Ribo-2′-deoxyribo hybrid dinucleoside monophosphates. Proton magnetic resonance studies of 3′,5′- and 2′,5′-uridylyl-2′-deoxythymidine

JAMES PEELING[1] AND FRANK E. HRUSKA
Department of Chemistry, University of Manitoba, Winnipeg, Man., Canada R3T 2N2
AND
PETER C. LOEWEN
Department of Microbiology, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

Received July 14, 1977

JAMES PEELING, FRANK E. HRUSKA, and PETER C. LOEWEN. Can. J. Chem. **56**, 522 (1978).

This ^{1}Hmr study initiates our examination of the conformations of dinucleoside monophosphates possessing ribo 2′- or 3′-nucleotidyl units linked to 5′-nucleotidyl units possessing the 2′-deoxyribo sugar. The syntheses of uridylyl-3′,5′-2′-deoxythymidine (3′,5′-UpdT) and its 2′,5′-isomer, 2′,5′-UpdT, were carried out with standard techniques. The ^{1}Hmr data were obtained at frequencies up to 270 MHz and used to derive the dominant conformation of the dimers in aqueous solution. Comparison with data for the component mononucleotides reveals that dimerization does not lead to drastic changes in the molecular conformation. Literature data for dimers possessing only the ribo sugar (3′,5′-UpU) and the 2′-deoxyribo sugar (3′,5′-d(TpT)) are also presented. The results indicate that, at least for our dipyrimidine dimers, the molecular conformation of a particular fragment is not critically dependent on the nature (ribo or 2′-deoxyribo) of the other nucleotide unit.

JAMES PEELING, FRANK E. HRUSKA, et PETER C. LOEWEN. Can. J. Chem. **56**, 522 (1978).

Cette étude par rmn ^{1}H marque le début de nos travaux sur les conformations de monophosphates de nucléosides possédant des unités ribo 2′- ou 3′-nucléotidyles qui sont liées à des unités 5′-nucléotidyles possédant un sucre 2′-déoxyribo. On a effectué des synthèses d'uridylyl-3′,5′ 2′-déoxythymidine (3′,5′-UpdT) et de son isomère 2′,5′-UpdT, en faisant appel à des techniques standards. On a obtenu des données de rmn ^{1}H à des fréquences allant jusqu'à 270 MHz et on les a utilisées pour déterminer la conformation prédominante des dimères en solution aqueuse. Une comparaison de ces données avec celles obtenues pour les composants mononucléotides indique que la dimérisation ne conduit pas à des changements importants dans la conformation moléculaire. On présente aussi des données provenant de la littérature au sujet de dimères ne possédant que le sucre ribo (3′,5′-UpU) et le sucre 2′-déoxyribo (3′,5′-d(TpT)). Les résultats indiquent, au moins pour nos dimères dipyrimidines, que la conformation moléculaire d'un fragment particulier ne dépend pas d'une façon critique de la nature (ribo ou 2′-déoxyribo) de l'autre unité nucléotide.

[Traduit par le journal]

Introduction

Conformational studies of oligonucleotides in solution have been directed mainly at molecules containing solely either the ribose or 2′-deoxyribose sugar (1–6) though structures with covalently linked ribo- and 2′-deoxyribonucleotides are known (7). Maurizot *et al.* (8) examined the temperature dependence of the circular dichroic spectra of the four possible hybrid dinucleoside-3′,5′-monophosphates of adenine, i.e., ApA, dApA, ApdA, and d(ApA),[2] with a view to elucidating the conformational role of a 2′-hydroxyl group, and suggested that the observed spectral differences were most likely due to an intramolecular hydrogen bond to the adjacent 3′-phosphate. Kondo *et al.* (9) carried out uv, cd, and proton nmr of these same molecules, and concluded that the mode and extent of intramolecular base stacking was influenced by the 2′-hydroxyl group but they suggested that its effect is exerted through steric hindrance.

In the earlier nmr studies of the hybrid dimers (9), spectral parameters obtainable from the bands of the base, the H_1' and H_2' protons were considered, and thus, though comments about base stacking and sugar pucker could be made, no information about the conformational situation about the $C_{4'}$—$C_{5'}$ and $C_{5'}$—$O_{5'}$ bonds was obtained. Here we present proton nmr data for the hybrid dimer, uridylyl-3′,5′-2′-deoxythymidine (3′,5′-UpdT) (Fig. 1) as well as for its isomer possessing the 2′,5′-phosphodiester linkage (2′,5′-UpdT). Dimers containing only the pyrimidine bases (U, T) were chosen for our initial studies; since base stacking is

[1]Post-doctoral fellow; present address: University of Petroleum and Minerals, Dhahran, Saudi Arabia.

[2]A 'd' outside the parenthesis indicates that both fragments are 2′-deoxyderivatives, d(ApA) for example. A 'd' immediately before a base symbol (A, T, etc.) indicates that that unit has the 2′-deoxy sugar. A ribose nucleoside fragment will not be prefixed.

