Re-investigation of the Effect of 2'-*O*-Methylation on Pyrimidine Nucleosides in Terms of the Pseudo-rotational Analysis of the Furanose Ring

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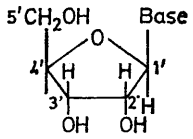
The scope of the pseudorotational analysis of the ribose ring has been investigated in terms of the limitations of the analysis imposed by the use of one Karplus relation for each molecular fragment and by the errors in the determination of the observed coupling constants. The method is illustrated by a re-investigation of the conformational properties of the pyrimidine nucleosides and the effect of 2'-*O*-methylation on the ribose ring. It is found that temperature changes and *O*-methylation do not alter the conformational parameters of the furanose ring but that they alter the equilibrium compositions.

THE conformational properties of the furanose ring (I) of nucleosides and nucleotides play an important role in determining the total conformation of the molecule.[1,2] The crystal structures of mononucleosides and nucleotides show that the ring is puckered and that the two envelope conformations, C(2')-*endo* and C(3')-*endo*, are



(I)

encountered about equally commonly.[1] The main puckered conformations are shown in Figure 1. The

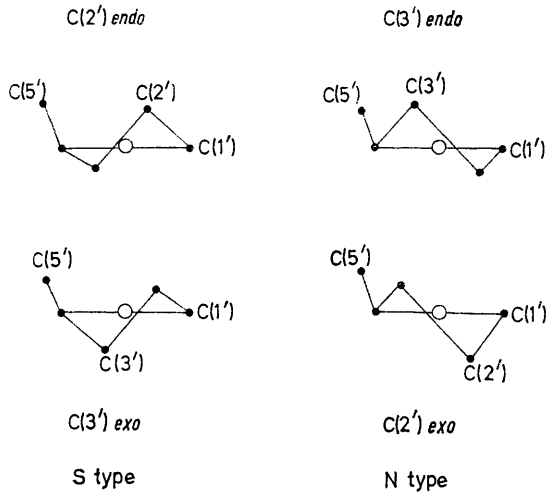


FIGURE 1 Representation of the conformations of the furanose ring

atoms C(2') and C(3') are displaced *ca.* 0·5 Å from the plane through the other atoms C(4')–O–C(1') and atoms lying on the same side of this plane as C(5') are designated *endo* and those on the other side *exo*.[1,3] The importance of the role of the conformation of the sugar ring is demonstrated for RNA and DNA in the solid state.[2] For example, it appears that the furanose ring in different RNA crystal forms exists in the C(3')-*endo*-conformation [4] whereas in B-DNA the sugar ring exists in the C(3')-*exo*-conformation and in A-DNA the C(3')-*endo* conformation occurs.[5]

The conformation of the furanose ring in solution was initially described in terms of the C(2')-*endo*- and C(3')-*endo*-conformations from an analysis of the observed proton spin-coupling constants between vicinal protons of the ribose and deoxyribose ring.[3,6,7] With the advent of higher magnetic fields and computed spectra to determine accurately all the vicinal coupling constants of the furanose ring, the conformation of the furanose ring has been qualitatively analysed in terms of a rapid equilibrium between the C(2')-*endo*- and C(3')-*endo* modes of puckering of the five-membered ring.[8-10] Recently the conformations of the furanose ring have been described in terms of the two pseudorotational parameters, the pseudorotational angle (P) and the angle of pucker (τ_m).[11] It was observed that the crystal structure data could be analysed in terms of the pseudorotational properties of the furanose ring existing in two narrow ranges of pseudorotational parameters (designated N and S type * conformers) which correspond approximately to the C(3')-*endo*- and C(2')-*endo*-conformations. It was also shown [12] that the observed vicinal proton spin-coupling constants of the ribose ring in various nucleosides and nucleotides in solution can be interpreted in terms of the pseudorotational parameters (P and τ_m) of the sugar ring and in terms of the N $\rightleftharpoons$ S equilibrium. Of the two methods it is expected that the pseudorotational analysis has greater scope for discussing the conformational properties of ribose and deoxyribose rings and for analysing perturbations of these conformations with such parameters as temperature and pH. For example, it has been shown that the pseudorotational

* Not to be confused with the *R,S* nomenclature.

