $J_{3'4'}$ or $J_{1'2'}$) of structurally similar pyrimidine nucleosides so one might expect that certain conformational properties of nucleosides are reflected in the properties of the exocyclic methylene group. It is argued that C(5')–O(5') bond rotation of nucleosides in aqueous solution is likely to have little effect on the magnetic anisotropy of the C(5') protons but that the nature of the base ring and the *syn* ⇌ *anti* equilibrium are likely to have a significant effect on the C(5') protons.

(a) C(5')–O(5') *Bond rotation.* Analysis of the effect of rotation about the C(5')–O(5') bond on the C(5') proton chemical shifts in any of conformers (I)—(III) can be made in terms of the relative populations of the three staggered conformations (IV)—(VI) as shown in Figure 3 (R = H).

Assuming that chemical shifts of protons in similar environments are the same then, from symmetry considerations, $\delta_{IV}^B = \delta_{IV}^C$, $\delta_V^B = \delta_{VI}^C$, and $\delta_V^C = \delta_{IV}^B$. Analogous to the C(4')–C(5') rotamer analysis it can be seen that equation (14) applies where the nomenclature,

$$(\delta^B - \delta^C) = (\delta_{VI}^B - \delta_{VI}^C)(p_{VI} - p_V) \qquad (14)$$

δ^B and δ^C, indicates the effect on C(5') proton chemical shifts.

There is some justification for the assumptions made in the analysis from the results for methylene groups in six-membered rings of pairs of similar compounds which correspond to conformers (V) and (VI), *i.e.* $(\delta_{IV}^C - \delta_{VI}^B) \sim (\delta_V^B - \delta_V^C)$. For example, it has been shown that α-D-xylopyranoside derivatives [41,42] exist in the $^4C_1$ conformation such that the C(5)–O(5) bond corresponds to conformer (VI) of Figure 3 with δH(-5*e*) ≡ δH(5'C) and δ(H-5*a*) ≡ δH(5'B) and values of δH(5'C) − δH(5'B) vary between 0.2 and 0.3 p.p.m. depending on solvent. On the other hand the β-D-arabinopyranoside derivatives [41,42] exist predominantly in the $^1C_4$ conformation with the C(5)–O(5) bond corresponding to conformer (V) such that δ(H-5*e*) ≡ δH(5'B) and δH(5*a*) ≡ δH(5'C) and values of δH(5'B) − δH(5'C) vary between 0.3 and 0.4 p.p.m. depending on the derivative and solvent. Similar behaviour is found for the C(6) methylene group of 4,6-*O*-o-nitrobenzylidene derivatives [43] of α- and β-D-glucopyranoside [conformer (VI), $(\delta_{VI}^C - \delta_{VI}^B)$ 0.58 p.p.m.] and of -D-galactopyranoside [conformer (V), $(\delta_V^B - \delta_V^C)$ 0.55—0.58 p.p.m.]. Although there are no ideal model systems for conformer (IV), molecules such as 1,5-anhydro-2,3-isopropylidene-β-D-ribofuranose [44] in which both lone-pairs of the bridging oxygen atom eclipse the methylene protons, indicate that protons symmetrically placed with respect to oxygen lone-pairs have similar chemical shifts, *i.e.* Δδ varies between 0.04 and 0.15 p.p.m. depending on solvent. Thus to a first approximation it is found that the assumptions used to derive equation (14) are valid.

It is possible to determine the relative values of the populations of conformers for rotation about the C(5')–O(5') bond from an analysis of ^3J(HCOH) observed for

* It should be noted that data for pyrimidine nucleosides in Table 4 plotted according to equation (13) using assignment B also gives an approximate straight line with an intercept of 15.2 Hz and $(\delta_I^B - \delta_I^C)$ −0.17 p.p.m.

[41] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 57.
[42] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 403.
[43] P. M. Collins and N. N. Oparaeche, *Carbohydrate Res.*, 1974, **33**, 35.
[44] T. B. Grindley and W. Szarek, *Carbohydrate Res.*, 1972, **25**, 187.

such molecules in dimethyl sulphoside (DMSO) solutions. The results for a series of nucleosides in DMSO observed previously [45,46] show that there is essentially free rotation about the C(5′)–O(5′) bond except for a slight preference for the *gg* rotamer for uridine.[46] Although similar measurements cannot be made for nucleosides in D_2O solution, it is assumed that the strong interaction between D_2O and the CH_2OH group results in essentially free rotation about the C(5′)–O(5′) bond as found for nucleosides in DMSO solution. Thus with $(p_{VI} - p_V)$ close to zero it can be seen from equation (14) that the contribution to the chemical shift difference of the methylene group for free rotation about the C(5′)–O(5′) bond is negligible.

