Pseudorotation of the Ribofuranose Ring. A Theoretical Study and a Comparison with Nuclear Magnetic Resonance Results

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The results of energy calculations by means of the consistent force field method performed for methyl β-D-ribofuranoside are presented. Two regions of stable pentose conformations are predicted, other than those observed for the ribose moiety in most nucleosides. Local energy minima are associated with different conformations of the hydroxy and hydroxymethylene groups. Models of the molecule in solution are proposed, for which proton coupling constants are calculated; a satisfactory agreement with experimental ^{1}H n.m.r. coupling constants is achieved. The influence of the exocyclic substituents and of the orientation of the hydroxy groups on the conformation of the furanose ring and on its interconversion by pseudorotation is discussed.

In nucleic acids one of the determinants of the secondary structure of the polynucleotide chain is the conformation of the furanose ring.[1-3] Recently several authors have discussed the flexibility of ribose and deoxyribose,[4-7] but they did not take into account all possible degrees of freedom of the systems studied.

A study by Gerlt and Youngblood[8] of the solution conformational preferences of methyl β-furanosides and their phosphates, which may serve as a model of ribose in RNA, has prompted us to perform energy calculations for methyl β-D-ribofuranoside (Me-ribose). The results of this study should help to answer the following questions: what is the influence of exocyclic hydroxy groups on the conformation of the pentose ring; is substitution at C-1 an important factor for the conformational behaviour of ribose?

An empirical potential function program developed by Rasmussen and his co-workers[9] for saccharides was used. Minimum energy states were regarded as models of an isolated molecule of Me-ribose. Out of all the stable conformations found, models of the molecule in solution were selected. For them proton coupling constants were calculated using a modified Karplus equation proposed by Altona and his co-workers.[10] Calculated coupling constants were compared with the experimental ^{1}H n.m.r. values.[8]

Methods

Figure 1 shows the structure, numbering scheme, and torsion angle conventions for methyl β-D-ribofuranoside. Cartesian coordinates for the molecule were generated by means of the program PENTAGON[11] with a built-in least-squares fitting procedure allowing the closure of the pentose ring. Endocyclic bond lengths and valence angles given by Arnott *et al.*[12] were used. Exocyclic groups were added, with standard bond lengths and angles.[13]

The initial pentose ring conformers were described by the phase angle of pseudorotation P = 0, 90, 180, and 270°, and the amplitude of pucker τ_m = 39°.[14] The initial conformation of the methoxy group was chosen to be *anti* (C-2–C-1–O-1–C-6 = 60°). The whole conformational range about the C-4–C-5 bond was examined; the exocyclic CH_2OH group was initially fixed in either γ^+ (C-3–C-4–C-5–O-5 = 60°), γ^t (180°), or γ^- (300°) orientation for each furanose ring conformation. The O-5–HO-5 bond adopted the *trans*-orientation with respect to the C-4–C-5 bond (C-4–C-5–O-5–HO-5 = 180°). All possible orientations of the O-2–HO-2 and O-3–HO-3 bonds were studied; the O-2–HO-2 and O-3–HO-3 bonds were initially fixed in either g^+, g^-, or t conformation with respect to the C-1–C-2 and C-2–C-3 bonds, respectively, for all ring conformations and all C-4–C-5 bond orientations examined, *i.e.* nine initial orientations of the O-2–HO-2 and O-3–HO-3 bonds were studied for each furanose ring conformation and each CH_2OH group conformation (Figure 2).

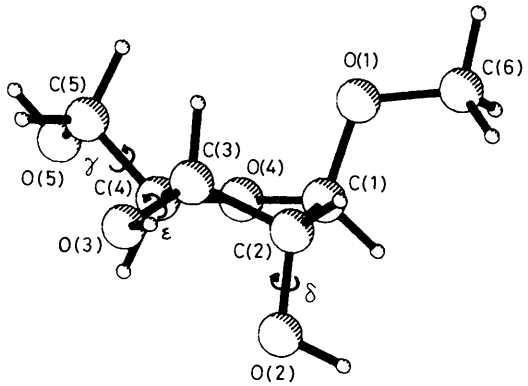

Figure 1. Numbering scheme and dihedral angle conventions in methyl β-D-ribofuranoside (Me-ribose)

Energy calculations were carried out with the aid of the consistent force field (CFF) program developed by Rasmussen.[9,15] The molecular strain energy is the sum of quadratic functions for bond and angle deformations, Pitzer rotational terms, Lennard-Jones 12-6 potentials for non-bonded interactions, and Coulomb terms for electrostatic interactions. Energy minimization with variation of all internal degrees of freedom was performed using the steepest-descent procedure, then the Newton or Davidon method. Minimization was considered finished when the norm of the gradient became less than 10^{-6} kJ mol^{-1}. Vibrational frequencies at equilibrium were found, and the enthalpy, entropy, free energy, and free enthalpy of the system were calculated.

The statistical weight σ_i associated with the minimum-energy conformer of free enthalpy G_i was evaluated as $\sigma_i = Z^{-1} \exp(-G_i/RT)$ where the partition function Z is given by $\sum_i \exp(-G_i/RT)$. The population of the N and S domains in the pseudorotational space was approximated by the summation of statistical weights σ_i over appropriate sets of discrete conformations. Rotameric distributions about C-4–C-5, C-2–O-2, and C-3–O-3 bonds were estimated in the same way.