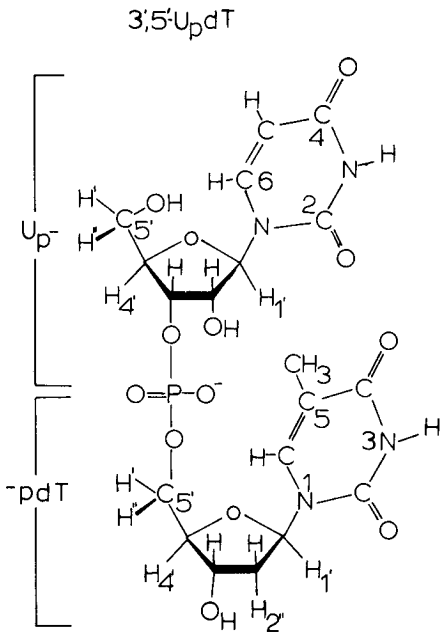

FIG. 1. Structural formula of uridylyl-(3',5')-2'-deoxythymidine or 3',5'-UpdT. Up- and -pdT refer to the 3'- and 5'-linked fragments, respectively.

expected to be less important here than in purine-containing dimers, the interpretation of the data is simplified but forms the basis for extension of the work to the more complex situation of stacked dimers.

Experimental

3',5'-UpdT and 2',5'-UpdT were prepared following the procedure of Lapidot and Khorana (10). Details of the preparation and purification are available upon request.

Proton spectra were obtained on a variety of spectrometers (Bruker WH-90DS, Manitoba; Bruker WH-270, Madison; Varian HA-100, Manitoba; Varian HR-220, Canadian 220 MHz Centre) operating in the CW or FT modes. Sodium-3-trimethylsilylpropionate, 2,2,3,3-d_4 (TSP) was used as an internal ^{1}Hmr reference. Solutions (0.05 *M* or less) were freeze-dried three times and dissolved in 100% D_2O. For the dimers the p*D* of the solution was adjusted to neutrality with NaOD and DCl; for the monomers the solution p*D* was adjusted to about 5.0. In all instances, then, the phosphate is monoanionic. Calibration of the spectra was carried out by standard methods. Spectral analysis was carried out using LAME (11) and computer simulated spectra were generated as a final test of the data. A sample spectrum of 3,5-UpdT is shown in Fig. 2.

The chemical shift (δ) and coupling constant (J) data for the *ribose* and *2'-deoxyribose* fragments of the hybrid dimers are listed in Tables 1 and 2, respectively. For purposes of comparison data for the mononucleotide, 5'dTMP, and the homodimers, 3',5'-UpU and 3',5'-d(TpT), are included in the appropriate table; the source of these data is indicated in the footnotes to the tables.

Results and Discussion

Spectral Assignment and Chemical Shifts

The assignment of the spectral bands of the Up- and -pdT fragments was accomplished by comparison with published spectra of mono- and oligonucleotides (1, 3, 12, 13). Following the work of Ritchie and Perlin (14) on selectively deuterated adenosine and that of Remin and Shugar (15) and Davies and Rabczenko (16), the low-field portion of the $H_{5'}$,$H_{5''}$ bands was assigned to $H_{5'}$ for all ribose and 2'-deoxyribose fragments (Fig. 3).

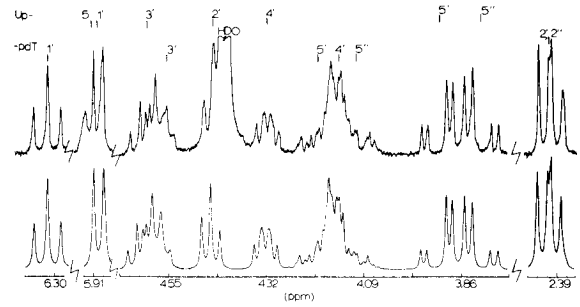

FIG. 2. (*a*) Single scan, continuous wave, 220 MHz spectrum of the ribose protons 3',5'-UpdT (p*D* = 6.8, 65°C, ~0.05 *M* in D_2O). Sweep width 250 Hz. (*b*) Computer simulated spectrum.

In the -pdT unit of 3',5'-UpdT there exists a substantial shift between the 5'- and 5''-hydrogens (0.13 ppm at 20°C; Table 2). In contrast $H_{5'}$ and $H_{5''}$ in -pdT of 2',5'-UpdT are nearly isochronous. Furthermore, the $\delta_{5'}$ and $\delta_{5''}$ values of -pdT (2',5'-isomer) are similar to those of the monomer 5'dTMP. This is not the case in either 3',5'-UpdT or 3',5'-d(TpT) where large deshielding effects (up to 0.19 ppm, relative to 5'dTMP) are evident in the $\delta_{5'}$ and $\delta_{5''}$ values of the -pdT units. Thus, incorporation of 5'-dTMP into a dimer induces magnetic nonequivalence in the $H_{5'}$,$H_{5''}$ pair if the second fragment is 3'-linked, the magnitude of the induced effect being dependent on the nature (ribo or 2'-deoxyribo) of the 3'-linked fragment. Ezra *et al.* (4) have seen an analogous situation for the ribose homodimers 2',5'-CpC and 3',5'-CpC. In the -pC fragment of the former the $H_{5'}$,$H_{5''}$ hydrogens are isochronous whereas in that of the latter they are separated by 0.22 ppm.

It is interesting to note that, whereas the $\delta_{5''}$ values for the -pdT units of 3',5'-UpdT and 3',5'-d(TpT) differ by only 0.03 ppm, the $\delta_{5'}$ values differ by a larger amount, 0.09 ppm. Thus, the $H_{5'}$ hydrogen on the -pdT fragment appears to be more sensitive to the nature (hydrogen or hydroxyl) of the *exo* substituent on the $C_{2'}$ carbon of the 3'-linked nucleotidyl fragment. This supports our assignment of the $H_{5'}$,$H_{5''}$ hydrogens in the -pdT fragment of both dimers and provides as well some conformational information. (See below).

