Conformation of the exocyclic 5'-CH_2OH in nucleosides and nucleotides in aqueous solution from specific assignments of the $H_{5'}$ and $H_{5''}$ signals in the NMR spectra

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Summary: Analyses of published NMR spectra for uridine, β-pseudouridine and their 3'-monophosphates, together with the deshielding effect of a phosphate group, have made possible specific assignments of the signals of the two 5' protons. This permits of a more accurate evaluation of the relative populations of the three CH_2OH conformers and, in turn, of a suitable choice of the constants in the Karplus relation applicable to this type of system. The results have been extended to a variety of other nucleosides and nucleotides, and to a consideration of the nature of the deshielding effect of a phosphate group on the ribose protons.

Of the six dihedral angles which delineate the conformation of a polynucleotide backbone, one is determined by the exocyclic 5'-CH_2OH of the furanose ring. The conformation of this exocyclic moiety relative to the carbohydrate ring may be studied by an analysis of the vicinal spin-spin coupling constants, $J_{4'-5'}$ and $J_{4'-5''}$, between the 4' and 5', and 4' and 5", protons in the NMR spectrum. A detailed description of this method, as well as its limitations, may be found in a paper by Blackburn et al. (1).

Analyses of such data for β-ψ (2), α-ψ (3), U (1), 4-SU (4), 3'-UMP (5), 3'-β-ψMP (5), dU (5), DHU (6), β-CAR (7)*, have shown that for all of these the exocyclic group CH_2OH undergoes rapid interconversion between three conformations about the exocyclic 4'-5' bond. Assuming that the interconversion period between the three forms is small relative to the lifetimes of the individual conformations, the values of the coupling constants may be formulated as weighted averages for the three conformers in terms of their populations, P_I, P_{II} and P_{III} (Fig. 1), as follows:

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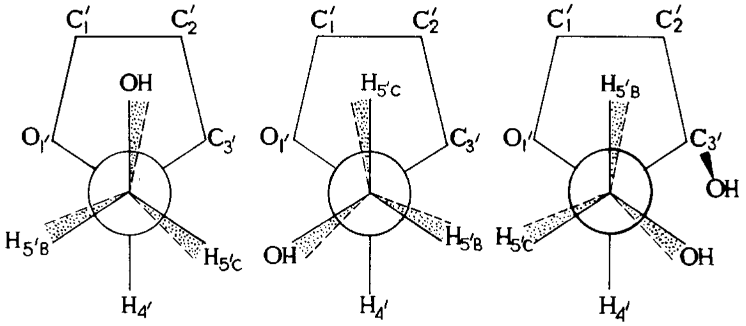
* The following abbreviations are used: ψ, pseudouridine; U, uridine, 4-SU, 4-thiouridine; CAR, cyanuric acid riboside; DHU 5,6-dihydrouridine; O, orotidine; dU, deoxyuridine; 5-EtdU, 5-ethyldeoxyuridine; araC, 1-β-(D-arabinofuranosyl)cytosine; araU, 1-β-(D-arabinofuranosyl)uracil; 6-Cl, 2'-deoxy-D-ribofuranosyl-6-chloropyridazon-(3).



I (GAUCHE- GAUCHE) II (GAUCHE-TRANS) III (TRANS-GAUCHE)

Fig. 1. Three possible conformations of the exocyclic CH_2OH group about the $C_{4'}-C_{5'}$ bond of nucleosides. The dashed lines indicate expected deviations from classical rotamers due to O-O repulsion.

$$\begin{aligned} J_{4'-5'b} &= P_I J_{Ib} + P_{II} J_{IIb} + P_{III} J_{IIIb} \\ J_{4'-5'c} &= P_I J_{Ic} + P_{II} J_{IIc} + P_{III} J_{IIIc} \\ P_I + P_{II} + P_{III} &= 1 \end{aligned} \tag{1}$$

From the assumed geometry of the individual conformers, the values of $J_{I'}, \ldots, J_{III''}$ may be calculated from the Karplus relation. Such calculations have been carried out on models consisting of classical rotamers, as well as for rotamers in which the dihedral angles are modified by 15° to take account of O-O repulsion (see Fig. 1). Both the constants proposed by Abraham et al. (8) and Lemieux (see ref. 1) have been applied for this purpose. It has been suggested by Lemieux (see ref. 1) that, with such systems, the coupling constants should be relatively large for $\phi = 180^\circ$, as much as 12 Hz.

