teraction for Me_2SO. Thus, while the magnitudes of the dipoles is the same for MeCN and Me_2SO, the actual distribution of the net atomic charges produces stronger interactions for Me_2SO. This is qualitatively revealed by the net atomic charges on the "electron donor" end of the molecules; the N atom in MeCN has only 0.185 negative charges, while the O atom of Me_2SO has 0.441 negative charges.[27]

Domain, Rinfret, and Benoit[28] on the basis of calorimetric measurements of the enthalpy of the reaction, in which gaseous HCl is dissolved in the given liquid solvent, have estimated the transfer enthalpies of the proton from the gas phase to the liquid solvent, $\Delta H_{tr}^{g\rightarrow Sl}(H^+)$. Their results are as follows: H_2O, -270.0; Me_2SO, -276.1; MeCN, -256.6 (all in kcal/mol). We have pointed out earlier[29] that the stepwise solvation enthalpies $(n-1,n)$ can on principle also be used for estimates of $\Delta H_{tr}^{g\rightarrow Sl}$ (ion). This can be done with eq 8, where $\Delta H_{n-1,n}(Ion(Sl)_n)$ are the

$$H_{tr}^{g\rightarrow Sl}(Ion) = \sum_{n=1}^{n=n}\Delta H_{n-1,n}(Ion(Sl)_n) - \sum_{n=2}^{n=n}\Delta H_{n-1,n}(Sl)_n \quad (8)$$

stepwise enthalpies for the ion solvent cluster while $\Delta H_{n-1,n}(Sl)_n$ are stepwise enthalpies for the neutral solvent cluster. Since for n large ("large" may mean less than 10 or 20) $\Delta H_{n-1,n}(Ion(Sl)_n) \approx \Delta H_{n-1,n}(Sl)_n$, one needs to know only the first several stepwise enthalpies for the ion and the neutral cluster to obtain an insight into the ion solvation energy. Unfortunately data for the neutral Me_2SO clusters are completely absent. More information is available for water from calculations.[30] As a rough approximation,[30] for $n = 1$ to $n = 3$, one can replace $\Delta H_{n-1,n}(Sl)_n$ with $-{}^1/_2\Delta H_{evap}(Sl)$, bearing in mind that for small n, $\Delta H_{n-1,n}(Sl)_n$ is much smaller than $-\Delta H_{evap}(Sl)$. With use of the available three $\Delta H_{n-1,n}$ in Table II for H_2O and Me_2SO and $\Delta H_{evap}(H_2O) = 10.5$ kcal/mol and $\Delta H_{evap}(Me_2SO) = 13.7$ cal/mol one obtains

$$\Delta H_{0,3}(H^+(H_2O)_n) + {}^2/_2\Delta H_{evap}(H_2O) = -212.4 \text{ kcal/mol}$$

$$\Delta H_{0,3}(H^+(Me_2SO)_n) + {}^2/_2\Delta H_{evap}(Me_2SO) = -252.9 \text{ kcal/mol}$$

Comparing this result with Benoit's values $\Delta H^{g\rightarrow H_2O}(H^+) = -270$ kcal/mol and $\Delta H^{g\rightarrow Me_2SO}(H^+) = -276.1$ kcal/mol, one finds that the first three solvent molecules account for ~80% of the total solvation of H^+ in water and for 92% of the same quantity for Me_2SO. As expected, the interactions with the first few solvent molecules make a preponderant contribution. Furthermore, for water where only 80% of the total is accounted, interactions with the ion will be contributing to much higher n than for Me_2SO. This difference of behavior is easily understood. Three-dimensional hydrogen bonding and the smallness of the water molecules contribute to strong net interactions of the ion with solvent molecules even at large n. On the other hand for Me_2SO, even if linear dipole chains like that in structure III do occur for ion clusters with large n, the large bulk of the Me_2SO molecules will prevent efficient stacking near the ion and thus lead to a relatively rapid fall off for the net binding energies to the ion. Comparing the large proton affinity difference of -42.7 kcal/mol between Me_2SO and H_2O with the modest difference of -6 kcal/mol for the transfer enthalpies of the proton of liquid Me_2SO and H_2O, one comes to the conclusion, that the greater solvation of the proton in liquid Me_2SO is completely due to the very much higher proton affinity of Me_2SO. While the initial two interactions $\Delta H_{1,2}$ and $\Delta H_{2,3}$ for Me_2SO and H_2O are of comparable magnitude, the net effect of all the higher n interactions is one of much poorer solvation by Me_2SO as compared to water.

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The Solution Conformational Preferences of the Sugar and Sugar Phosphate Constituents of RNA and DNA

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Abstract: The analysis of the proton NMR spectra of stereospecifically deuterated (*S*)-tetrahydrofuranmethanol, methyl β-D-ribofuranoside, methyl β-D-2-deoxyribofuranoside, and the 5-phosphates of the sugar acetals leads to several important and previously unrecognized observations about the determinants of the solution conformations of nucleosides and nucleotides: (1) in the unsubstituted sugars and their phosphates, the preferred furanoside ring conformation is the 3′-endo pucker and not the 2′-endo pucker observed in most nucleosides and nucleotides; (2) in the unsubstituted sugars and their phosphates, the gauche–gauche rotamer about the C_4–C_5 bond does not predominate as is found in the nucleosides and nucleotides but rather the two rotamers in which the vicinal C–O bonds are gauche to one another (gauche–gauche and gauche–trans) are about equally favored; (3) the observed predominant occurrence of the gauche–gauche rotamer about the C_4–$C_{5'}$ bond in nucleosides and nucleotides is presumably the result of an interaction between the substituent on $C_{5'}$ and the heterocyclic base, which is made more favorable by a shift in the furanoside ring conformation; and (4) the rotameric distribution about the C_5–O_5 bonds in the sugar acetal phosphates is the same as that observed in nucleosides and nucleotides. These observations suggest that the nature of the furanoside ring conformation and the rotameric distributions about the C_4–$C_{5'}$ and C_5–$O_{5'}$ bonds may not be as intimately interdependent as previously believed.