In order to compare theoretical calculation results with

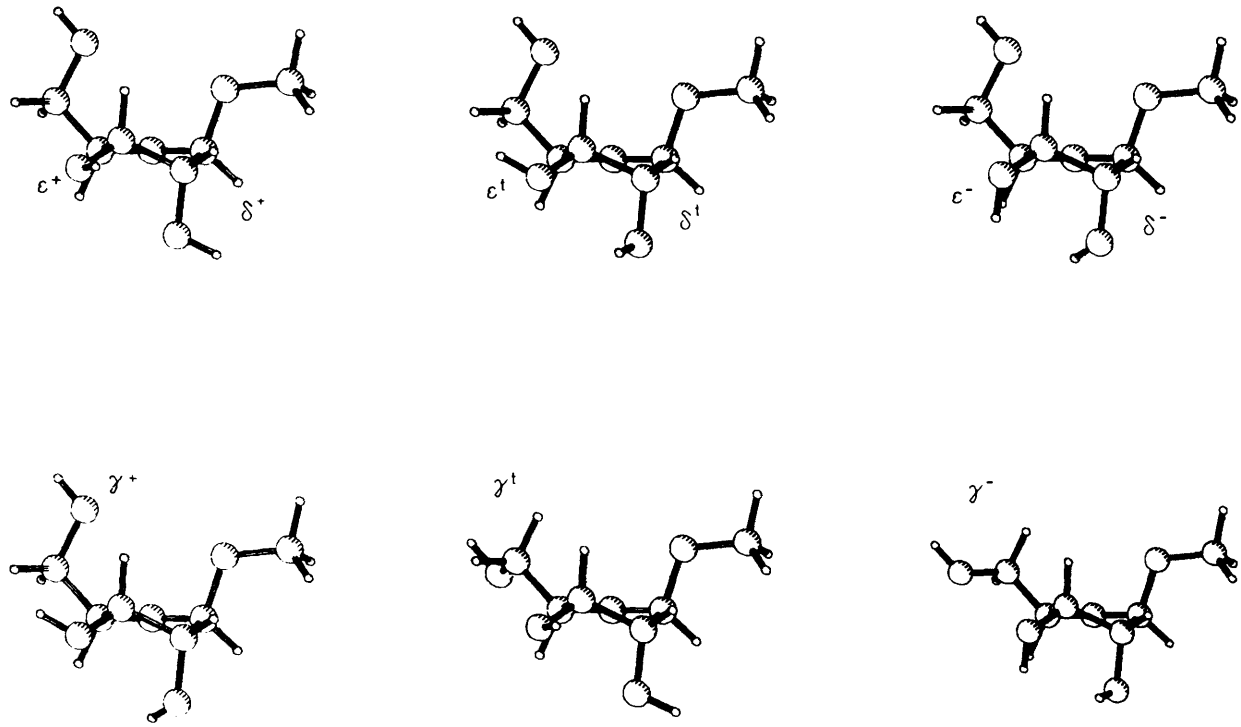

Figure 2. Representation of an N-type conformer of the furanose ring, showing the conformations about the C-2–O-2 and C-3–O-3 bonds, and three typical rotamers about the C-4–C-5 bond examined in this study

experimental n.m.r. data, vicinal proton–proton coupling constants were calculated using the modified Karplus equation proposed by Haasnoot *et al.*,[10] which includes a correction for substituent electronegativities. Only conformers with 'free' HO atoms which may participate in intermolecular hydrogen bonds were taken into account in this comparison in order to simulate solution conditions. Conformers in which intramolecular 5-OH ··· O-4 hydrogen interactions were found, or in which the O-2–HO-2 or O-3–HO-3 bond adopted a g^- conformation, were rejected.

The J_{12}, J_{23}, and J_{34} coupling constants were calculated from equation (1) where θ is the H–C–C–H torsion angle, ξ_i is equal to +1 or −1 according to the orientation of the *i*th α-substituent, and $\Delta\chi_i$ is the relative electronegativity of the *i*th substituent. The summation is over all the α-substituents attached to the carbon atoms. $\Delta\chi_i$ is defined by equation (2) where the summation is over all the substituents bound to the α-substituent.

$$^3J_{HH} = 13.24\cos^2\theta - 0.91\cos\theta + \sum_i \Delta\chi_i[0.53 - 2.41\cos^2(\xi_i\theta + 15.5|\Delta\chi_i|)] \quad (1)$$

$$\Delta\chi_i = \Delta\chi_i\ (\alpha\text{-substituent}) - 0.19\,\Sigma\Delta\chi_i(\beta\text{-substituent}) \quad (2)$$

The J_{45} and $J_{45'}$ coupling constants were calculated from equation (3) where the summation is over all the α-substituents bound to the carbon atoms, and $\Delta\chi_i$ is calculated with no correction for the electronegativity of β-substituents. Substituent electronegativities were taken from Huggins.[16]

$$^3J_{HH} = 13.22\cos^2\theta - 0.99\cos\theta + \sum_i \Delta\chi_i[0.87 - 2.46\cos^2(\xi_i\theta + 19.9|\Delta\chi_i|)] \quad (3)$$

The vicinal proton coupling constants observed for Me-ribose[8] were compared with average $^3J_{HH}$ values evaluated from equation (4) where the summation is over appropriate sets of conformations.

$$^3J_{HH} = \sum_i {}^3J_{HH}{}^i\,\sigma_i \quad (4)$$

Results

The first part of the Results section presents the results of energy calculations for all the stable conformers of methyl β-D-ribofuranoside found in this study, which represent predicted conformations of an isolated molecule. In the second part the results for 'solution' conformers are exposed. The class of 'solution' conformers has been differentiated between all stable conformers found according to the orientation of the exocyclic O–H bonds: molecular forms in which the O–H bonds were directed outside from the molecule and in which the hydroxy groups could participate in intermolecular hydrogen bonds with other chemical entities were selected to represent Me-ribose in solution.