The $H_{2'}$ and $H_{2''}$ of the -pdT units of 3',5'-UpdT,

TABLE 1. Proton chemical shifts (δ) and coupling constants (J)[a] of 2′UMP, 3′UMP, 3′,5′-UpU, and the ribose protons of 2′,5′-UpdT and 3′,5′-UpdT in D_2O

Parameter	3′UMP	Up- 3′,5′-UpU[b] 20°C	Up- 3′,5′-UpdT 20°C	Up- 3′,5′-UpdT 65°C	2′UMP (23°C)	Up- 2′,5′-UpdT 20°C	Up- 2′,5′-UpdT 65°C
$\delta_{1'}$	5.96	5.89	5.85	5.90	6.02	6.06	6.03
$\delta_{2'}$	4.46	4.46	4.46	4.45	4.75	4.73	4.76
$\delta_{3'}$	4.57	4.55	4.55	4.60	4.37	4.39	4.37
$\delta_{4'}$	4.31	4.34	4.33	4.32	4.15	4.14	4.14
$\delta_{5'}$	3.93	3.94	3.94	3.91	3.87	3.87	3.87
$\delta_{5''}$	3.85	3.85	3.84	3.83	3.81	3.80	3.80
δ_6	7.91	7.93	7.95	7.86	7.84	7.81	7.77
δ_5	5.92	5.87	5.86	5.91	5.91	5.74	5.81
$J_{1'2'}$	4.9	4.2	4.3	4.6	5.7	4.5	5.5
$J_{2'3'}$	5.2	5.2	5.1	4.8	5.4	4.6	5.8
$J_{3'4'}$	5.2	5.3	5.6	4.9	4.4	4.1	4.9
$J_{4'5'}$	2.9	2.4	2.7	2.9	3.0	3.1	2.7
$J_{4'5''}$	4.2	4.0	3.3	4.0	4.7	3.8	4.7
$J_{5'5''}$	−12.8	−13.1	−13.1	−13.0	−12.7	−13.7	−12.7
J_{56}	8.2	8.1	8.2	8.1	8.1	7.7	8.1
$J_{P2'}$	—	—	—	—	8.4	9.4	8.9
$J_{P3'}$	8.2	8.2	8.3	7.8	—	—	—
Σ[c]	7.1	6.4	6.0	6.9	7.7	6.9	7.4

[a]Chemical shifts reported in ppm from TSP. Coupling constants in Hz. Estimated error: J, ±0.2 Hz; δ, ±0.01 ppm.
[b]Data from ref. 3; pD = 7.4, 0.05 M.
[c]$\Sigma = J_{4'5'} + J_{4'5''}$.

3′,5′-d (TpT) and 5′dTMP are nearly isochronous. Furthermore, $\delta_{2'}$ and $\delta_{2''}$ fall in the narrow range from 2.37 to 2.41 ppm from TSP. This constancy of the $\delta_{2'}$ values on the one hand and of $\delta_{2''}$ on the other, for these three molecules, is perhaps predictable from space-filling models of the dimers in either the extended (unstacked) conformation or in the right-handed stacked arrangement. In both conformations the $C_{2'}$ hydrogens on -pdT are distant from the 3′-linked unit.

In contrast with 5′dTMP and the 3′,5′-dimers, a substantial chemical shift (0.06 ppm) exists between the $H_{2'}$ and $H_{2''}$ protons of the -pdT fragment of 2′,5′-UpdT, a consequence of the differential shielding effect induced by the formation of the 2′,5′-phosphodiester bond. Thus, relative to 3′,5′-UpdT, $H_{2'}$ and $H_{2''}$ of the 2′,5′-isomer are shifted upfield by 0.10 and 0.04 ppm, respectively. (Our assignment of the $H_{2'}$ and $H_{2''}$ hydrogens is based on the observation that $J_{2'3'}$ is generally larger than $J_{2''3'}$ (1, 12, 13).) This shielding effect is not simply explained. It could be due to changes in the sugar-base torsion angle (χ) (4, 17) of the -pdT unit which determines the disposition of $H_{2'}$ and $H_{2''}$ relative to the magnetically anisotropic 2-keto oxygen of the base. (In this regard it is relevant to point out that $H_{1'}$ is also shifted (upfield) in -pdT of 2′,5′-UpdT relative to the 3′,5′-isomer.) The need for change in χ could in turn be traced to the stacking properties of the 2′,5′- and 3′,5′-isomers, but further comment here would require extensive cd measurements.

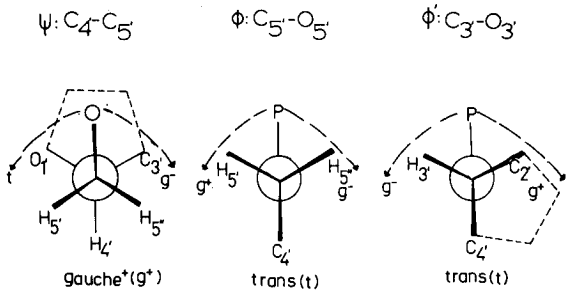

FIG. 3. Newman projections of the dominant conformations viewed along the $C_{4'}$—$C_{5'}$, $C_{5'}$—$O_{5'}$, and $C_{3'}$—$O_{3'}$ bonds. Definitions according to ref. 18.