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[10] F. E. Hruska, in ' Conformation of Biological Molecules and Polymers. Proceedings of the 5th Jerusalem Symposium,' eds. F. D. Bergman and B. Pullman, Academic Press, London, 1973.
[11] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1972, **94**, 8205.
[12] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1973, **95**, 2333.

analysis can be applied to both the ribose and deoxyribose ring of nucleosides [12] and nucleotides.[12,13]

The 100 and 220 MHz ^{1}H n.m.r. spectra of uridine (U), cytidine (C), and their 2′-*O*-methyl derivatives (Um) and (Cm) in aqueous solution have recently been measured at several temperatures.[14] The spectra were completely analysed and the observed chemical shifts and coupling constants were discussed in terms of the overall conformations of the molecules. It was concluded that 2′-*O*-methylation has little effect on the conformation of the pyrimidine mononucleosides in an aqueous environment. The conformation of the ribose ring in each of these nucleosides was analysed in terms of a dynamic equilibrium between the C(2′)-*endo*- and C(3′)-*endo*-puckering conformations. It was inferred from the small changes in $J_{1',2'}$ and $J_{3',4'}$ of Um and Cm relative to U and C that a slight shift occurs toward the C(3′)-*endo*-puckering mode and that methylation has no large effect on the barrier to interconversion or upon the equilibrium composition. It is obvious that the conformational properties of nucleosides need to be analysed more precisely in order to substantiate quantitatively such conclusions.

In this work some of the limitations of the pseudorotational analysis of the conformational properties of ribose rings is investigated for pyrimidine nucleosides in aqueous solution. In particular, the limits on the application of such an analysis resulting from the error in observed coupling constants is discussed using, as an example, the effect of 2′-*O*-methylation on the conformational properties of the ribose ring determined from proton spin-coupling constants of the pyrimidine nucleosides and their 2′-*O*-methyl derivatives previously published.[14] The results show that the pseudorotational analysis substantiates quantitatively the previous qualitative conclusion that 2′-*O*-methylation has little effect on the conformational properties of the pyrimidine mononucleosides in aqueous solution.[14] On the other hand, the results indicate that there are changes in equilibrium composition of the furanose ring on 2′-*O*-methylation which are consistent with the expected behaviour of introducing a substituent in the 2′-position.

The application of the pseudorotational analysis depends on constant values of $J_{2',3'}$ and $(J_{1',2'} + J_{3',4'})$ being observed for a set of molecules in order to determine the parameters of the Karplus relation [15] in the form (1) where A and B are constants.[12] It can be seen

$$J = A \cos^2 \phi - B \cos \phi \qquad (1)$$

from the observed J values in the Table that for solutions of the molecules at about room temperature the average $J_{2',3'}$ is given by $\bar{J}_{2',3'}$ 5·3 (±0·1) Hz and the average sum of $J_{1',2'}$ and $J_{3',4'}$ is $(\bar{J}_{1',2'} + \bar{J}_{3',4'})$ 10·1 (±0·15) Hz. As the expected error in the determination of each vicinal proton spin-coupling constant for nucleosides in aqueous solution is *ca.* ±0·1 Hz, it can be seen that 2′-*O*-methylation has little effect on the adjacent vicinal proton spin-coupling constants ($J_{2',3'}$) and that the same average $\bar{J}_{2',3'}$ can be used for both the nucleosides and their 2′-*O*-methyl derivatives. A similar conclusion can be made for $(J_{1',2'} + J_{3',4'})$ where no marked trends are apparent between the nucleosides and their 2′-*O*-methyl derivatives. The average values are similar to those found previously by Altona and Sundaralingam [12] for a variety of nucleosides and nucleotides, *i.e.*, $\bar{J}_{2',3'}$ 5·1, $(\bar{J}_{1',2'} + \bar{J}_{3',4})$ 10·1 Hz. It was shown [12] that these J values could be analysed to give a Karplus relation of the form (1) with $A = 10{\cdot}5$ and $B = 1{\cdot}2$. One equation was used to relate J with ϕ for each molecular fragment of the ribose ring. It is expected that different modified Karplus relations are necessary to deal with different molecular fragments because of the effect of the electronegativity of attached substituents [16-18] but, at present, such refinements are not available for nucleosides and so, in this work, the Karplus relation will be used for each molecular fragment of the ribose ring with values of constants $A = 10{\cdot}5$ and $B = 1{\cdot}2$ in equation (1).