The Isolated Molecule.—Bond lengths and bond angles. The endocyclic bond lengths and endocyclic bond angles, as well as the exocyclic C–O bond lengths found in the stable conformers of Me-ribose, are presented in Table 1. Table 1 also displays a comparison of the molecular geometries simulated in this work with the bond lengths and angles found in the crystal structures of several carbohydrate compounds.[17–19]

Furanose ring conformations. (1) γ^+ Rotamer about the C-4–C-5 bond. Two distinct domains of stable ring conformations of Me-ribose have been found: one domain occupies part of the *N* quadrant of the pseudorotation wheel (Figure 3), the second domain occurs in the *S* quadrant.

Eight stable conformers with *N*-type ring puckering have been discovered. The missing ninth conformer had initially 2-OH and 3-OH groups in δ^t and ε^+ conformations, respectively; the orientation of the O-2–HO-2 bond was changed in energy

Table 1. Internal co-ordinates of methyl β-D-ribofuranoside and comparison with crystallographic data for furanoside fragments

	Methyl β-D-ribofuranoside[a]	Methyl α-D-lyxofuranoside[b]	Methyl α-D-galactofuranoside[c]	β-D-Furanoside fragments of nucleic acid constituents[d]
(a) Bond lengths (Å)				
O-4–C-1	1.410—1.417	1.427 (8)	1.424 (3)	1.411 (11)
C-1–C-2	1.512—1.528	1.541 (11)	1.520 (3)	1.529 (12)
C-2–C-3	1.516—1.526	1.516 (11)	1.517 (4)	1.527 (12)
C-3–C-4	1.516—1.533	1.513 (10)	1.530 (3)	1.526 (11)
C-4–O-4	1.412—1.426	1.449 (9)	1.454 (3)	1.449 (10)
C-1–O-1	1.421—1.424	1.382 (11)	1.395 (3)	
C-2–O-2	1.423—1.426	1.407 (9)	1.416 (3)	1.415 + 0.010 cos (P − 9 π/10)
C-3–O-3	1.422—1.428	1.416 (12)	1.420 (3)	1.426 (12)[e]
(b) Bond angles (°)				
O-4–C-1–C-2	106.2—110.2	105.6 (5)	103.4 (2)	106.1 + 1.9 cos (2P + 8 π/5)
C-1–C-2–C-3	98.6—103.2	102.9 (6)	102.4 (2)	102.3 + 1.4 cos (2P + 6 π/5)
C-2–C-3–C-4	100.2—102.6	99.5 (6)	103.9 (2)	102.9 + 0.7 cos (2P + 4 π/5)
C-3–C-4–O-4	105.8—109.6	103.9 (6)	105.7 (2)	104.7 + 1.8 cos (2P + 2 π/5)
C-4–O-4–C-1	106.1—108.8	108.7 (5)	109.6 (2)	107.7 + 2.4 cos 2P

[a] This work. [b] Ref. 18. [c] Ref. 19. [d] Ref. 17. [e] Mean value for 3′-phosphates.

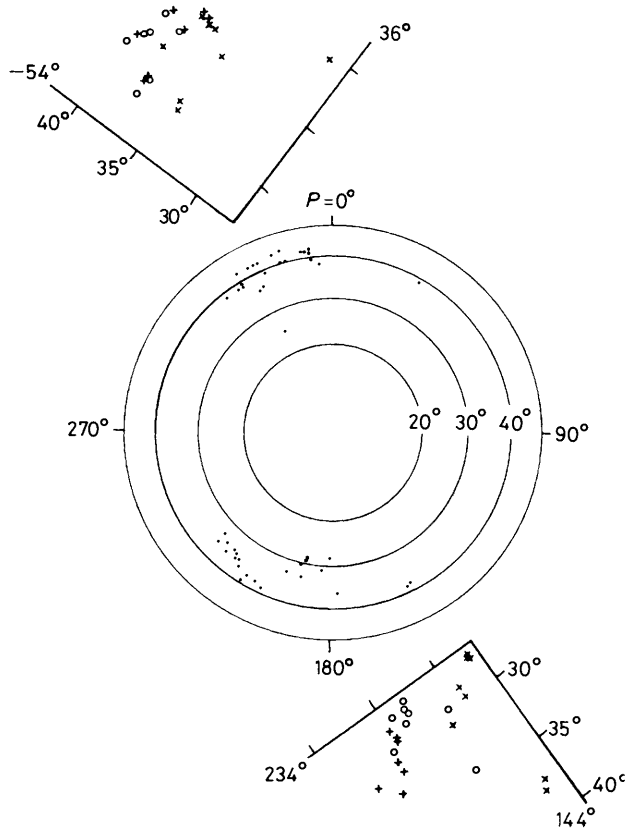

Figure 3. Pseudorotational wheel representation of the furanose ring showing all the stable states found for methyl β-D-ribofuranoside. The two occupied sectors have been depicted on a larger scale for more detail: ×, γ^+ rotamer; ○, γ^t rotamer; +, γ^- rotamer