The Sugar-base Torsion Angle (χ)

A variety of methods have been employed for evaluating χ. One method is based on the characteristic shielding changes observed for the $H_{2'}$ and $H_{3'}$ protons when, in the *syn* conformation, the 2-keto oxygen is located over the sugar ring (19, 20). Following the reasoning outlined (19), we propose on the basis of the chemical shifts (Table 2) that the *syn* ⇆ *anti* balance is not drastically altered by incorporation of the monomers into the dimer molecules. The general consensus is that in solution pyrimidine nucleosides prefer the *anti* conformation but the *syn* can be significantly populated (2).

TABLE 2. Proton chemical shifts and coupling constants[a] for 5′dTMP, 3′5′-d(TpT), and the 2′-deoxyribose fragment of 2′,5′-UpdT and 3′,5′-UpdT

		-pdT				
		3′,5′-d(TpT)[b]	3′,5′-UpdT		2′,5′-UpdT	
Parameter	5′dTMP[b] 30°C	18°C	20°C	65°C	20°C	65°C
$\delta_{1'}$	6.34	6.34	6.33	6.31	6.28	6.27
$\delta_{2'}$	2.37[c]	2.38[c]	2.39[c]	2.40[c]	2.30	2.31
$\delta_{2''}$	2.38[c]	2.39[c]	2.40[c]	2.41[c]	2.36	2.38
$\delta_{3'}$	4.58	4.60	4.59	4.57	~4.54[d]	~4.52[d]
$\delta_{4'}$	4.17	4.13	4.16	4.16	~4.08[e]	~4.07[e]
$\delta_{5'}$	4.06[c]	4.16	4.25	4.20	~4.08[e]	~4.07[e]
$\delta_{5''}$	4.05[c]	4.09	4.12	4.12	~4.08[e]	~4.07[e]
δ_6	7.77	7.71	7.75	7.68	7.71	7.66
$\delta_5(CH_3)$	1.93	1.91	1.91	1.92	1.92	1.93
$J_{1'2'}$	7.0[f]	6.9	6.2	6.9	6.8	6.9
$J_{1'2''}$	7.0	6.9	6.2	6.9	6.8	6.7
$J_{2'2''}$[g]	−13.8	−14.1	−14.2	−14.2	−13.2	−14.5
$J_{2'3'}$	4.8	5.8	5.4	5.2	6.4	7.6
$J_{2''3'}$	4.8	5.8	5.4	5.2	5.7	4.5
$J_{3'4'}$	2.9	4.0	3.2	3.9	—[i]	—
$J_{4'5'}$	3.8	2.5	2.4	2.8	—	—
$J_{4'5''}$	3.8	4.0	4.0	4.2	—	—
$J_{5'5''}$[g]	−12.1	−12.1	−11.2	−11.5	—	—
$J_{CH_3,6}$	1.1	1.3	1.0	1.2	1.0	1.0
$J_{P4'}$	1.6	1.6	1.7	1.8	—	—
$J_{P5'}$	5.0	4.0	4.0	5.0	—	—
$J_{P5''}$	5.0	4.0	3.8	5.7	—	—
Σ[h]	7.6	6.5	6.4	7.0	—	—
Σ'	10.0	8.0	7.8	10.7	—	—

[a]Chemical shifts in ppm from TSP. Coupling constants in Hz. Estimated error: J, ±0.2 Hz; δ, ±0.01 ppm.
[b]Data from ref. 14; 5′dTMP: 0.1 M, pD 5.8; 3′,5′-d(TpT), 0.1 M, pD = 7.0.
[c]The 0.01 ppm is arbitrary if $\delta_{2'} \simeq \delta_{2''}$ or $\delta_{5'} \simeq \delta_{5''}$.
[d]Taken from centre of broad $H_{3'}$ band; featureless since $\delta_{4'} \simeq \delta_{5'} \simeq \delta_{5''}$.
[e]Taken from centre of doublet $H_{4'}$, $H_{5'}$, $H_{5''}$ band.
[f]Only sum of connected couplings is significant.
[g]Arbitrary if $\delta_{2'} \simeq \delta_{2''}$ or $\delta_{5'} \simeq \delta_{5''}$.
[h]$\Sigma = J_{4'5'} + J_{4'5''}$; $\Sigma' = J_{P5'} + J_{P5''}$.
[i]Not measurable.

Sugar Pucker

The conformation of a ribose or 2′-deoxyribose ring, described in terms of the pseudorotational parameters (P), the amplitude of pucker (τ_m), and the populations of the 2′*endo* (S) and 3′*endo* (N) puckers in the N ⇆ S blend (21), can be evaluated from the magnitudes of the vicinal couplings ($J_{1'2'}$ etc.) (22, 23). In Table 3 are presented the % 3′*endo* values for the entries in Table 1, calculated according to Davies and Danyluk (22).

A striking feature of the $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ data (Table 1) is their similarity in the monomer, 3′UMP, and the 3′-linked uridylyl fragments of 3′,5′-UpU and 3′,5′-UpdT. Clearly, the sugar pucker of the 3′-linked uridine fragment is not critically dependent on whether its 5′-linked pyrimidine nucleotidyl partner is a ribose or 2′-deoxyribose derivative. The data in Table 3 show a slight bias for the 3′*endo* conformation which is little affected by temperature change.