NMR spectroscopy has permitted the determination of the conformational preferences of a large number of nucleosides, mononucleotides, and oligonucleotides in aqueous solution.[2] The most widely used experimental method has been to measure the vicinal coupling constants (J_{HCCH} and J_{POCH}) and relate these to the values of the intervening dihedral angle by application of the appropriate Karplus equation. Although there has been some disagreement as to the precise values of the parameters to be used in the Karplus equation, the results obtained by a large number of investigators are in qualitative agreement. In almost all nucleosides and mononucleotides, the furanoside ring has been observed to prefer the 2′-endo puckered conformation and the rotameric distribution about both the C_4–$C_{5'}$ and C_5–$O_{5'}$ bonds favors the gauche–gauche conformations. Determination of the

(1) NIH Research Career Development Awardee, CA-00499, 1978-1983.
(2) For an excellent recent review of the voluminous literature, see D. B. Davies, *Prog. Nucl. Magn. Reson. Spectrosc.*, **12**, 135 (1978).

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orientation of the heterocyclic base relative to $C_{5'}$ has proven to be much more difficult; however, it is generally agreed that both the purine and pyrimidine bases prefer the anti conformation. On the basis of these conformational preferences, various proposals have been advanced about the interrelationships of the conformational features and the "rigidity"[3] or "flexibility"[4] of the monomeric units when they are incorporated into the more complex oligo- and polynucleotide structures.

Curiously, an examination of the literature on the conformations of nucleosides and nucleotides reveals that there are essentially no data available concerning the preferred solution conformations of either furanosides or furanoside phosphates which are not substituted at C_1 with a heterocyclic base. There are two probable explanations for this lack of rather basic and important conformational information. First, the simplest nucleoside and nucleotide analogues, 1-amino-β-D-ribofuranoside and its 2-deoxy analogue and their 5-phosphates, are unstable in aqueous solution.[5] Second, the NMR spectra of ribose and 2-deoxyribose are exceedingly complicated, even at high field, due to the fact that these exist in aqueous solution as mixtures of furanoside and pyranoside forms and of α- and β-anomers.[6] Although only the furanoside forms are possible for the 5-phosphates of these sugars, their spectra are also complex due to the mixture of anomers.[6] Levitt and Warshel recently reported the results of an examination of the energetics of furanose ring puckering in 1-amino-β-D-ribofuranoside and the 2-deoxy analogue.[7] Their method was theoretical, using a molecular mechanics program which calculated conformational energy as the sum of the usual potential energy contributions. The molecular coordinates which they considered were constrained to be those which described the pseudorotational pathway of the furanoside ring. Their results indicated in these model compounds that (1) the 3'-endo conformation is about 0.2 kcal/mol more stable than the 2'-endo conformation, (2) other conformations were energetically feasible, and (3) the energy barrier to pseudorotation is low. Thus, the most flexible component of nucleotide conformation appears to be the pseudorotational pathway of the furanoside ring; attempts to calculate the conformational preferences of nucleotides should consider this previously neglected conformational determinant.

In order to provide experimental data regarding the conformational preferences of the furanoside rings in nucleic acids, chemically and configurationally stable models are required. Methyl β-furanosides fulfill this requirement. Although these compounds do not possess nitrogen functionality at C_1, comparison of the experimentally determined conformational preferences with those predicted for the 1-amino furanosides should suggest whether this structural difference is an important factor in determining the conformations of nucleotides. In addition, the data obtained for the methyl furanosides should provide a more detailed understanding of the interrelationships between the conformational degrees of freedom in the more complex derivatives. The structures of the methyl β-furanosides, their 5-phosphates, and the specifically deuterated (*S*)-tetrahydrofuranmethanol used in this study for chemical shift assignments are shown in Figure 1.

An analysis of the vicinal coupling constants for methyl β-D-ribofuranoside, methyl β-D-2-deoxyribofuranoside, and their 5-phosphates reveals in contrast to nucleosides and nucleotides that the furanoside rings of these molecules do not prefer the 2'-endo conformation and that the rotameric distribution about the C_4–C_5 bond does not favor the gauche–gauche rotamer. However, the rotameric distribution about the C_5–O_5 bond in the phosphates is the same as that found in nucleotides.