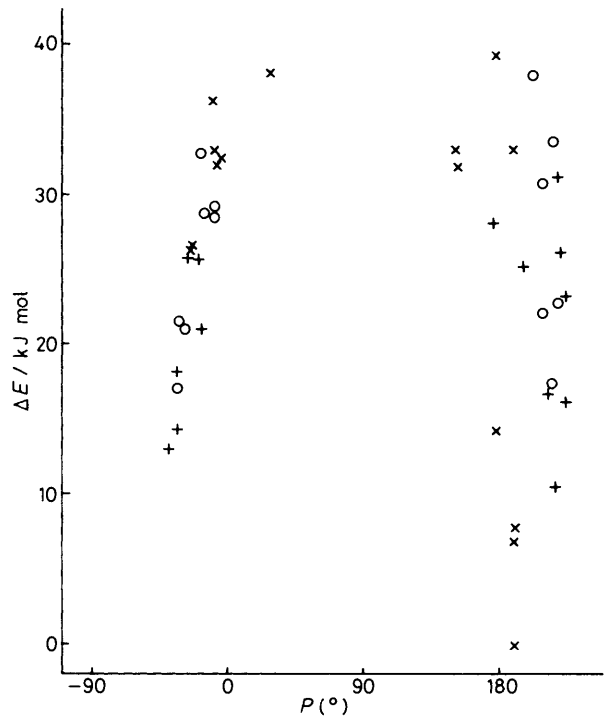

Figure 4. Potential energy of stable states of an isolated molecule of methyl β-D-ribofuranoside: ×, γ^+ rotamer; ○, γ^t rotamer; +, γ^- rotamer

minimization to δ^-. In the N domain of ring puckering three ranges of conformations may be distinguished: one group occurs at P = 333—337° (there are three conformers, all of them with δ^- conformation, adopting the C-1-*endo*-C-2-*exo* conformation); one group is described by P = 350—355° (there are four conformers with C-2-*exo* or C-3-*endo*-C-2-*exo* geometry); in the third group there is only one conformer with P = 29.0° (C-3-*endo* geometry), and δ^t, ε^-, and β^t orientation of the O-2–HO-2, O-3–HO-3, and O-5–HO-5 bond, respectively. The amplitude of pucker τ_m varies in the described range of P values from 35.3° for P = −26.8° (δ^- and ε^+ conformation in this case) to 40.9° for P = −10.4° (δ^+ and ε^t orientation).

In the S quadrant of the pseudorotation wheel eight stable states were found. As in the N range of ring puckering, the conformer in which the initial orientation of the 2-OH and 3-OH groups was δ^t and ε^+ passed during energy minimization to a state in which the O-2–HO-2 and O-3–HO-3 bonds adopted the δ^t and ε^- conformation, respectively; it may be noted that

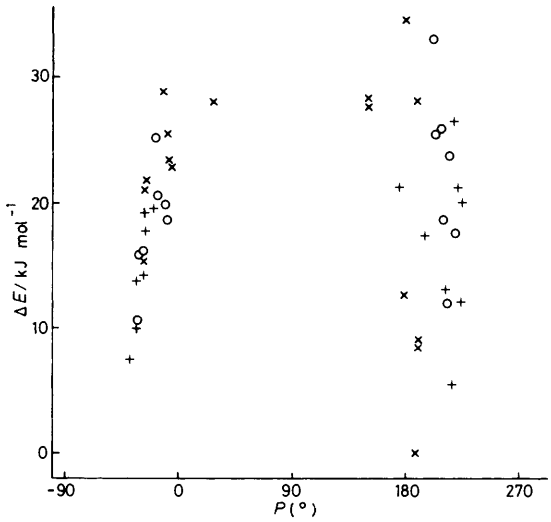

Figure 5. Free enthalpy of stable states of an isolated molecule of methyl β-D-ribofuranoside: ×, γ^+ rotamer: ○, γ^t rotamer; +, γ^- rotamer

this final configuration of the hydroxy groups in the *S*-type conformation is symmetrical with the *N*-type one.

Two domains of *P* values may be seen in the *S* quadrant: $P = 152°$ (there are two conformers with C-2-*endo*-C-1-*exo* geometry; the conformation about the C-2–O-2 and C-3–O-3 bond is δ^- and ε^+ in one of them, and δ^- and ε^+ in the other), and $P = 180$—$192°$ (there are six conformers with C-2-*endo*-C-3-*exo* geometry).

In four cases, namely ($P = 180.3°$, $\tau_m = 28.2°$, δ^t, ε^t), ($P = 190.1°$, $\tau_m = 28.1°$, δ^t, ε^-), ($P = 190.7°$, $\tau_m = 28.1°$, δ^+, ε^-), and ($P = 191.2°$, $\tau_m = 32.8°$, δ^-, ε^-), rotation about the C-5–O-5 bond occurred during energy minimization, leading from the initial β^t rotamer to the final β^+ rotamer (β *ca.* 60° in all cases). In the β^+ region the HO-5 hydrogen atom is situated over the furanose ring; the O-4 ··· O-5 distance is *ca.* 2.9 Å in all four β^+ conformers, suggesting the possibility of forming of an intramolecular hydrogen bond. This interaction may be enhanced by the flattening of the furanose ring to $\tau_m = 28.1$—32.8°.

τ_m varies in the *S* region from 28.1° for a ring with an intramolecular hydrogen bond to 38.3° for a conformer with $P = 152.8°$ and the δ^-, ε^t, and β^t configurations.

The state with $P = 190.1°$, $\tau_m = 28.1°$, $\delta = 163.8°$, $\varepsilon = -53.5°$, and $\beta = 60.7°$, has the lowest energy and free enthalpy (Figures 4 and 5). The energy and free enthalpy of all other *S*-type conformers with no O-4 ··· O-5 hydrogen bond is greater by 31.8—39.3 and 27.8—34.7 kJ mol^{-1}, respectively, than that of the minimum-energy state. The presence of an O-4 ··· O-5 hydrogen bond decreases the energy and free enthalpy difference relative to the lowest energy state to 7.0—14.3 and 8.6—12.6 kJ mol^{-1}, respectively.