With few exceptions, 2′-deoxyribose derivatives display a preference for the 2′*endo* pucker, both in the crystal and solution states (18, 24). The -pdT fragment of 3′,5′-UpdT is not, in this respect, exceptional. Calculations (Table 3) show contributions of 32 and 39% from the 3′*endo* pucker at 20 and 65°C, respectively, results which are similar to those of the -pdT unit of 3′,5′-d(TpT) and to that of the monomer, 5′dTMP. Thus the pucker of a -pdT unit is not critically dependent on the nature (ribo or 2′-deoxyribo) of its 3′-linked pyrimidine nucleotidyl partner. (At 20°C the magnitude of the sum $J_{1'2'} + J_{1'2''}$ is 12.4 and 13.8 Hz for the -pdT units of 3′,5′-UpdT and 3′,5′-d(TpT), respectively. Thus, some dependence on the nature of the 3′-linked unit is indicated; however, it is difficult to assess since only the sum, and not the individual couplings, could be obtained owing to the chemical shift equivalence of $H_{2'}$ and $H_{2''}$.)

The data for the 2′-phosphorylated uridine fragments provide some contrast. For 2′UMP there is a shift, relative to 3′UMP, in the 3′*endo* bias (Table 3).

TABLE 3. Calculated rotamer populations

	T(°C)	% 3′*endo*	% $g^+(\psi)$	% $t(\phi)$
2′- or 3′-Linked uridylyl				
3′UMP[a]	18	52	59	—
3′-Up- (3′,5′-UpU)[b]	20	56	66	—
3′-Up- (3′,5′-UpdT)	20	57	70	—
3′-Up- (3′,5′-UpdT)	65	51	61	—
2′UMP	23	44	53	—
2′Up- (2′,5′-UpdT)	20	48	61	—
2′Up- (2′,5′-UpdT)	65	47	56	—
5′-Linked-2′-deoxythymidylyl				
5′dTMP[c]	30	29	54	71
-pdT[c] (3′,5′-d(TpT))	18	40	65	81
-pdT (3′,5′-UpdT)	20	32	66	82
-pdT (3′,5′-UpdT)	65	39	60	68
-pdT (2′,5′-UpdT)	20	—	—	—
-pdT (2′,5′-UpdT)	65	—	—	—

[a]Reference 23.
[b]Reference 4.
[c]Reference 14.

Furthermore, dimerization of 2′UMP to yield the Up- unit of 2′,5′-UpdT results at 20°C in a decrease in $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$. This decrease in $J_{2'3'}$ is indicative of an increase in the amplitude of pucker (21) and is not observed upon dimerization of 3′UMP. Dimerization also leads to a slight increase in the 3′*endo* contribution (Table 3). Elevation of the temperature to 65°C leads to an increase of 1.2 Hz in the magnitude of $J_{2'3'}$, indicating a decrease in the τ_m value of 65°C. This pronounced decrease in τ_m with increasing temperature is not observed for the Up- unit of 3′,5′-UpdT (Table 3) or of 3′,5′-UpU (3). Ezra *et al.* (4) have found that 2′,5-dimerization of 2′CMP to yield 2′,5′-CpC leads to an increase in $J_{2'3'}$ (decrease in τ_m) at 20°C, results which contrast with our results for 2′,5′-UpdT. It seems therefore, that a systematic study of the influence of the base, sugar, temperature, etc., is warranted for the 2′,5′-isomers.

Conformation about the $C_{4'}$—$C_{5'}$ (ψ) and $C_{5'}$—$O_{5'}$ (ϕ) Bonds

Contributions from $g^+(\psi)$ and $t(\phi)$ conformers (Fig. 3), calculated[3] by the usual methods, are listed in Table 3. The following comments can be made about the $C_{4'}$—$C_{5'}$ conformation of the uridine units. (*a*) The data define a range from 53 to 70%, indicating a bias for $g^+(\psi)$ over $t(\psi)$ and $g^-(\psi)$ in all instances. (*b*) 3′-Elongation of 3′UMP with either a U fragment or a dT fragment enhances % $g^+(\psi)$ (7–11%). Thus, the ψ conformer distribution is not strongly dependent on the nature (ribo or 2′-deoxyribo) of the 5′-nucleotidyl unit. (*c*) Relative to 3′UMP the bias for $g^+(\psi)$ is reduced in 2′UMP. This reduction is probably a reflection of the shift in the 3′*endo* ⇆ 2′*endo* balance (Table 3) which is known (24) to influence the ψ conformer distribution. Elongation of 2′UMP with a dT unit (2′,5′-UpdT) leads to a slight increase in % $g^+(\psi)$. It is interesting that our 2′,5′- and 3′,5′-dimerization effects are in line with observations on 2′,5′- and 3′,5′-CpC (4); in both isomers dimerization leads to an increase in % $g^+(\psi)$.

Ezra *et al.* (4) have noted a significant magnetic nonequivalence for $H_{5'}$ and $H_{5''}$ of -pC of 3′,5′-CpC and that this pair of hydrogens are, along with $H_{4'}$ of -pC, essentially isochronous in the 2′,5′-isomer. In like fashion, we observe magnetic nonequivalence for $H_{5'}$ and $H_{5''}$ of -pdT of 3′,5′-UpdT but a collapse of $H_{5'}$, $H_{5''}$, and $H_{4'}$ in the 2′,5′isomer. Thus, in this respect, a parallel behavior exists for the 5′-nucleotidyl units of the CpC and UpdT isomers. An unfortunate consequence of this spectral collapse is that the couplings required for discussion of the ϕ,ψ conformational features of the 2′,5′-isomers cannot be obtained.