Experimental Section

^{1}H NMR spectra were recorded at 30 °C and 270 MHz by using the Bruker spectrometer of the Southern New England High Field NMR Facility at Yale University; this instrument is equipped for simultaneous proton and phosphorus decoupling capabilities. Chemical shifts are reported in parts per million and are referenced relative to internal DSS. Melting points were obtained in open capillary tubes with a Hoover melting point apparatus and are corrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter at ambient temperature. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

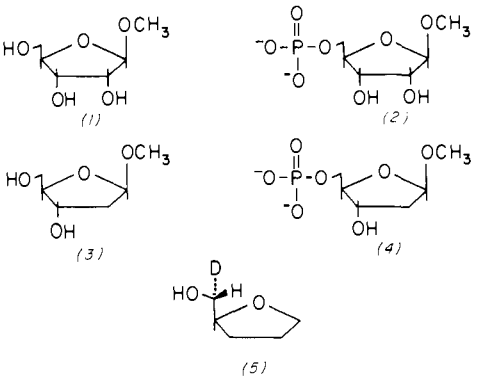

Figure 1. Structures of the compounds studied by NMR. The compounds shown are: (**1**) methyl β-D-ribofuranoside; (**2**) methyl β-D-ribofuranoside 5-phosphate; (**3**) methyl β-D-2-deoxyribofuranoside; (**4**) methyl β-D-2-deoxyribofuranoside 5-phosphate; (**5**) (*S*)-tetrahydrofuranmethanol-d_{5R}.

D-Ribose, racemic tetrahydrofuranmethanol, and diphenyl phosphorochloridate were purchased from Aldrich. Horse liver alcohol dehydrogenase and D-2-deoxyribose were obtained from Sigma. Chromatopure oxidized NAD^+ was the product of P-L Biochemicals. Ethanol-d_6 (95% in D_2O, 99% *d*) was obtained from Stohler Isotope Chemicals. Deuterium oxide was from Merck, Sharp, and Dohme. All other reagents were the best grades commercially available.

Methyl β-D-Ribofuranoside and Methyl β-D-Ribofuranoside 5-(Bis(cyclohexylammonium) phosphate) were prepared according to the literature procedures[8,9] and were the kind gift of Mr. Jeffrey A. Coderre of this laboratory.

Methyl β-D-2-Deoxyribofuranoside. Methyl β-D-2-deoxyribofuranoside 3,5-bis(4-nitrobenzoate) was prepared according to the procedure of Ness et al.[10] The anomeric configuration of this material was assigned on the basis of its negative optical rotation. The 4-nitrobenzoate groups were removed in methanolic sodium methoxide, and after completion of the reaction, as judged by TLC, the basic solution was neutralized with Amberlite IR-120 (H^+). After removal of the ion exchange resin and solvent, the residue was suspended in water and the crystalline methyl 4-nitrobenzoate was removed by filtration. Evaporation of the solvent afforded a pale yellow syrup which was not subjected to further purification. On the basis of the 270-MHz proton NMR spectrum, this material was judged to be anomerically pure. The anomeric configuration of this material was judged to be β, based on its significantly negative optical rotation ($[\alpha]_{5893}$ 72° (*c* 1.03, H_2O)).

Methyl β-D-2-Deoxyribofuranoside 5-(Bis(triethylammonium) phosphate). The methyl β-D-2-deoxyribofuranoside was phosphorylated with diphenylphosphorochloridate according to the procedure described for phosphorylation of methyl β-D-ribofuranoside.[9] The oily diphenyl ester was hydrogenolyzed in ethanolic solution in the presence of Adams' catalyst. After removal of the catalyst, the acidic solution was neutralized with cyclohexylamine. The residue resulting from evaporation of the solvent resisted attempts at purification by crystallization. This material was applied to a column of DEAE-Sephadex A-25 (HCO_3^-), and the product was eluted with a linear gradient of 0 to 0.4 M triethylammonium bicarbonate. The fractions containing the monoester, as judged by total phosphate analyses of the fractions, were pooled and concentrated. On the basis of its proton NMR spectrum, this material was the desired phosphate ester.

(*S*)-Tetrahydrofuranmethanol. A racemic mixture of tetrahydrofuranmethanol was resolved according to the literature procedure by fractional crystallization of the brucine salt of tetrahydrofuranmethanol hydrogen phthalate.[11] The phthalate was decomposed by base-catalyzed

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hydrolysis to yield the dextrarotatory ($[\alpha]_{5893}$ 1.85° (neat)) (*S*)-tetrahydrofuranmethanol.

(*S*)-Tetrahydrofuranmethanol-d_{5R}. (*S*)-Tetrahydrofuranmethanol (0.94 g, 0.009 mol) and 1.98 mL (0.019 mol) of ethanol-d_6 were dissolved in 150 mL of 0.032 M sodium pyrophosphate, pH 8.8, prepared in D_2O. Horse liver alcohol dehydrogenase (about 8 mg) and 35 mg of NAD^+ were added, and the solution was stirred at room temperature. The enzyme was observed to slowly precipitate, and after removal of the precipitated protein, an additional 5 mg of enzyme was added. This process was repeated until integration of the proton NMR spectrum of the reaction mixture indicated that equilibration of ethanol deuterium with the tetrahydrofuranmethanol hydrogen was complete. After 12 days, the protein was removed by heating the reaction mixture in a boiling water bath for 5 min followed by centrifugation. The alcohols were salted out of solution by addition of 157 g of anhydrous K_2CO_3 and then extracted into ether.[12] Evaporation of the ether and ethanol yielded 0.29 g (0.0028 mol) of (*S*)-tetrahydrofuranmethanol-d_{5R}.