In the *N* domain the energy is greater by 21.0 (for $P = -26.8°$, $\tau_m = 35.3°$, δ^-, ε^+, β^t) to 38.1 kJ mol^{-1} (for $P = 29.0°$, $\tau_m = 39.6°$, δ^t, ε^-, β^t). The free enthalpy is greater by 15.5 (for $P = -26.8°$, $\tau_m = 35.3°$, δ^-, ε^+, β^t) to 29.0 kJ mol^{-1} (for $P = -10.4°$, $\tau_m = 40.9°$, δ^+, ε^t, β^t).

(2) γ^- Conformer about the C-4–C-5 bond. In the *N* domain of the phase angle of pseudorotation *P* there are seven stable conformations which differ in the orientation of the 2- and 3-hydroxy groups. The conformer in which the 2- and 3-OH groups adopted initially the δ^t and ε^+ conformation, respectively, was 'relaxed' during energy minimization to a state with δ^- and ε^+ conformation, as was the case for γ^+ rotamers. The other conformer missing with an initial δ^t and ε^- configuration changed its pucker from *N* to *S* during energy minimization with no rotation about the C-2–O-2 and C-3–C-3 bonds.

The seven *N*-type stable ring conformations are shifted towards the C-1-*endo*-C-2-*exo* state, with $P = 322$—343°. The amplitude of pucker τ_m varies from 37.7 to 41.6°. In this domain the lowest-energy conformer is described by $P = -37.8°$, $\tau_m = 37.7°$, δ^t, ε^+, and β^t; its energy is higher by 13.0 kJ mol^{-1} than the energy of the global minimum-energy state, and its free enthalpy is higher by 7.7 kJ mol^{-1} than the free enthalpy of the reference conformer. The energy difference for the other six *N*-type conformers ranges from 14.3 (for $P = -31.8°$, $\tau_m = 37.9°$, δ^-, ε^t, and β^t) to 25.9 kJ mol^{-1} (for $P = -26.5°$, $\tau_m = 40.9°$, δ^+, ε^-, and β^t). The free enthalpy difference varies from 10.0 (for $P = -31.8°$, $\tau_m = 37.9°$, δ^-, ε^t, and β^t) to 19.7 kJ mol^{-1} (for $P = -19.0°$, $\tau_m = 41.6°$, δ^+, ε^t, and β^t).

In the *S* region of the pseudorotation wheel eight stable conformations were found. There is no stable ring conformation with the δ^- and ε^+ configuration of the 2- and 3-hydroxy groups; the initial C-2-*endo*-C-3-*exo* conformer with δ^- and ε^+ geometry underwent a conversion similar to that described above during energy minimization, changing its pucker to *N* with no rotation of the hydroxy groups.

A distinctive feature of the γ^- conformational range is the presence of a stable *S*-type ring conformer with a δ^t and ε^+ configuration of the 2- and 3-hydroxy groups. Such a conformer has not been found for any other rotamers about the C-4–C-5 bond. In this case $P = 177.4°$, $\tau_m = 35.7°$, $\delta = 178.0°$, $\varepsilon = 55.2°$, and $\beta = -178.6°$.

In the *S* domain six stable conformations gather at $P = 214$—227° (C-4-*endo*-C-3-*exo* geometry), one is defined by $P = 177.4°$ (C-2-*endo*-C-3-*exo* geometry), and one is described by $P = 197.3°$ ($\tau_m = 31.8°$, δ^t, ε^t, β^t) (C-3-*exo* geometry). The amplitude of pucker τ_m varies from 31.8 to 35.7°.

The energy of the *S*-type ring conformers is greater by 10.6 (for $P = 217.8°$, $\tau_m = 33.9°$, δ^t, ε^-, β^t) to 31.1 kJ mol^{-1} (for $P = 219.7°$, $\tau_m = 33.4°$, δ^+, ε^t, β^t) than the energy of the reference state. The free enthalpy difference ranges from 5.6 to 26.5 kJ mol^{-1}.

(3) γ^t Conformer about the C-4–C-5 bond. In both *N* and *S* domains *P* values corresponding to the stable ring conformations are more scattered than those obtained for γ^+ and γ^- rotamers (Figure 3).

In the *N* quadrant of the pseudorotation wheel seven stable states have been found. No conformer with δ^t and ε^+ configuration of the 2- and 3-hydroxy groups was observed; as for γ^+ and γ^- rotamers, the initial (δ^t, ε^+) conformation was changed to (δ^-, ε^+) by energy minimization. The initial *N*-type ring conformer with δ^t and ε^- orientation of the O-2–HO-2 and O-3–HO-3 bonds underwent a conversion similar to the one described in the previous section, with a change of pucker from C-3-*endo*-C-2-*exo* to C-4-*endo*-C-3-*exo* and no rotation about the C-2–O-2 and C-3–O-3 bonds.

In the *N* domain the phase angle of pseudorotation *P* describing the stable ring conformations ranges from 327 to 352°. The amplitude of pucker τ_m varies from 38.1 to 41.7°.

The energy difference relative to the global minimum (Figure 4) is 13.7 (for $P = -32.7°$, $\tau_m = 38.1°$, δ^-, ε^+, β^t) to 29.4 kJ mol^{-1} (for $P = -17.0°$, $\tau_m = 41.7°$, δ^+, ε^t, β^t). The free enthalpy is greater by 10.8 kJ mol^{-1} to 25.3 kJ mol^{-1} (Figure 5).