Pseudorotational parameters of pyrimidine ribosides

	pD	Temp. (K)	Spin-coupling constants (Hz) [a] 1′,2′	2′,3′	3′,4′	Σ [c]	Pseudorotational parameters [b] P_N (°)	$^N\tau_m$(°)	P_S (°)	$^S\tau_m$ (°)	$^NJ_{1',2'}$ (Hz)	$^NJ_{3',4'}$ (Hz)	%N [d]
U	7·0	296	4·8	5·2	5·4	10·2	16	35	167	38	0·15	10·05	53
Um	7·0	301	4·0	5·4	5·9	9·9	11	35	171	37	0·1	9·8	60
C	8·0	296	4·0	5·2	6·0	10·0	13	35	170	37	0·1	9·9	60
Cm	8·0	301	3·6	5·4	6·7	10·3	17	36	165	38	0·15	10·15	66
Mean				5·3		10·1	14		168				
Mean error				(±0·1)		(±0·1)	(±2)		(±2)				
U	*e*	353	5·1	5·2	5·1	10·2	16	35	165	38	0·15	10·05	50
Um	*e*	333	4·2	5·6	5·6	9·8	9	34	173	37	0·05	9·75	57
C	*e*	338	4·2	5·5	5·9	10·1	14	35	168	38	0·1	10·0	59
Cm	*e*	333	3·8	5·5	6·3	10·1	14	35	168	38	0·1	10·0	63
Mean				5·45		10·05	13		168				
Mean error				(±0·15)		(±0·15)	(±2)		(±2)				

[a] Ref. 14. [b] $^NJ_{1',2'} = {}^SJ_{3',4'}$ and $^NJ_{3',4'} = {}^SJ_{1',2'}$. [c] $\Sigma = (J_{1',2'} + J_{3',4'})$. [d] Error in each value = ±1. [e] Not measured.

The method by which the pseudorotational parameters can be determined from observed proton vicinal coupling constants of the ribose ring has been outlined [12] though the scope and limitations of the method were not discussed. In particular, the limitations of the analysis as a result of errors in observed coupling constants or of using one Karplus relation for each molecular fragment were not investigated. In order to facilitate the deter-

[13] D. B. Davies and S. S. Danyluk, in preparation.
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mination of the pseudorotational parameters of the ribose ring of different nucleosides under a variety of conditions, values of the parameters P, $J_{1',2'}$, and $J_{3',4'}$ were computed for τ_m varying in integral steps from 25 to 50 using the one set of parameters for the Karplus relation for each molecular fragment. For each τ_m, the two solutions of parameters P, *etc.*, corresponded to the relevant parameters for the N and S type conformations, *i.e.*, P_N, P_S, ${}^NJ_{1',2'}$, ${}^NJ_{3',4'}$, *etc.* The variation in each of these parameters with $(J_{1',2'} + J_{3',4'})$ is shown in Figures 2 and 3.