(*S*)-Tetrahydrofuranmethanol-d_{5R} Bis(cyclohexylammonium) Phosphate. The deuterated alcohol was phosphorylated with diphenylphosphorochloridate in pyridine solution. The product was partitioned between ether and dilute aqueous hydrochloric acid solution, and the ether solution was washed with water and 5% sodium bicarbonate solution. After removal of the solvent, the oily residue was hydrogenolyzed in absolute ethanol in the presence of Adams' catalyst. After removal of the catalyst, the acidic solution was neutralized with cyclohexylamine, and the precipitated bis(cyclohexylammonium) salt was recrystallized from absolute ethanol. A satisfactory elemental analysis could not be obtained for this material, presumably due to the loss of cyclohexylamine during drying in vacuo.

NMR Spectroscopy. Proton NMR spectra were obtained on 0.010 M samples of the furanosides and their phosphates; the pH of each phosphate ester sample was adjusted to pH 9 with sodium deuteroxide to assure complete ionization of the phosphate. The resonances were assigned on the basis of selective proton decoupling experiments by assuming that the resonance for the anomeric proton would be the farthest downfield. For methyl β-D-ribofuranoside and its 5-phosphate, coupling constants and chemical shifts were derived by iterative fitting of the spectra, using the program LACX. For methyl β-D-2-deoxyribofuranoside and its 5-phosphate, coupling constants and chemical shifts could be obtained directly by a first-order analysis of the NMR spectrum as evidenced by comparison of the spectrum computed with LAOCN3 and the actual spectrum.

Results and Discussion

Tetrahydrofuranmethanol. Since alcohol dehydrogenase is known to catalyze the oxidation of primary alcohols by abstraction of the pro-*R* hydrogen, preparation of stereospecifically deuterated alcohols is possible by equilibration of the unlabeled alcohol with deuterated ethanol in the presence of small amounts of NAD^+ and enzyme.[13] Using this approach, we synthesized (*S*)-tetrahydrofuranmethanol-d_{5R} so that assignment of the proton resonances of the primary alcohol carbon would be possible (it is necessary to perform this equilibration on the optically active alcohol since the pro-*R* hydrogens in the two enantiomers are not magnetically equivalent). The primary alcohol hydrogen portions of the proton NMR spectra of racemic tetrahydrofuranmethanol and (*S*)-tetrahydrofuranmethanol-d_{5R} are shown in Figure 2. Integration of the ABX spectra reveals that the upfield hydrogen was exchanged by alcohol dehydrogenase, allowing this proton to be assigned as the pro-*R* hydrogen in the chiral alcohol. The coupling constants for these protons to the methine hydrogen in the tetrahydrofuran ring are $J_{H_R,H} = 6.5$ Hz and $J_{H_S,H} = 3.5$ Hz. Application of the Karplus equation used for estimation of the rotameric distribution about the C_4–$C_{5'}$ bond in nucleosides[2] gives the following relative populations of the three possible rotamers about the exocyclic C–C bond: gauche–gauche, 0.40; gauche–trans, 0.45; trans–gauche, 0.15 (Table I). Thus, in this simplest analogue of the tetrahydrofuran ring in nucleosides, the two rotamers in which the C–O bonds are gauche are favored.

The observed rotameric distribution about the exocyclic C–C bond in tetrahydrofuranmethanol demonstrates that the rotameric distribution about this bond cannot be described in terms of a symmetrical threefold torsional potential of the form

$$E_{tors} = V_3/2(1 + \cos 3\omega)$$

where V_3 is the energy barrier separating the three stable rotamers and ω is the dihedral angle describing the rotation about the bond. Rather, the description of the torsional potential should include an additional term

$$E_{tors} = V_2/2(1 - \cos 2\omega) + V_3/2(1 + \cos 3\omega)$$

where the presence of the additional V_2 potential allows description of the observation that the gauche rotamers predominate. Given the rotameric distribution observed for tetrahydrofuranmethanol, the appropriate value of V_2 to be included is about −1.0 kcal/mol. The magnitude of the V_2 potential deduced from these experimental data is in close agreement with that deduced for the conformational equilibrium of 5-methoxytrimethylene phosphate in aqueous solution.[14] At the present time the origin of this

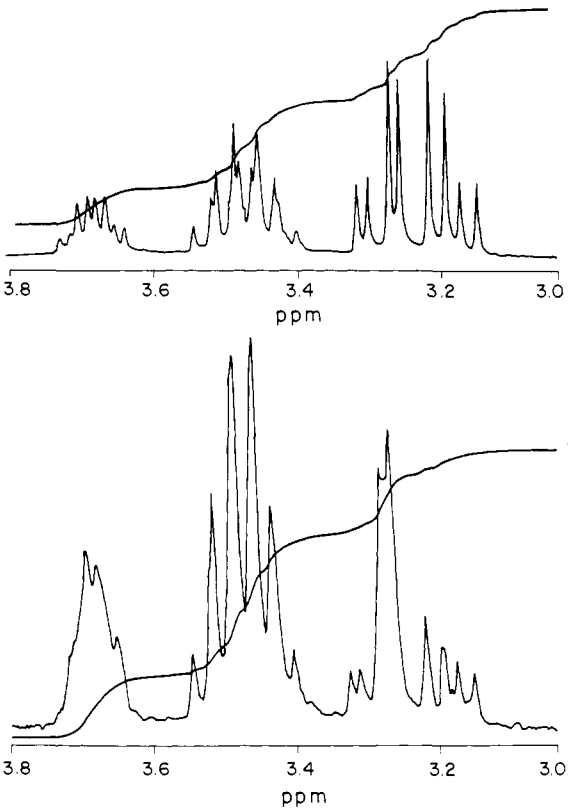

Figure 2. 270 MHz ^1H NMR spectra of (top) (*S*)-tetrahydrofuranmethanol and (bottom) (*S*)-tetrahydrofuranmethanol-d_{5R} in D_2O. From the integral the extent of deuteration is 61%.