In the *S* quadrant seven stable ring conformations have been found with $P = 203$—221° (C-4-*endo*-C-3-*exo* geometry). The two missing forms initially involved (δ^t, ε^+) and (δ^-, ε^+) conformational combinations. The (δ^t, ε^+) form was 'relaxed' during energy minimization to the (δ^t, ε^-) state, yielding a conformation symmetrical to the one in the *N* domain. The unstable (δ^-, ε^+), conformer passed to *N*-type state (C-1-*endo*-C-2-*exo*) with no rotation about the C-2–O-2 and C-3–O-3

Table 2. Conformational populations of an isolated molecule of methyl β-D-ribofuranoside (in %)

Conformation about the C-4–C-5 bond		γ^+		γ^-		γ^t				
Conformation about the C-2–O-2 bond	Conformation about the C-3–O-3 bond	*N*	*S*	*N*	*S*	*N*	*S*	Σ*N*	Σ*S*	Σ*N* + Σ*S*
δ^+	ε^+	0.01	0.00	0.06	0.01	0.04	0.01	0.11	0.02	0.13
δ^+	ε^-	0.00	2.47	0.03	0.56	0.02	0.06	0.05	3.09	3.14
δ^+	ε^t	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.03
δ^-	ε^+	0.15	0.00	3.57		0.99		4.71	0.00	4.71
δ^-	ε^-	0.01	1.88	0.28	0.38	0.11	0.04	0.40	2.30	2.70
δ^-	ε^t	0.02	0.00	1.37	0.02	0.12	0.00	1.51	0.02	1.53
δ^t	ε^+				0.01				0.01	0.01
δ^t	ε^-	0.00	78.16		8.15		0.62	0.00	86.93	86.93
δ^t	ε^t	0.01	0.48	0.24	0.07	0.02	0.00	0.27	0.55	0.82
$\Sigma P_\gamma^N, \Sigma P_\gamma^S$		0.20	82.99	5.58	9.20	1.30	0.73	7.08	92.92	
$\Sigma P_\gamma^N + \Sigma P_\gamma^S$		83.19		2.03		14.78				

Table 3. Equilibrium values of pseudorotational parameters *P* and τ_m in methyl β-D-ribofuranoside (°)

	P_N	τ_m^N	P_S	τ_m^S
Isolated molecule	−33.8	38.1	193.0	28.8
Molecule 'in solution'	−17.0	40.4	199.4	32.8

bonds. In the *S* range the amplitude of pucker τ_m varies from 35.1 to 38.7°.

The energy difference relative to the global minimum (Figure 4) ranges from 17.3 (for $P = 214.9°, \tau_m = 35.3°, \delta^t, \varepsilon^-, \beta^t$) to 37.9 kJ mol^{-1} (for $P = 203.4°$, $\tau_m = 38.3°$, δ^+, ε^t, β^t). The free enthalpy difference is 12.0—33.1 kJ mol^{-1}.

Conformation about the C-1–O-1 *bond.* In the stable states of Me-ribose the conformation about the C-1–O-1 bond is in the *anti* range with respect to the O-4–C-1 bond, but the value of the torsional angle in the *N* and *S* puckered forms differs slightly: the C-2–C-1–O-1–C-6 angle varies from 95 to 100° in *N*-type states, and from 80 to 86° in *S*-type states, with two exceptions: for ($P = 152°$, $\tau_m = 38.3°$, γ^+, δ^-, ε^t, β^t) C-2–C-1–O-1–C-6 = 62°, and for ($P = 192°, \tau_m = 30.2°, \gamma^+, \delta^+, \varepsilon^+, \beta^t$) C-2–C-1–O-1–C-6 = 72°.

Relative populations of stable conformers. Table 2 displays the conformational populations of the isolated molecule, and Table 3 shows the equilibrium values of pseudorotational parameters *P* and τ_m calculated for *N*- and *S*-type conformers. The following conclusions may be drawn from an inspection of Tables 2 and 3.

The predominant furanose ring conformation in Me-ribose occurs in the *S* region of the pseudorotation wheel. The *N*-type states centre at $P_N = -34°$, and the *S*-type states at $P_S = 193°$. The amplitude of pucker τ_m is greater by *ca.* 9° in the *N* than in the *S* domain.

The dominant conformation about the C-4–C-5 bond was found to be γ^+ (83%). The γ^- and γ^t regions are less energetically favoured, with relative populations equal to 15 and 2%, respectively.

The predominant conformation of the 2-hydroxy group in *N*-type furanose ring conformers is δ^-, followed by δ^t and δ^+. In the *S* region of ring conformations the δ^t rotamer is the most richly populated, followed by δ^+ and δ^-.

The dominant conformation of the 3-hydroxy group in *N*-type furanose ring conformers is ε^+, followed by ε^t and ε^-. In *S*-type ring conformers the ε^- rotamer is the most richly populated, followed by ε^t and ε^+.

The Molecule in Solution.—'Solution' conformations of the furanose ring. Out of all the stable final molecular conformations found 18 have been differentiated in which O–H bonds in exocyclic hydroxy groups were orientated outside the molecule and in which OH groups could participate in intermolecular hydrogen bonds with other chemical entities. The special conformations include ($\delta^+, \varepsilon^+, \beta^t$), ($\delta^+, \varepsilon^t, \beta^t$), ($\delta^t, \varepsilon^+, \beta^t$), and ($\delta^t, \varepsilon^t, \beta^t$) rotamers and serve as models of Me-ribose in solution.