The experimentally determined values of $J_{4'-5'}$ and $J_{4'-5''}$ may be assigned to the values $J_{4'-5'b}$ and $J_{4'-5'c}$ in the following two manners (see Fig. 1):

(A) 4'-5' ------ 4'-5'b
4'-5" ------ 4'-5'c

(B) 4'-5' ------ 4'-5'c
4'-5" ------ 4'-5'b

To date, however, no criteria exist for the unequivocal assignment of $H_{5'b}$ and $H_{5'c}$ from the corresponding signals in the NMR spectra. It has consequently become common practise to present (A) and (B) as equally

Table 1: Chemical shifts at temperatures indicated of ribose protons in uridine and β-pseudouridine and their 3'-monophosphates. Data taken from Schleich et al. (5).

Compound	1'	2'	3'	4'	5'	5"
U 23°C	5.901	4.340	4.221	4.127	3.907	3.803
3'-UMP 23°C	5.927	4.400	4.496	4.228	3.898	3.852
U 80°C	5.901	4.348	4.222	4.122	3.890	3.798
3'-UMP 88°C	5.906	4.411	4.541	4.229	3.905	3.855
β-ψ 30°C	4.674	4.279	4.141	4.009	3.840	3.726
3'-β-ψMP 23°C	4.715	4.341	4.433	4.116	3.850	3.785
β-ψ 70°C	4.668	4.260	4.128	3.993	3.835	3.719
3'-β-ψMP 75°C	4.714	4.334	4.455	4.111	3.853	3.785

likely alternatives.

It is clear from Eq. 1 and Fig. 1 that the population gauche-gauche (P_I) is invariant with respect to the choice (A) or (B). By contrast the populations gauche-trans (P_{II}) and trans-gauche (P_{III}) are markedly dependent on the method of assignment.

It will now be shown that an analysis of the chemical shifts for the pentose protons of nucleosides and their 3'-monophosphates leads to the elimination of one of the two alternatives (A) and (B). Table I presents the values of δ for the ribose protons of U and 3'-UMP, as well as for β-ψ and 3'-β-ψMP, taken from the data of Schleich et al. (5). Note the marked influence of the phosphate group, reflected in the deshielding of the ribose protons in its vicinity, as compared to the same protons in the nucleosides. Particularly interesting is the influence of the phosphate on the chemical shifts of $H_{5'}$ and $H_{5''}$.

The deshielding effect of a 5'-phosphate on a purine H-8 or a pyrimidine H-6 has been noted for a number of nucleoside-5'-phosphates (9,10). The more marked effect observed at pD 7.4, as compared to pD 5.9, clearly indicates that the degree of secondary phosphate ionization (pK 6.1-6.4 for various nucleotides) influences the extent of deshielding, the mechanism of which must consequently be at least partially electrostatic in nature (cf. ref. 10).

The NMR spectral data in Table I shows that the extent of deshielding for a given ribose proton decreases with displacement of the latter from the phosphate, e.g. $\Delta\delta_{3'} > \Delta\delta_{4'} > \Delta\delta_{2'} > \Delta\delta_{1'}$. It follows that the deshielding effect of a 3'-phosphate on $H_{5'}$ and $H_{5''}$ will depend on the relative displacements of these two protons from the phosphate.

Comparison of the data for U and 3'-UMP, and β-ψ and 3'-β-ψMP, demonstrates that $H_{5''}$ is more shielded (0.05-0.06 ppm) in the nucleosides than in the nucleotides; whereas the chemical shifts of the $H_{5'}$ protons in both pairs are very similar (differences varying from 0.000--0.015). In all four compounds there is a clear preference for the rotamer gauche-gauche, so that $H_{5'c}$, in contrast to $H_{5'b}$, spends a major proportion of its time in the vicinity of the phosphate group. Schleich et al. (5) and Mantsch & Smith (11), on the basis of measured coupling constants ^{32}P-^{1}H and ^{13}C-^{31}P, have estimated the relative populations of the phosphate group rotamers about the bond C-O; the results point to a some preference for the rotamer $C_{3'}$-$C_{4'}$ trans to $O_{3'}$-P.*

In the rotamer gauche-trans, $H_{5'b}$ is closer to the phosphate group than $H_{5'c}$; nonetheless the population of this conformer is about 2 to 3-fold lower according to the assignment (A) and 5 to 30-fold less with assignment (B). In the rotamer trans-gauche, both $H_{5'b}$ and $H_{5'c}$ are well outside the range of the phosphate group.