Table I. Coupling Constants[a] and Rotameric Distributions[b] about the C_4–C_5 Bonds

compd	$J_{4,5R}$	$J_{4,5S}$	P_{gg}	P_{gt}	P_{tg}	V_2[c]
tetrahydrofuranmethanol	6.5	3.5	0.40	0.45	0.15	−1.0
methyl β-D-ribofuranoside	6.6	3.3	0.38	0.44	0.18	−0.9
methyl β-D-ribofuranoside 5-phosphate	6.3	4.2	0.38	0.39	0.23	−0.7
methyl β-D-2-deoxyribofuranoside	7.0	4.3	0.32	0.45	0.23	−0.7

[a] In Hz; estimated uncertainty ±0.4 Hz. [b] Calculated from the equations $1.3P_{gg} + 2.7P_{gt} + 11.7P_{tg} = J_{4,5R}$, $1.3P_{gg} + 11.5P_{gt} + 5.8P_{tg} = J_{4,5S}$, and $P_{gg} + P_{gt} + P_{tg} = 1$. P_{gg} is the mole fraction of the gauche–gauche rotamer, P_{gt} is the mole fraction of the gauche–trans rotamer, and P_{tg} is the mole fraction of the trans–gauche rotamer. [c] Potential parameter for representation of the occurrence of gauche–gauche and gauche–trans rotamers; in kcal/mol.

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Table II. Coupling Constants[a] for Methyl β-D-Ribofuranoside, Methyl β-D-2-Deoxyribofuranoside, and Their 5-Phosphates

compd	$J_{1,2}$	$J_{1,2'}$	$J_{2,3}$	$J_{2',3}$	$J_{3,4}$	$J_{4,5R}$	$J_{4,5S}$	$J_{P,5}$
methyl β-D-ribofuranoside	0.8		4.7		6.5	6.6	3.3	
methyl β-D-ribofuranoside 5-phosphate[b]	1.6		5.0		6.1	6.3	4.2	5.2, 5.6
methyl β-D-2-deoxyribofuranoside	2.5	5.8	6.6	6.2	4.1	7.0	4.3	
methyl β-D-2-deoxyribofuranoside 5-phosphate[b]	2.9	5.1	6.6	5.7	4.0	Σ = 11.8		Σ = 11.8

[a] In Hz; estimated uncertainty ±0.4 Hz. [b] No coupling between P and H_4 could be detected.

phenomenological V_2 potential is uncertain. Experiments currently in progress in this laboratory are determining whether the magnitude of this potential is solvent dependent, as was found in the case of 5-methoxytrimethylene phosphate, or is caused by a "gauche effect"[15] inherent to this arrangement of polar bonds.

In any event, the observation of this V_2 potential in tetrahydrofuranmethanol (and in the methyl furanosides and their phosphates to be discussed later in this section) does suggest that molecular mechanics calculations directed toward predicting the conformations of nucleosides and nucleotides in aqueous solution should employ such a potential energy contribution.

The rotameric distribution about the C_4–C_5 bond in the phosphorylated alcohol might also be expected to show a similar need for a V_2 potential to accurately describe its behavior in aqueous solution. Our attempts to demonstrate a preference for the gauche rotamers in this phosphate monoester have thus far been unsuccessful due to coincidences of resonances in the spectrum at 270 MHz.

Methyl β-D-Ribofuranoside and Its 5-Phosphate. The proton NMR spectra of methyl-β-D-ribofuranoside and its 5-phosphate are shown in Figure 3; the vicinal proton coupling constants derived for these molecules by use of the computer program LACX are listed in Table II.

For the vast majority of ribonucleosides and monoribonucleotides that have been examined by NMR methods, it has been found that the sum of $J_{1',2'}$ and $J_{3',4'}$ is approximately equal to 10 Hz and that $J_{1',2'}$ is larger than $J_{3',4'}$.[2] Davis and Danyluk have proposed that the equilibrium constant between the 2′-endo and 3′-endo conformational families may be represented by the ratio $J_{1',2'}/J_{3',4'}$.[16] Therefore, most ribonucleosides and nucleotides prefer the 2′-endo sugar puckering. Inspection of the data in Table II for the ribofuranoside and its phosphate indicates that $J_{1,2}$ is much smaller than $J_{3,4}$, suggesting that in these molecules the sugar prefers the 3′-endo type of puckering. Thus, the introduction of the heterocyclic base causes a shift in the sugar puckering from 3′-endo to 2′-endo. In the case of the ribofuranoside and its phosphate, the sum of $J_{1,2} + J_{3,4}$ equals approximately 7 Hz, a value significantly smaller than that found in nucleosides and nucleotides; this difference is presumably the result of the presence of the more electronegative oxygen substituent at C_1 in the sugars. (The small value for this sum cannot be explained by unusual flattening or puckering of the ring, since the value for $J_{2,3}$ is normal.) The percentage of 3′-endo in these compounds is found to be about 60% with the use of the value of $J_{3,4}$ as a measure of the 3′-endo contribution to the conformation. It should be noted that Levitt and Warshel calculated a similar conformational bias toward the 3′-endo pucker in 1-amino-β-D-ribofuranoside,[7] suggesting that our use of the methyl acetals does not influence the preferred sugar conformation. The conformational bias that we observed for these compounds implies a free-energy difference of only about 0.2 kcal/mol between the 3′-endo and 2′-endo puckers, a value which is in excellent agreement with that derived by Levitt and Warshel (0.2 kcal/mol).