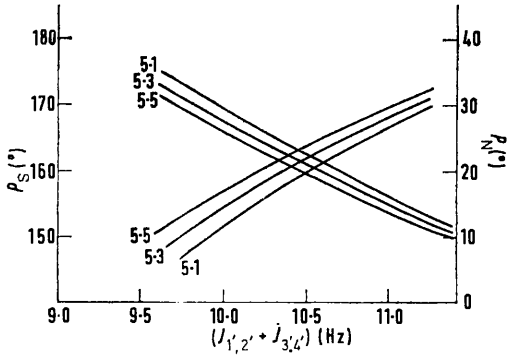

FIGURE 2 Variation of calculated pseudorotational angles (P_N and P_S) with $(J_{1',2'} + J_{3',4'})$ at different values of $J_{2',3'}$, *i.e.* 5·1, 5·3, and 5·5 Hz

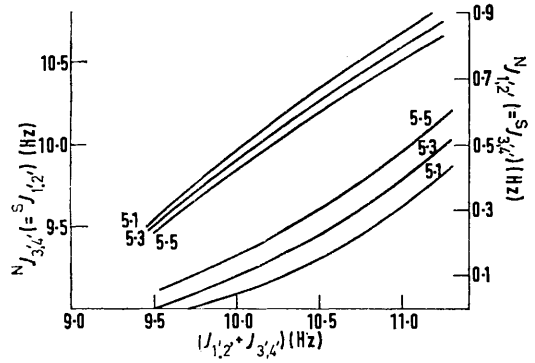

FIGURE 3 Variation of calculated vicinal proton spin coupling constants for ' pure ' N and S type conformers with $(J_{1',2'} + J_{3',4'})$ at different values of $J_{2',3'}$, *i.e.*, 5·1, 5·3, and 5·5 Hz

It should be noted that in Figure 3 the variations of ${}^NJ_{1',2'}$ and ${}^SJ_{3',4'}$ are the same and that ${}^NJ_{3',4'}$ and ${}^SJ_{1',2'}$ follow the same curve. This behaviour results from the symmetry in the N and S conformations expressed by the torsional angles $\phi_{1',2'}$ and $\phi_{3',4'}$ and the fact that a single Karplus curve was used for both molecular fragments.

Calculations of each of these parameters were made for different values of $J_{2',3'}$ in order to determine the error in the calculated parameters arising from the errors in the determination of the observed spin coupling constants. Examples of these graphs are shown in Figures 2 and 3 for $J_{2',3'}$ with values 5·1, 5·3, and 5·5 Hz. The curves drawn for $J_{2',3'}$ 5·3 Hz were used for subsequent determination of the pseudorotational parameters and equilibrium compositions, as this was the average value observed for the pyrimidine nucleosides and their 2'-*O*-methyl derivatives in aqueous solution.

The values of P_N and P_S interpolated directly from the appropriate curve in Figure 2 for different observed $(J_{1',2'} + J_{3',4'})$ are listed in the Table. As τ_m is only a slowly varying function with $(J_{1',2'} + J_{3',4'})$, the values of τ_m were interpolated by inspection of the calculated values of P and the appropriate values are listed in the Table. The equilibrium composition of the N $\rightleftharpoons$ S equilibrium was calculated from the observed coupling constants, assuming that the calculated pseudorotational parameters represented the ' pure ' N and S type conformations.[12] The observed coupling constants are the weighted averages of the coupling constants in the two conformations [equations (2) and (3) where X_N is the

$$J_{1',2'}(\text{obs}) = X_N\,{}^NJ_{1',2'} + (1 - X_N)\,{}^SJ_{1',2'} \quad (2)$$

$$J_{3',4'}(\text{obs}) = X_N\,{}^NJ_{3',4'} + (1 - X_N)\,{}^SJ_{3',4'} \quad (3)$$

fractional population of the N type conformer]. Values of ${}^NJ_{1',2'}$ $(= {}^SJ_{3',4'})$ and ${}^NJ_{3',4'}$ $(= {}^SJ_{1',2'})$ were determined for each molecule from Figure 3 and equations (2) and (3) were solved for X_N. The calculated values of %N type conformations are listed in the Table.