(1) γ^+ Rotamer about the C-4–C-5 bond. Three stable ring conformations have been found in the *N* domain of the pseudorotational space, with *P* ranging from −10 to −4° (C-3-*endo*-C-2-*exo* geometry), and the amplitude of pucker from 38.1 to 40.9°. The conformation of the hydroxy groups is ($\delta^+, \varepsilon^+, \beta^t$), ($\delta^+, \varepsilon^t, \beta^t$), and ($\delta^t, \varepsilon^t, \beta^t$).

In the *S* range there are only two stable forms, both with δ^+ orientation of the O-2–HO-2 bond: ($\delta^+, \varepsilon^+, \beta^t$) and ($\delta^+, \varepsilon^t, \beta^t$). $P = 192.0°$ and $\tau_m = 30.2°$ for the former state, $P = 184.0°$ and $\tau_m = 30.2°$ for the latter.

(2) γ^- Rotamer about the C-4–C-5 bond. Three stable states have been found in the *N* domain with ($\delta^+, \varepsilon^+, \beta^t$), ($\delta^+, \varepsilon^t, \beta^t$), and ($\delta^t, \varepsilon^t, \beta^t$) conformation of the hydroxy groups. *P* ranges from −25 to −17°, τ_m ranges from 40.2 to 41.6°.

In the *S* domain four stable conformations have been discovered, described by $P = 177$—223° and $\tau_m = 31.8$—35.7°. An *S*-type ring conformer with γ^- orientation of the CH_2OH group is the only one which has been found to be stable with ($\delta^t, \varepsilon^+, \beta^t$) conformation; it is described by $P = 177.4°$ and $\tau_m = 35.7°$.

(3) γ^t Rotamer about the C-4–C-5 bond. There are three *N*-type stable ring conformations with γ^t orientation of the exocyclic CH_2OH group. They include ($\delta^+, \varepsilon^+, \beta^t$), ($\delta^+, \varepsilon^t, \beta^t$), and ($\delta^t, \varepsilon^t, \beta^t$) rotamers. *P* varies between −17 and −8°, τ_m ranges from 40.3 to 41.7°.

The same three conformations of the hydroxy groups have been found to be stable in the *S* range of the pseudorotation wheel. The stable conformers have $P = 206$—216° and $\tau_m = 35.2$—38.3°.

Energy and free enthalpy distribution and conformational populations in Me-ribose in solution. The lowest-energy and free enthalpy solution conformer of Me-ribose found is defined by $P = -17.2°$, $\tau_m = 40.2°$, $\gamma^- = -56.4°$, $\delta^t = -166.2°$, $\varepsilon^t = -166.9°$, $\beta^t = 177.6°$. *N*-type ring conformers have energies higher by 4.7—15.2 kJ mol^{-1} than the most favoured form (Figure 6). The energy of *S*-type states is greater by 4.2—18.2 kJ mol^{-1} than that of the global minimum. Free enthalpy differences range from 3.6 to 14.6 kJ mol^{-1} for *N*-type

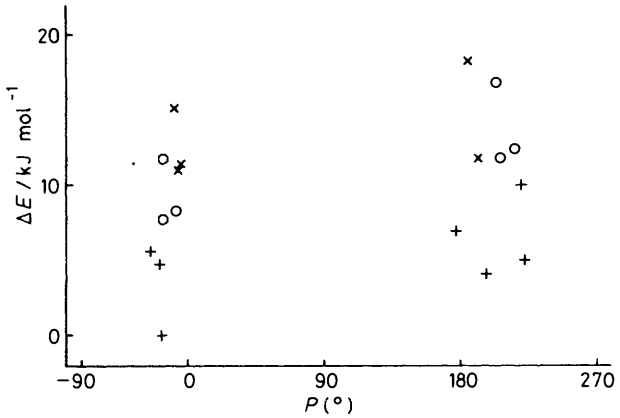

Figure 6. Potential energy of stable states of models for methyl β-D-ribofuranoside in solution: ×, γ^+ rotamer; ○, γ^t rotamer; +, γ^- rotamer

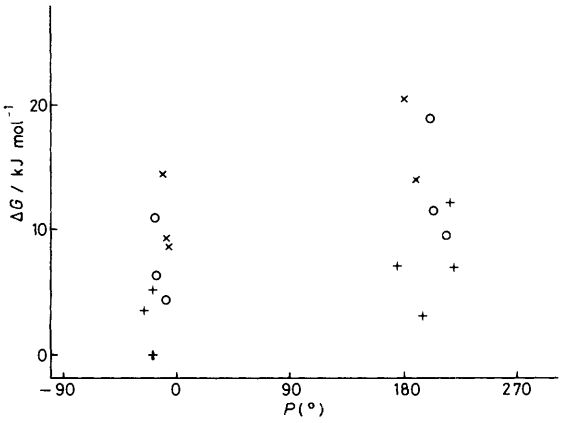

Figure 7. Free enthalpy of stable states of models for methyl β-D-ribofuranoside in solution: ×, γ^+ rotamer; ○, γ^t rotamer; +, γ^- rotamer

conformers, and from 3.0 to 20.3 kJ mol^{-1} for *S*-type conformers (Figure 7).

The predominant ring conformation in solution of Me-ribose occurs in the *N* region of the pseudorotational wheel (Table 4). The *N*-type forms centre at $P = -17.0°$ (Table 3), and have an average amplitude of pucker $\tau_m = 40.4°$. The average value of *P* in the *S* domain is 199.4°, while the average amplitude of pucker is smaller than in the *N* region and equal to 32.8°.