In ribonucleosides and ribonucleotides, the free energy bias toward the 2′-endo pucker is about 0.4 kcal/mol. Thus, our data and those for the sugar substituted by a heterocyclic base indicate that the perturbation in pucker free energy is only about 0.6 kcal/mol. Apparently, an interaction between the base and

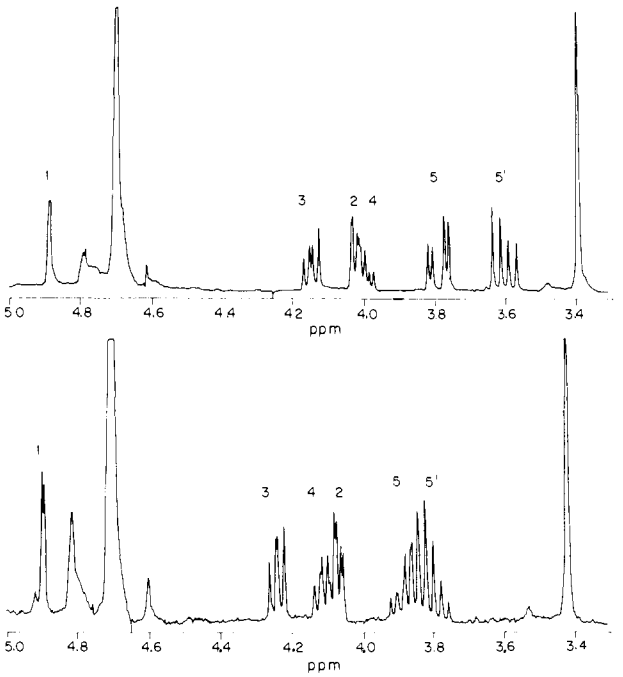

Figure 3. 270 MHz ^1H NMR spectra of (top) methyl β-D-ribofuranoside and (bottom) methyl β-D-ribofuranoside 5-phosphate in D_2O. The spectrum of the phosphate monoester is coupled to phosphorus.

substituent on $C_{5'}$ of the sugar is responsible for this free-energy difference. That this experimentally determined free-energy difference is not very large illustrates the conclusion made by Levitt and Warshel[7] that equilibration between the possible puckered conformations in sugar is facile and provides an important contribution to the preferred conformations of nucleosides and nucleotides.

The coupling constant analysis which we have employed in the treatment of our data considers the relative populations of the 2′-endo and 3′-endo *conformational families*. The work of Levitt and Warshel predicts that these families will be composed of a continuum of conformations which will have pseudorotational phase angles similar to those associated with the 2′-endo and 3′-endo conformations which describe the calculated energy minima. In principle it would be desirable to quantitate the range of pseudorotational angles which these families populate in solution; in practice this type of analysis is not now possible since it would require an accurate Karplus equation for each C–C bond and a smaller uncertainty in the experimentally determined coupling constants than is routinely possible. Thus, our data and conclusions can be used only to illustrate that in solution the energy difference between the 2′-endo and 3′-endo families is small and that it is sensitive to the presence of a heterocyclic base at C_1. A demonstration that the energy barrier to pseudorotational equilibration between these conformational families is low cannot be easily performed by NMR experiments, if the energy barrier calculated by Levitt and Warshel (0.5 kcal/mol) is correct.

A comparison between the values for $J_{4,5}$ and $J_{4,5'}$ found for the ribofuranosides and those reported for nucleosides and nucleotides indicates that the introduction of the heterocyclic base results in a significant change in the rotameric distribution about the C_4–C_5 bond of the sugar. The sum of the two vicinal proton coupling constants in nucleosides and nucleotides is about 6 Hz,[2]

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whereas the sums we observe for the ribofuranoside and its phosphate are each about 10 Hz. The increase in sum qualitatively indicates a substantial depopulation of the gauche–gauche rotamer about this bond as compared to the nucleosides and nucleotides. A tentative quantitative estimate of the rotameric distribution about this bond in our compounds can be obtained if we assume that the upfield resonance of the primary alcohol protons can be assigned to the pro-*R* proton of the D sugar as was experimentally found for *S*-tetrahydrofuranmethanol; based on the structural similarities of the ribofuranoside itself and the less substituted alcohol, this should be a reasonable assumption. From the fractional populations which are listed in Table I, we again find nearly equal populations of the two gauche rotamers and a smaller contribution by the rotamer in which the C–O bonds are trans. The actual distribution may correspond to a slightly smaller value for the V_2 potential required to describe this conformational preference than was necessary for the tetrahydrofuranmethanol; however, the uncertainty in the coupling constant analyses plays the major role in the accuracy of the estimate of this potential (the coupling constants have an estimated uncertainty of 0.4 Hz). From our data we can provide no detailed explanation for the change in rotameric distribution about the C_4–C_5 bond upon introduction of the base, although molecular mechanics calculations and X-ray crystal structure data suggest that the rotameric distributions about this bond and the glycosidic bond in nucleosides and nucleotides may be interdependent by virtue of electrostatic and/or hydrogen bonding interactions between the substituent on $C_{5'}$ and the base.