Angle of Pucker τ_m.—For both the N and S conformations the angle of pucker for the ribose ring of the pyrimidine nucleosides and their 2'-*O*-methyl derivatives are approximately the same as those found previously for nucleosides and nucleotides in the solid state [11] or in solution [12] and for polyribonucleotides in the solid state.[11] The results in the Table suggest that 2'-*O*-methylation has little effect on the puckering of the ribose ring but that there is a slightly greater puckering in the S type conformations (${}^S\tau_m$ *ca.* 38°) than in the N type conformations (${}^N\tau_m$ *ca.* 35°). This behaviour is found for solutions of these molecules both at room temperatures and at elevated temperatures. For $J_{2',3'}$ 5·3 Hz a variation in τ_m of ± 1 corresponds to a variation of $\pm 0{\cdot}3$ Hz in $(J_{1',2'} + J_{3',4'})$. Thus the differences in ${}^N\tau_m$ and ${}^S\tau_m$ are greater than the expected variation caused by the error in observed coupling constants.

It should be noted that the difference in ${}^N\tau_m$ and ${}^S\tau_m$ depends on the assumptions made in the analysis rather than reflecting real differences in conformation. The determination of each pseudorotational parameter depends on the comparison of the calculated sum $(J_{1',2'} + J_{3',4'})$ with the observed value for each molecule; the sum is dominated by $J_{1',2'}$ for S type conformations and by $J_{3',4'}$ for N type conformations. As ${}^3J_{H,H}$ decreases with increasing electronegativity of attached substituents,[16-18] the use of one Karplus relation for each molecular fragment overestimates the value of $J_{1',2'}$ compared to $J_{3',4'}$. Thus the value of ${}^S\tau_m$ is expected to be greater than ${}^N\tau_m$ as ${}^SJ_{1',2'}$ dominates the sum of the former and ${}^NJ_{3',4'}$ dominates the sum of the latter in which ${}^NJ_{1',2'}$ is close to zero as shown in Figure 3. The magnitude of the effect can be approximately correlated with the expected difference in electronegativity of attached substituents of the molecular fragments C(3')–C(4') and C(1')–C(2'). In the S type conformations with $J_{2',3'}$ 5·3 Hz the difference of three units in ${}^S\tau_m$

corresponds to a difference of 1·2 Hz in $(J_{1',2'} + J_{3',4'})$ which, in turn, results from differences of 0·3 Hz in $J_{3',4'}$ and 0·9 Hz in $J_{1',2'}$. Although the effect of the electronegativity of the nitrogen atom in the primidine ring on $J_{1',2'}$ of ribose rings is not known, it is expected by comparison with other C–H and C–N bonds that the difference in electronegativity could account for variations in J of this magnitude.[17,18] It has also been shown [13] for pyrimidine ribosides in the solid state that the average values of ${}^{N}\tau_m$ and ${}^{S}\tau_m$ determined from the results of many crystal structures [11] are equal. Thus the expected variation in τ due to the effect of different substituents on ${}^{3}J_{H,H}$ indicates that ${}^{N}\tau_m$ and ${}^{S}\tau_m$ of the pyrimidine nucleosides and their 2′-*O*-methyl derivatives are also equal in solution. Consequently, differences in ${}^{N}\tau_m$ and ${}^{S}\tau_m$ cannot be ascribed to conformational features until reliable Karplus relations have been determined for each molecular fragment.

Pseudorotational Angle P.—The calculated values of P for the pyrimidine nucleosides lie within those found for nucleosides and nucleotides in the crystalline state,[11] *i.e.*, P_N (9—17°) and P_S (165—173°). There are no consistent trends in P_N or P_S between a pyrimidine nucleoside and its 2′-*O*-methyl derivative; the behaviour for U and Um is the reverse of that for C and Cm.

It can be shown from the set of curves in Figure 2 that an observed error of ±0·1 Hz in $J_{2',3'}$ corresponds to the interpolated values of P_N and P_S varying by 1°; interpolated values of P are rounded to the nearest integer. It is also found that an expected variation of +0·2 Hz in observed values of $(J_{1',2'} + J_{3',4'})$ correspond to variations in P of ±3°. With such expected errors in P due to error in observed coupling constants, it can be seen that the differences between U and C and between their 2′-*O*-methyl derivatives are within the error limits of this analysis. This conclusion is substantiated by the fact that the average values of P_N and P_S (at room temperatures or elevated temperatures) are the same within the average error calculated for each set of results.