It follows from the foregoing that only assignment (A) is consistent with the selective influence of the phosphate on the chemical shifts of $H_{5'b}$ and $H_{5'c}$.

Acceptance of assignment (A) leads to some interesting conclusions regarding the values of the constants in the Karplus relation, with respect to the exocyclic CH_2OH group. The appreciable difference in sensitivity of the chemical shifts of the $H_{5'}$ and $H_{5''}$ protons to introduction of a 3'-phosphate (5 to 10-fold greater for $H_{5''}$ than for $H_{5'}$) implies that $H_{5''}$ spends a longer time in the vicinity of the phosphate than $H_{5'}$, i.e. the population of the gauche-gauche rotamer is higher than that of gauche-trans. Since the population ratio for the two conformers, P_I/P_{II}, is dependent on the constants applied in the Karplus relation, it is pertinent to examine the nature of this dependence.

In the case of uridine the ratio P_I/P_{II}, calculated by means of the constants proposed by Lemieux (1), is about 3. When the constants of Abraham et al. (8) are applied, the ratio is about 2. Similarly, in other instances, the ratio P_I/P_{II} is appreciably lower, from 1.5 to

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* Two possible conformations exist for the 50° dihedral angle H-C-O-P: (i) $C_{3'}$-$C_{2'}$ gauche to $O_{3'}$-P and $C_{3'}$-$C_{4'}$ trans to $O_{3'}$-P; (ii) $C_{3'}$-$C_{2'}$ trans to $O_{3'}$-P and $C_{3'}$-$O_{4'}$ gauche to $O_{3'}$-P.

5-fold, with the constants of Abraham et al. (8). These results provide a supplementary argument for acceptance of the constants of Lemieux as more appropriate to this type of system. It should be recalled that the parameters proposed by Abraham et al. (8) for five-membered furanose rings were derived from an empirically developed procedure for application of the Karplus relation to six-membered carbohydrate rings (12); there is no real justification for applying these same parameters to the exocyclic CH_2OH group, the geometry of which is hardly comparable to that of a furanose ring.

The $H_{5'}$ and $H_{5''}$ spectra for the above nucleosides, and for a number of additional ones listed in Table 2, all exhibit two regularities: (i) $\delta_{H_{5'}} > \delta_{H_{5''}}$; (ii) $J_{4'-5'} < J_{4'-5''}$. It appears, therefore, reasonable to conclude that for all of these the more shielded proton is identifiable as $H_{5'c}$.

Calculations of the relative populations of the forms <u>gauche-gauche</u> <u>gauche-trans</u> and <u>trans-gauche</u> (Table 2) were carried out as follows: coupling constants for each conformer were calculated from the relation (13), $J(\phi) = A + B\cos\phi + C\cos(2\phi)$, using the constants $A = 5.1$ Hz, $B = -1.5$ Hz, $C = 5.4$ Hz $[J(180^\circ) = 12$ Hz, $J(0^\circ) = 9$ Hz, $J(90^\circ) = -0.3$ Hz.$]$ It was assumed that conformations falling in the range between the accepted classical form and that allowing for O-O repulsion (shaded area in Fig. 1) are equally probable; it follows that

$$J(\phi_1,\phi_2) = \frac{\int_{\phi_1}^{\phi_2} J(\phi)\,d\phi}{\phi_2 - \phi_1}$$

The ranges of the dihedral angles for the accepted conformers are 45°-60°, 60°-75°, 165°-180°, i.e. each conformer is uniformly distributed over a range of 15°. The calculated conformer populations are shown in Table 2, which includes also data derived from as yet unpublished spectra of EtUdR (14) and various O'-methyl derivatives of araC and araU (15,16).

The foregoing analysis is being extended to include calculations of the energies of the different rotamers; the origin of the observed rules $\delta_{H_{5'}} > \delta_{H_{5''}}$ and $J_{4'-5'} < J_{4'-5''}$; the interaction of the exocyclic CH_2OH with the aglycone; and comparisons with crystallographic data. Furthermore, it should be emphasized that improved data regarding the conformation of the exocyclic group may have additional useful applications, e.g. in assessments of the specificities of various enzymes, such as kinase, deaminases, polymerases.

Table 2: Coupling constants for exocyclic CH_2OH protons and calculated population distributions (±5-10%) of the three conformers, for a variety of nucleosides and nucleotides, at room temperature unless otherwise indicated. Figures and letters in brackets are reference numbers, or table footnotes.