The observation that nucleosides and nucleotides prefer the gauche–gauche orientation about the C_4–$C_{5'}$ bond does not in itself constitute evidence that the torsional potential about this bond should include a V_2 contribution. We have performed molecular mechanics calculations which are able to predict the conformational preference about this bond with an accuracy consistent with the coupling constant estimates which are used to deduce the rotameric distribution.[6] The results which we have obtained indicate that small differences in the relative populations of the gauche–trans and trans–gauche rotamers occur when the results of calculations employing only a V_3 potential and employing both V_2 and V_3 potentials are compared. For example, in calculations with AMP, when only the V_3 potential is used, the relative populations of the three rotamers are predicted to be gauche–gauche, 0.64; gauche–trans, 0.23; and trans–gauche, 0.13; when both the V_2 potential deduced in this study and a V_3 potential are used, the relative populations of the three rotamers are predicted to be gauche–gauche, 0.72; gauche–trans, 0.24; and trans–gauche, 0.04. (In each case the base is predicted to be essentially exclusively anti.) Thus, evidence for use of a V_2 potential could be obtained from studies on nucleotides only by the ability to distinguish between the predicted small differences in the gauche–trans and trans–gauche rotamers, and it is doubtful that the coupling constants used to estimate these populations experimentally are known with sufficient accuracy to make such a distinction.

The proton NMR spectrum of the methyl ribofuranoside phosphate also permits evaluation of the coupling between the phosphorus nucleus and the protons on C_5 and, therefore, the preferred rotameric distribution about the C_5–O_5 bond. These coupling constants are essentially equal, and their sum is 10.8 Hz. From this sum and the equations developed by Sarma,[17] this bond prefers the trans conformation to the extent of about 70%, with the two possible gauche arrangements being equally favored and about 15% populated. Thus, even though the rotameric distribution about the C_4–C_5 bond and the sugar pucker differ in this molecule from that which is observed in ribonucleotides, the conformational preference about the C_5–O_5 bond is the same. Thus these three conformational degrees of freedom do not appear to be strongly interdependent.

Methyl β-D-2-Deoxyribofuranoside and Its 5-Phosphate. The proton NMR spectra of methyl β-D-2-deoxyribofuranoside and its 5-phosphate are shown in Figure 4; the vicinal proton coupling constants derived from these molecules by first-order analysis of the spectra are listed in Table II.

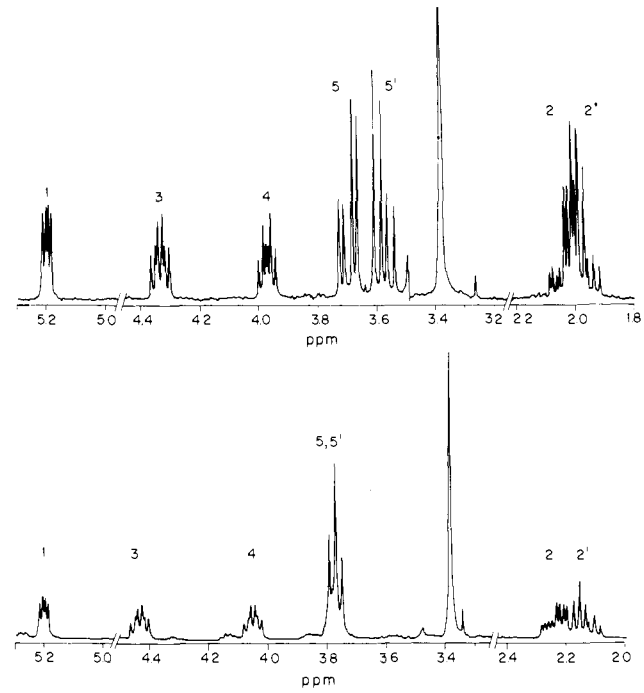

Figure 4. 270 MHz ^{1}H NMR spectra of (top) methyl β-D-2-deoxyribofuranoside and (bottom) methyl β-D-2-deoxyribofuranoside 5-phosphate in D_2O. The spectrum of the phosphate monoester is coupled to phosphorus.

An entirely consistent description of the sugar pucker is not as straightforward for the deoxyribofuranosides as was the situation for the ribo sugars. Whereas the values found for $J_{3,4}$ would suggest that the 2'-endo conformation is favored in these molecules (the observed value is 4 Hz as compared to the values of 6 Hz found in the previously discussed ribofuranosides), the values for $J_{2,3}$ and $J_{2',3}$ can be best explained only by predominant population of the 3'-endo pucker (the values for $J_{2,3}$ and $J_{2',3}$ are nearly identical) as can the values for $J_{1,2}$ and $J_{1,2'}$ (significantly different values). Thus, it is probable that the sugar ring does preferentially populate the 3'-endo type of pucker. If this description of the sugar pucker is correct, the protons on C_2 can be assigned, with the pro-*R* proton being downfield of the pro-*S* proton. The uncertainty associated with the assignment of the sugar pucker, which is caused by an unexpectedly small value for $J_{3,4}$, does not allow a precise estimate of the free-energy difference between the two types of pucker. (It should be noted that in 2'-deoxyribonucleosides and nucleotides, the preferred pucker is the 2'-endo type based on unequal values for $J_{2,3}$ and $J_{2',3}$ and nearly equal values for $J_{1,2}$ and $J_{1,2'}$.)