(b) syn ⇌ anti *Equilibrium.* It was suggested by Hruska *et al.*[6] that pyrimidine nucleosides exhibiting certain structural features preferentially exist in the *anti*-conformation in aqueous solution and these molecules conform to the $\Sigma(= J_{4'5'} + J_{4'5''})/\Delta\delta$ correlation. Other pyrimidine nucleosides which preferentially exist in or mimic the *syn*-conformation do not exhibit the same correlation, *i.e.* for the same $\Sigma, \Delta\delta(syn) > \Delta\delta(anti)$. The discrepancy in C(5′) proton chemical shift differences is likely to result from the magnetic anisotropy of the base-ring 2-keto-group in the *syn*-conformation though other properties such as different ribose ring conformations may contribute to the effect. Similar differential effects on ribose ring proton chemical shifts have been noted for base-rings of pyrimidine nucleosides [47] and cyclic purine-3′,5′-mononucleotides [48] in *syn*- and *anti*-conformations. From measurements [49] of ^5J[H(5)*H*-(1′)] of uridine (0.41 Hz) and deoxyuridine (0.45 Hz) and ^4J[H(6),H(1′)] of β-pseudouridine [50] (0.8 Hz), together with chemical shift arguments it was suggested that the base-rings exist in a predominantly *anti*-conformation. This conclusion was substantiated for ^{13}C enriched uridine by observation of ^3J[C(2)H(1′)] 2.3 Hz which was interpreted in terms of a specific *anti*-conformation [51] similar to that found in the solid state.[52] On the other hand, it has been shown [19] by N.O.E. experiments and CNDO molecular orbital calculations that both *syn*- and *anti*-conformations of β-pseudouridine (which also conforms to the Hruska correlation [6]) are found to exist in rapid equilibrium with roughly equal populations.[50] As base-rings of nucleosides exist in a *syn* ⇌ *anti* equilibrium and the equilibrium position is likely to vary for different nucleosides, it can be seen that C(5′) proton chemical shifts depend not only on the C(4′)–C(5′) rotamer distribution but also on the base-ring equilibrium. As a result of these considerations it can be seen that the assumptions used to derive equation (13) need further analysis. Nevertheless the derivation of an equation which characterises the δ–J correlation provides a basis on which results for different molecules and for different solvents can be rationalised. For example, a difference in behaviour between purine and pyrimidine nucleosides is expected to result from the marked magnetic anisotropy of the former base-ring compared to the latter. Although there are insufficient data on purine nucleosides to make a meaningful plot according to equation (13), it is found that the limited data supplemented by the present measurements as summarised in Table 4 exhibit a correlation between $(J_{4'5'} + J_{4'5''})$ and $\delta H(5') - \delta H(5'')$ shown in Figure 4. The intercept is similar to that found for pyrimidine nucleosides whereas the slope indicates that $(\delta_I^B - \delta_I^O)$purine $< (\delta_I^O - \delta_I^B)$pyrimidine. The observed linear correlation and common intercept suggest that, within the limits of the available data, the assumptions used to derive equation (13) apply to purine as well as pyrimidine nucleosides. Although there is limited data on the *syn* ⇌ *anti* equilibrium properties of purine nucleosides, the significant N.O.E. between H(8) and H(1′) (17—20%) compared to H(2) and H(1′) (3—4%) of adenosine, inosine, and deoxyadenosine in dilute D_2O solutions indicates that the base-ring exists predominantly in the *anti* conformation.[53] Each of these molecules exhibits the Hruska correlation [6] as shown in Figure 4. Further work on purine nucleosides is needed to define the relationship between J and δ according to equation (13) before such observations are assigned to conformational properties of these systems. Nevertheless the assignment of the C(5′) methylene protons and derivation of the correlation between J and δ for nucleosides provides a basis to compare the conformational properties of purine and pyrimidine nucleosides. The method can be extended to compare measurements for nucleosides in different solvents and to understand the conformational behaviour of nucleotides.

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