However, we may make the following comments about the $C_{4'}$—$C_{5'}$ (ψ) and $C_{5'}$—$O_{5'}$ (ϕ) conformer distributions for the -pdT unit of 3′,5′-UpdT. (*a*) The $g^+(\psi)$ conformer is dominant in 5′dTMP (Table 3). (*b*) The magnitude of % $g^+(\psi)$ is enhanced to about 65% when (at 20°C) 5′dTMP is incorporated into 3′,5′-UpdT and 3′,5′-d(TpT). With dimerization of 5′dTMP there occurs also an increase in % t (ϕ) regardless of the nature (Up- or dTp-) of the 3′-unit. It is, then, clear that neither the ϕ nor the ψ conformer distribution of -pdT is dependent on the nature of the sugar moiety of the 3′-unit. This, of course, need not be the case for purine derivatives nor for that matter in pyrimidine dimers in the fully stacked conformation. (*c*) Increasing temperature leads to decreases in % $g^+(\psi)$ and % $t(\phi)$. This is also the case for -pdT of 3′,5′-d(TpT) (4) and -pU of 3′,5′-UpU (3). It seems, however, that Σ' is somewhat more sensitive to temperature in 3′,5′-UpdT than in the other dimers, increasing by 3.7 Hz in the 20–65°C interval. In the 18–65°C range Σ' increases by about only 1 Hz for -pdT of 3′,5′-d(TpT) (14) and by 0.9 Hz for -pU of 3′,5′-UpU in the 20–85°C interval (3). Thus, the ϕ orientation is not entirely insensitive to the nature of the 3′-linked sugar.

The long-range $^4J_{P4'}$ couplings provide information about the $C_{4'}$—$C_{5'}$ and $C_{5'}$—$O_{5'}$ conformer distribution (26, 27) although, owing to their small size, they may be less reliable than Σ and Σ' as conformational probes. However, the magnitudes of the observed $^4J_{P4'}$ couplings for the 5′-nucleotidyl fragments (1.6–1.8 Hz, Table 2) are in line with our observations based on Σ and Σ', namely, that $g^+(\psi)$ and $t(\phi)$ are the dominant conformations.

[3]% $g^+(\psi) = (13 - \Sigma)/10$ where $\Sigma = J_{4'5'} + J_{4'5''}$ and % $t(\phi) = (25 - \Sigma')/21$ where $\Sigma' = J_{P5'} + J_{P5''}$ (12, 25).

Conformation about $C_{3'}$—$O_{3'}$ and $C_{2'}$—$O_{2'}$

Information about the conformations about these bonds in 3′- and 2′-nucleotidyl units may be obtained from $^3J_{P3'}$ and $^3J_{P2'}$. On the basis of steric considerations and a wealth of X-ray data (18), the $g^+(\phi')$ conformation (Fig. 3) may be excluded from consideration. However, the $H_{3'}$ ($H_{2'}$) and P atoms are *gauche* in the two remaining possibilities, $g^-(\phi')$ and $t(\phi')$, and thus no unambiguous distinction between the two can be made on the basis of ^1H–^{31}P couplings alone. They provide, however, the following important information about our 3′(2′)-uridylyl units if we assume the applicability of eq. 1 (25).

$$[1] \qquad {}^3J_{PH}\,(\text{Hz}) = 18.1\cos^2\theta - 4.8\cos\theta$$

where θ is the dihedral angle in the relevant H—C—O—P fragment. Note that $\theta = 60°$ corresponds to the classical $g^-(\phi')$ and $t(\phi')$ staggered conformers whereas $\theta = 0$ corresponds to the conformation in which $H_{2'}$ (of the 2′-nucleotidyl units) and $H_{3'}$ (of the 3′-nucleotidyl units) eclipse the phosphorus atom, i.e. $\phi' = -120°$.

The $^3J_{P3'}$ and $^3J_{P2'}$ values (Table 1) are significantly larger than expected for the classical $g^-(\phi')$ and $t(\phi')$ conformers (ca. 2 Hz) and since the $g^+(\phi')$ conformation is excluded (expected $^3J_{P3',2'} = 23$ Hz), a substantial bias towards the $\phi' = -120°$ situation is indicated. In this conformer which separates the g^- and t ranges, the phosphorus and $H_{2'}$($H_{3'}$) atoms are eclipsed ($\theta = 0°$) and a large (13.3 Hz) ^1H–^{31}P coupling is expected. Using eq. 4 we obtain $\theta \simeq 35°$ for 2′- and 3′-UMP and the Up- units of 3′,5′UpU and 3′,5′UpdT from the low temperature data, corresponding to ϕ' angles of -85 and $-155°$ in the g^- and t conformer range, respectively. Increasing temperature leads to a small decrease in $^3J_{P3'}$ and an increase (ca. 1°) in θ for the 3′-units of the dimers. For Up- of 2′,5′-UpdT $\theta \simeq 30°$, corresponding to $\phi' = -90$ and $-150°$. It appears then that in the 2′,5′-dimer the $C_{2'}$—$O_{2'}$ bond is somewhat more constrained towards the eclipsed ($\phi' = -120°$) situation than is the $C_{3'}$—$O_{3'}$ bond in the 3′,5′-isomer. (A similar trend has been noted (4) for the Cp- units of 2′,5′-CpC ($^3J_{P2'} = 9.1$ Hz) and 3′,5′CpC ($^3J_{P3'} = 8.3$ Hz).) Thus 3′,5′-dimerization appears to have little influence on $^3J_{P3'}$, regardless of the nature (ribo or 2′-deoxyribo) of the 5′-nucleotidyl unit. 2′,5′-Dimerization appears to bias the angle about the $C_{2'}$—$O_{2'}$ bond towards the $\phi' = -120°$ situation but again the nature of the 5′-linked sugar is unimportant.