An analysis of the rotameric distribution based upon the chemical shift assignments described for tetrahydrofuranmethanol also allows the conclusion that the rotameric distribution about the C_4–C_5 bond in the deoxyfuranoside has similar populations of the gauche–gauche and gauche–trans rotamers and a smaller population for the trans–gauche rotamer (Table I). No estimate of the rotameric population about this bond can be made for the deoxyribofuranoside phosphate, since the chemical shifts of the methylene protons are identical; however, that the sum of the $J_{4,5}$ coupling constants is similar to that found for the unphosphorylated sugar suggests that a similar rotameric distribution is possible. Thus, at least in the case of the deoxy sugar, the rotational potential energy for this bond can best be described by consideration of a V_2 potential contribution.

The sum of the vicinal coupling constants between the phosphorus nucleus and the methylene protons may be evaluated from the spectrum of the deoxyribofuranoside phosphate and, as in the case of the ribo sugar, the predominant conformation about the C_5–O_5 bond is trans.

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Conclusions

The analysis of the NMR spectra of specifically deuterated tetrahydrofuranmethanol, methyl β-D-ribofuranoside, methyl β-D-2-deoxyribofuranoside, and the sugar acetal 5-phosphates has permitted some important conclusions to be reached about the solution conformations of nucleosides and nucleotides: (1) in the unsubstituted sugars and their phosphates, the preferred sugar pucker is the 3′-endo and not the 2′-endo, demonstrating that the heterocyclic base has an important influence on this flexible portion of the structure; (2) in the unsubstituted sugars and their phosphates, the gauche–gauche rotamer about the C_4–C_5 bond does not predominate as in the nucleosides and nucleotides, but rather the two rotamers in which the vicinal C–O bonds are gauche to one another are about equally favored, and this rotational distribution can be described by consideration of a phenomenological V_2 potential; (3) the observed predominant occurrence of the gauche–gauche rotamer about the C_4–$C_{5'}$ bond in nucleosides and nucleotides is presumably the result of an interaction between the substituent on $C_{5'}$ and the heterocyclic base, further favoring the gauche–gauche rotamer and which perhaps can occur only by a shift in the 2′-endo-3′-endo equilibrium; and (4) the rotameric distribution about the C_5–O_5 bonds in furanoside 5-phosphates is the same as is observed in nucleotides, even though the sugar ring pucker and rotameric distribution about the C_4–C_5 bond are different, indicating that these conformational degrees of freedom in nucleosides and nucleotides may not be intimately interdependent.

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Conformational Study of 1,2-Dimethylhexahydropyridazine by Variable-Temperature Photoelectron Spectroscopy[1]

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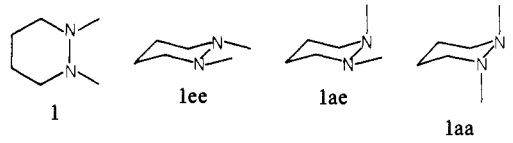
Abstract: Variable-temperature photoelectron spectroscopy (VTPES) measurements show that the diequatorial (lone pairs diaxial) conformation of 1,2-dimethylhexahydropyridazine is 1.20 ± 0.08 kcal/mol more stable than the axial,equatorial (lone pairs gauche) conformation. This energy difference is only 0.4 kcal/mol in solution, and the larger value in the vapor phase is attributed to the greater polarity of the axial,equatorial conformation.

Introduction

The photoelectron (PE) spectra of hydrazines show two low-energy ionization bands, caused by ionization from the symmetric and antisymmetric lone-pair combination orbitals. The separation of these bands (Δ) has been found to be sensitive to the lone-pair–lone-pair dihedral angle θ,[3,4] varying from about 2.3 eV when θ is near 0 or 180° and the lone-pair interaction is large to about 0.5 eV when θ is near 90°. The PE spectrum of 1,2-dimethylhexahydropyridazine (**1**) shows three bands in the lone-pair region,[3b,4b] two large ones separated by 2.3 eV, with a smaller band between them. The relatively rigid chair six-membered ring causes **1** to exist in conformations with well-defined θ, **lee** (θ ~ 180°), **lae** (θ ~ 60°), and (in principle) **laa** (θ ~ 60°), depending on the relative configurations of the two nitrogens. The large bands were assigned to conformation **lee** and the smaller band to the higher energy lone-pair ionization of **lae**/**laa**; the lowest energy **lee** and **lae**/**laa** bands overlap and are not resolved. When Gaussian curves were fitted to the PE spectrum of **1**, it was estimated that the bands due to **lee** were about three times as intense as the **lae**/**lee** peaks.[4c] Thus **lee** was claimed to predominate the conformational mixture for **1**, but the possibility of significantly different band size for different conformations precluded accurate determination of the relative amounts of conformations.



It was later shown by variable-temperature ^{13}C NMR work[5] that although **lee** does predominate in solution, K_{eq} = [**lee**]/[**lae**] is only about 1.4 at room temperature, with a $\Delta E°$ of −0.4 kcal/mol. In this paper we report measurement of the vapor phase $\Delta E°$, using the method of variable-temperature PE spectroscopy (VTPES),[6] so that the solution and vapor-phase conformational

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