Compound	$J_{4'-5'}$	$J_{4'-5''}$	gauche-gauche P_I(%)	gauche-trans P_{II}(%)	trans-gauche P_{III}(%)
U (5)	2.9	4.4	58	22	20
U 80°C(5)	3.3	4.6	52	25	23
3'-UMP (5)	3.0	4.0	62	17	21
3'-UMP 88°C(5)	3.6	4.5	49	25	26
β-ψ 30°C(2)	3.2	4.6	53	25	22
β-ψ 70°C(2)	3.4	4.8	48	28	24
3'-β-ψMP (5)	3.0	4.6	55	24	21
3'-β-ψMP 75°C(5)	3.5	4.7	48	27	25
4-SU (4)	3.0	4.3	58	21	21
β-CAR 30°C(7)	3.2	6.2	34	43	23
DHU (6)	3.6	4.8	46	28	26
O 50°C(17)	3.0	6.1	38	41	21
α-ψ (3)	2.4	5.7	49	36	15
dU (5)	3.4	5.1	45	31	24
β-5-EtdU (a)	3.4	4.8	48	28	24
α-5-EtdU (a)	4.0	5.1	38	32	30
dC (b)	3.3	5.2	45	32	23
2'-FdU (19)	2.0	4.0	73	15	12
6-Cl (β) (20)	4.3	6.4	20	48	32
6-Cl (α) (20)	3.45	4.6	50	25	25
5'-O-Me-araC (a)	3.8	5.4	37	35	28
2'-O-Me-araC (a)	2.1	5.3	35	44	21
2',3',5'-tri-O-Me-araC (a)	5.35[c]	5.35[c]	20	-	-
araC (a)	3.3	5.8	38	39	23
araU (a)	3.2	5.5	42	35	23

(a) Detailed analyses of the complete NMR spectra of these compounds will be presented elsewhere.

(b) J values taken from spectra in ref. (18).

(c) Average.

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References

1. Blackburn B.J., Grey A.A., Smith I.C.P. and Hruska F.E. (1970) Can.J.Chem. 48, 2866
2. Hruska F.E., Grey A.A. and Smith I.C.P. (1970) J.Amer.Chem.Soc. 92, 4088
3. Grey A.A., Smith I.C.P. and Hruska F.E. (1971) J.Amer.Chem.Soc. 93, 1765
4. Hruska F.E., Ogilvie K.K., Smith A.A. and Wayborn H. (1971) J.Amer. Chem.Soc. 49, 2449
5. Schleich T., Blackburn B.J., Laper R.D. and Smith I.C.P. (1972) Biochemistry 11, 137
6. Deslauriers R., Lapper R.D. and Smith I.C.P. (1971) J.Amer.Chem. Soc. 49, 1279
7. Dugas H., Blackburn B.J., Robins R.K., Deslauriers R. and Smith I.C. P. (1971) J.Amer.Chem.Soc. 93, 3468
8. Abraham R.J., Hall L.D., Hough L. and McLauchlan K.A. (1962) J.Chem.Soc. 3699
9. Ts'o P.O.P., Kondo N.S., Schweizer M.P. and Hollis D.P. (1969) Biochemistry 8, 997
10. Schweizer M.P., Broom A.D., Ts'o P.O.P. and Hollis D.P. (1968) J.Am.Chem.Soc. 90, 1042
11. Mantsch H.H. and Smith I.C.P. (1972) BBRC 46, 808
12. Lenz and Heeschen (1961) J.Polymer.Sci. 51, 247
13. Karplus M. (1963) J.Amer.Chem.Soc. 85, 2870
14. Swierkowski M. and Shugar D. (1969) J.Med.Chem. 12, 533
15. Darżynkiewicz E., Kuśmierek J.T. and Shugar D. (1972) BBRC 46, 1734
16. Giziewicz J., Kuśmierek J.T. and Shugar D. (1972) J.Med.Chem. in press.
17. Hruska F.E. (1971) J.Amer.Chem.Soc. 93, 1795
18. Fraser-Reid B., Radatus B. J.Amer.Chem.Soc. 93, 6342
19. Cushley R.J., Codington J.F. and Fox J.J. (1968) Can.J.Chem., 46, 1131
20. Zschunke A., Nuhn P., Heller D. and Wagner G. (1971) Z.Chem. 11, 68