The absolute values of P_N and P_S depend on the assumptions made in the pseudorotational analysis. Following the discussion of the effect on ${}^{N}\tau_m$ and ${}^{S}\tau_m$ of using one Karplus relation for both molecular fragments, a similar analysis shows that the value of P_S is likely to be greater than that listed in the Table whereas the value of P_N is not likely to differ significantly. An estimate of the maximum variation in P_S can be made by assuming that the difference in spin-coupling constants between the vicinal protons of the C(3′)–C(4′) and C(1′)–C(2′) molecular fragments is a nominal 0·9 Hz (corresponding to that calculated for the difference in ${}^{N}\tau_m$ and ${}^{S}\tau_m$). Using the curve for $J_{2',3'}$ 5·3 Hz in Figure 2, it can be seen that the value of P_S might increase by up to 10°. It can be concluded that within the present accuracy of determination of proton spin-coupling constants of nucleosides in aqueous solution the pseudorotational properties of the ribose ring of pyrimidine nucleosides are similar to each other and are not affected by 2′-*O*-methylation.

Equilibrium Compositions.—The present results listed in the Table show a preferred N type conformation for the pyrimidine nucleosides and their 2′-*O*-methyl derivatives. The value for U and C are similar to those calculated previously [12] using an approximate calculation involving the observed $J_{1',2'}$ only. The slight preference for the N type correlates with the observed N type conformations of RNA in the solid state,[11] *i.e.*, C(3′)-*endo*-⇄ C(2′)-*exo*-conformations.

Using the expected measuring error of ±0·2 Hz in the sum $(J_{1',2'} + J_{3',4'})$ the approximate error limits in ${}^{N}J_{1',2'}$ and ${}^{N}J_{3',4'}$ were determined from the graphs in Figure 3. These variations led to an expected error of ±1 in the percentage equilibrium compositions of the ribose ring. The results in the Table show that there is a significant difference in the equilibrium composition of the two pyrimidine nucleosides at the same temperature, which is reflected by the equilibrium compositions of their 2′-*O*-methyl derivatives. The effect of 2′-*O*-methylation is to increase by 7% the preference for the N type conformer in the equilibrium compositions for both the uridine and cytidine nucleosides.*

The %N values at different temperatures indicate that the effect of temperature tends to equalise the composition of the N ⇌ S equilibrium of the ribose ring. It appears that equal populations of the N and S type conformers occur for the ribose ring of U in aqueous solution at 353 °C. The trend is also found for the other nucleosides where an increase in temperature leads to a decrease in the preferred N type conformation. The equilibrium compositions of the ribose ring of each nucleoside and its derivative can also be expressed in terms of the thermodynamic parameters. However, as n.m.r. measurements have been made on solutions at different pD values and at different temperatures, such calculated thermodynamic parameters cannot lead to meaningful results for comparison of these molecules.

It has been shown that the vicinal proton spin coupling constants of the ribose ring of the pyrimidine nucleosides and their 2′-*O*-methyl derivatives in aqueous solution can be analysed in terms of the pseudorotational properties of the five-membered ring and the composition of the N ⇌ S equilibrium. Within the limits of this analysis, it has been shown that both temperature and 2′-*O*-methylation have little effect on the pseudorotational parameters of the ribose ring but they alter the equilibrium composition.

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* The origin of the effect is not clear but it correlates with the expected behaviour of substitution of the hydroxy-group with the more bulky hydroxymethyl group at C(2′) of the ribose ring. The base ring exists in a *syn* ⇌ *anti* equilibrium. In the S type conformations of the furanose ring, the rotation of the methyl group can hinder the rotation of the base ring by steric repulsion with either the H(6) or O(2) atom of the base ring. The interaction between these groups cannot occur with the furanose ring in the N type conformations. On this basis, the greater preference for the N type conformation compared to the S conformation for the 2′-*O*-methyl-pyrimidine nucleosides compared to the pyrimidine nucleosides can be rationalised.