The bias towards the $\phi' = -120°$ eclipsed conformer is consistent with X-ray data. Thus, in Sundaralingam's review (18), we note that for 3′-nucleotides (five entries) the ϕ' values lie in the range from -91 to $-165°$, with one entry, 3′AMP, having a ϕ' value of $-123°$, just 3° from the $H_{3'}$–P eclipsed situation.

In general, the $^3J_{P3'}$ values for the Up- units are considerably larger than those measured for dTp- units (6.8 Hz for dTp- of 3′,5′-d(TpT) (4) and 6.1–6.4 Hz for the dTp- units of 3′,5′-d(TpTp) (16) and 3′,5′-d(TpTpA) (1)). Thus, the bias towards the eclipsed conformer is less pronounced in the 2′-deoxyribo units ($\theta \simeq 44°$; $\phi' = -76, -164°$). This greater bias for $\phi' = -120°$ in the ribo units is most likely due to steric interactions involving the 2′-hydroxyl group. Support for this contention is provided by the observation (28) that 2′-*O*-methylation of the Ap- unit of ApA results in an increase of 1.4 Hz in $^3J_{P3'}$.

The similarity in the $^3J_{P3'}$ values suggests that the ϕ' conformer distribution is similar for the Up- units of 3′,5′-UpdT, and 3′,5′-UpU and for 3′UMP itself. This suggestion is supported by the chemical shift data. In the $g^-(\phi')$ conformer $H_{4'}$ is near to the charged nonester oxygens of the phosphate while $H_{2''}$ is distant; in $t(\phi')$, the converse is true. Thus, changes in the $g^-(\phi') \rightleftarrows t(\phi')$ blend should be manifest in $\delta_{2''}$ and $\delta_{4'}$. However, in Table 1 we note a remarkable constancy for these species in, on the one hand, $\delta_{2''}$, and, on the other hand, $\delta_{4'}$, which is inconsistent with major differences in the conformer weighting.

Conformation about the $O_{3'}$—P and $O_{5'}$—P Bonds

Our measured coupling constants cannot provide information on the orientations about the internucleoside O—P bonds. Recently, however, variations in the $H_{5'}$ shift of the 5′-unit of 3′,5′-ribodimers have been related (3) to the proximity of $H_{5'}$ to the 2′-hydroxyl of the 3′-unit. When the $O_{3'}$—P and $O_{5'}$—P bond are g^-, as in the right handed (g^-g^-) stacked conformation, van der Waals contact between these atoms is possible and appears to lead to a specific deshielding of $H_{5'}$ (3). These effects are best discussed in terms of the dimerization shifts, Δ, defined as the shift of a proton in the dimer relative to its shift in the corresponding mononucleotide, with positive values indicating increased shielding at the dimer level. For the -pdT fragments we obtain from the low temperature data in Table 2: $\Delta_{5'}$ ($\Delta_{5''}$ in brackets) in ppm: -0.10 (-0.04), -0.19 (-0.07), -0.02 (-0.03) for 3′,5′-d(TpT), 3′,5′-UpdT, and 2′,5′-UpdT, respectively. For 3′,5′-UpdT a significant deshielding effect upon dimerization is noted for the $H_{5'}$, that for $H_{5''}$ being greatly attenuated. Following the reasoning outlined (3), we conclude that $H_{5'}$ is located in the vicinity of the 2′-OH of the Up- unit for a significant fraction of the time and

therefore, that the g^-g^- conformer makes a significant (but quantitatively undeterminable here) contribution to the time-averaged conformation of 3′,5′-UpdT. It is important to point out, however, that a large (ca −0.10 ppm) $\Delta_{5'}$ value is observed for -pdT of 3′,5′-d(TpT), but the effect is certainly smaller than that noted for the mixed dimer. Clearly factors other than the proximity of the 2′-OH of the 3′-unit can contribute to the $\Delta_{5'}$ values and thus the interpretation of these Δ values in quantitative terms will be difficult. Interestingly, the $\Delta_{5'}$ and $\Delta_{5''}$ values are small (less than −0.03 ppm) for the 2′,5′-isomer. In the g^-g^- conformer of a 2′,5′-dimer $H_{5'}$ does not approach any position of the furanose moiety of the 3′-unit and thus the appearance of this conformer may not be reflected in the $\Delta_{5'}$. However, with molecular models one may construct a compact, g^-g^- stacked arrangement of a 2′,5′-dimer in which $H_{5'}$ is placed in juxtaposition with the magnetically anisotropic 2-keto oxygen of the pyrimidine base on the 3′-unit. The minimal value of $\Delta_{5'}$ for 2′,5′-UpdT cannot, therefore, be unambiguously interpreted; it could be a reflection of the virtual exclusion of the g^-g^- arrangement or it may be a consequence of an insensitivity of $\Delta_{5'}$ to the orientation of the O—P bonds.

Comparison of some other chemical shift data (Table 1) provides some evidence for base-base interaction in the 2′,5′-dimer. Thus, in particular, the large (0.17 ppm) upfield shift (relative to 3′UMP) experienced by the 5-hydrogen of the uracil base is only reasonably explained if some stacking of the U and T bases occurs. For the 3′,5′-isomer an upfield shift (0.06 ppm) for the H_5 hydrogen of the uracil is observed, in line with the evidence given above for some contribution from the g^-g^- conformer. That the H_5 dimerization shift is larger for the 2′,5′-UpdT isomer could be a consequence of more extensive base stacking as well as to alternations in the spatial arrangement of the bases in the stack.

Conclusions

The ^{1}Hmr data of mixed dimers 3′,5′-UpdT and 2′,5′-UpdT are compared with data for the component mononucleotides, 3′UMP, 2′UMP, and 5′dTMP, and for the dimers 3′,5′-UpU and 3′,5′-d(TpT), and discussed in terms of their molecular conformations in aqueous solution. For the greater part the conformations (sugar pucker and the $C_{4'}$—$C_{5'}$, $C_{5'}$—$O_{5'}$, and $C_{3'}$—$O_{3'}$ conformer distributions) of the 3′- and 5′-linked dimer units are similar to those of the corresponding mononucleotides. Furthermore, the sugar phosphate conformation of a particular nucleotidyl unit (3′- or 5′-linked) does not appear to be critically dependent upon the nature (ribo or 2′-deoxyribo) of the other unit. This conclusion is, of course, restricted to our dipyrimidine dimers which exist to a large extent in an extended rather than stacked orientation.

Acknowledgements

This research was supported by operating grants from the National Research Council of Canada and Rh Institute of Manitoba. We thank the NRCC, the University of Manitoba, and its Faculty of Graduate Studies for capital grants for purchase of a Bruker WH-90 spectrometer. We thank Dr. A. A. Grey of the Canadian 220 MHz centre and Dr. W. A. Gibbons of the University of Wisconsin (Madison) for obtaining 220 MHz and 270 MHz spectra, respectively. We are grateful to Dr. Donald Wood for his generous aid in the spectral analysis, and to Mr. A. George and W. Niemczura for obtaining spectra for 2′UMP.

1. C. Altona, J. H. Van Boom, and C. A. G. Haasnoot. Eur. J. Biochem. **71**, 557 (1976).
2. C. Chachaty, T. Yokono, T.-D. Son, and W. Guschlbauer. Biophys. Chem. **6**, 151 (1977).
3. C.-H. Lee, F. S. Ezra, N. S. Kondo, R. H. Sarma, and S. S. Danyluk. Biochemistry, **15**, 3627 (1976).
4. F. S. Ezra, N. S. Kondo, C. F. Ainsworth, and S. S. Danyluk. Nucl. Acid Res. **3**, 2549 (1976).
5. N. S. Kondo and S. S. Danyluk. Biochemistry, **15**, 756 (1976).
6. F. S. Ezra, C. H. Lee, N. S. Kondo, S. S. Danyluk, and R. H. Sarma. Biochemistry, **16**, 1977 (1977).
7. A. L. Lehninger. Biochemistry. 2nd ed. Worth Publishers, New York, NY. 1975. p. 909.
8. J. C. Maurizot, J. Brahms, and F. Eckstein. Nature, **222**, 559 (1969).
9. N. S. Kondo, K. N. Fang, P. S. Miller, and P. O. P. Ts'O. Biochemistry, **11**, 1991 (1972).
10. Y. Lapidot and H. G. Khorana. J. Am. Chem. Soc. **85**, 3852 (1963).
11. C. W. Haigh and J. M. Williams. J. Mol. Spectrosc. **32**, 398 (1969).
12. D. J. Wood, F. E. Hruska, and K. K. Ogilvie. Can. J. Chem. **52**, 3353 (1974).
13. D. J. Wood, K. K. Ogilvie and F. E. Hruska. Can. J. Chem. **53**, 2781 (1975).
14. R. G. S. Ritchie and A. S. Perlin. Carbohydr. Res. **55**, 121 (1977).
15. M. Remin and D. Shugar. Biochem. Biophys. Res. Commun. **48**, 636 (1972).
16. D. B. Davies and A. Rabczenko. J. Chem. Soc. Perkin Trans. II, 1703 (1975).
17. J. Prestegard and S. I. Chan. J. Am. Chem. Soc. **91**, 2843 (1969).
18. M. Sundaralingam. *In* Proceedings international symposium on the conformation of biological molecules and polymers, symposia on quantum chemistry and biochemistry. Vol. 5. *Edited by* B. Pullman and E. D. Bergmann. Academic Press, Jerusalem. 1973. p. 417.
19. H. Dugas, B. J. Blackburn, R. K. Robins, R. Des-

LAURIERS, and I. C. P. SMITH. J. Am. Chem. Soc. **93**, 3468 (1971).
20. J. CADET, R. DUCLOMB, and C. TAIEB. Tetrahedron Lett. 3455 (1975).
21. C. ALTONA and M. SUNDARALINGAM. J. Am. Chem. Soc. **95**, 2333 (1973).
22. D. B. DAVIES and S. S. DANYLUK. Biochemistry, **14**, 543 (1975).
23. T. SCHLEICH, B. J. BLACKBURN, R. D. LAPPER, and I. C. P. SMITH. Biochemistry, **11**, 137 (1972).
24. F. E. HRUSKA. *In* Proceedings international symposium on the conformation of biological molecules and polymers, symposia on quantum chemistry and biochemistry. Vol. 5. *Edited by* B. Pullman and E. D. Bergmann. Academic Press, Jerusalem. 1973. p. 345.
25. C. H. LEE and R. H. SARMA. Biochemistry, **15**, 697 (1976).
26. R. H. SARMA, R. J. MYNOTT, D. J. WOOD, and F. E. HRUSKA. J. Am. Chem. Soc. **95**, 6457 (1973).
27. P. J. COZZONE and O. JARDETZKY. Biochemistry, **15**, 4860 (1976).
28. H. SINGH, M. H. HERBUT, C. H. LEE, and R. H. SARMA. Biopolymers, **15**, 2167 (1976).