The conformational behaviour about the C-4–C-5 bond is presented in Table 4. The γ^- region is the most richly populated, followed by γ^t, and in only 3% of the conformers does the γ^+ rotamer occur.

The 2-hydroxy group occurs mainly in the δ^t conformation in both *N* and *S*-type states. The ε^t region is also the most populated in both *N*- and *S*-type forms.

Coupling constants in Me-ribose in solution. Proton–proton coupling constants were calculated using the modified Karplus equation with H–C–C–H dihedral angles found in minimum-energy conformers which serve as models of Me-ribose in solution. The results are presented in Table 5, which displays the calculated $^3J_{HH}$ values for 'pure' *N* and *S* states, for 'pure' γ^+, γ^t, and γ^- states, and the average values found by population analysis.

Discussion

We first discuss the results obtained for all stable conformers found for Me-ribose. These conformers are considered to be hypothetical stable states in an isolated molecule. In the second part, the results for conformers which were selected as models of the molecule in solution are examined; these conformers were chosen among all the stable forms found according to their capacity of forming intermolecular hydrogen bonds.

The results obtained for Me-ribose indicate that stable furanose ring conformations in this molecule occur in the *N* and *S* regions of the pseudorotational wheel. With each region there is associated a certain number of local energy-minimum conformations, corresponding to different orientations of exocyclic bonds. The two-domain distribution of stable conformations found for Me-ribose is similar to the two-state conformational preference observed in nucleosides and nucleotides.

The Isolated Molecule.—Bond lengths and angles. The accuracy of simulation of the geometrical features of the molecule may be considered a test for the reliability of the method used. From Table 1 it may be seen that satisfactory agreement between most bond lengths and angles obtained for Me-ribose in this study and data derived from crystallography for related carbohydrates has been achieved. The values found

Table 4. Conformational populations of models for methyl β-D-ribofuranoside in solution (%)

Conformation about the C-4–C-5 bond		γ^+		γ^-		γ^t				
Conformation about the C-2–O-2 bond	Conformation about the C-3–O-3 bond	*N*	*S*	*N*	*S*	*N*	*S*	Σ*N*	Σ*S*	Σ*N* + Σ*S*
δ^+	ε^+	1.41	0.17	11.03	2.86	7.77	1.03	20.21	4.06	24.27
δ^+	ε^t	0.13	0.01	5.46	0.35	0.56	0.02	6.15	0.38	6.53
δ^t	ε^+				2.83				2.83	2.83
δ^t	ε^t	1.12		47.02	14.12	3.64	0.47	51.78	14.59	66.37
$\Sigma P_\gamma{}^N, \Sigma P_\gamma{}^S$		2.66	0.18	63.51	20.16	11.97	1.52	78.14	21.86	
$\Sigma P_\gamma{}^N + \Sigma P_\gamma{}^S$		2.84		83.67		13.49				

Table 5. Vicinal coupling constants $^3J_{HH}$ in methyl β-D-ribofuranoside in solution (in Hz)

Coupling constants calculated for 'pure' conformational states

$J_{12}{}^N$	$J_{23}{}^N$	$J_{34}{}^N$	$J_{12}{}^S$	$J_{23}{}^S$	$J_{34}{}^S$	$J_{45}{}^+$	$J_{45'}{}^+$	$J_{45}{}^-$	$J_{45'}{}^-$	$J_{45}{}^t$	$J_{45'}{}^t$
1.2	5.2	7.5	4.2	5.9	0.8	2.8	0.7	10.6	5.6	1.5	8.6

Comparison of calculated and experimental coupling constants

	J_{12}	J_{23}	J_{34}	J_{45}	$J_{45'}$
$J^{calc.}$	1.8	5.4	6.0	5.9	4.3
$J^{exp.}$	0.8	4.7	6.5	6.6	3.3

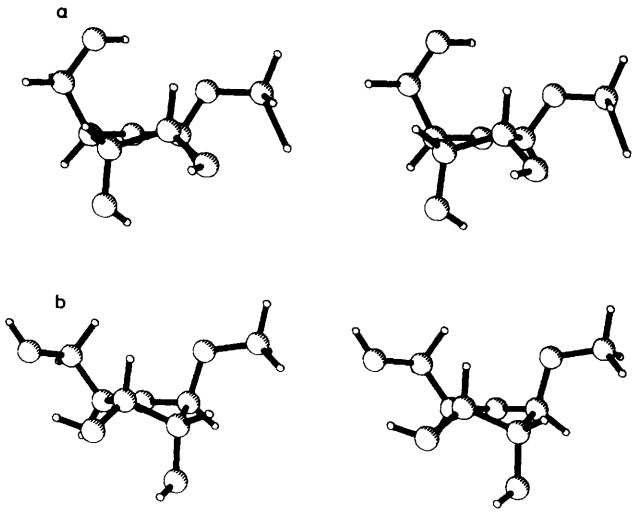

Figure 8. Stereoview of the lowest free enthalpy conformer of a, an isolated molecule; b, a solution model of methyl β-D-ribofuranoside

for Me-ribose do not differ significantly from those observed in methyl-α-D-lyxofuranoside[18] and methyl-α-D-galactofuranoside,[19] nor from the geometrical parameters derived from *X*-ray structures of 178 ribo-, 2′-deoxyribo- and arabinonucleosides.[17]

Two bond lengths calculated for Me-ribose differ from those found in the compared structures: C-4–O-4 and C-1–O-1. The C-4–O-4 bond is predicted to be only slightly longer than the C-1–O-4 bond, while this difference is more pronounced in the compared carbohydrates. Also the predicted C-1–O-1 bond length does not differ from the C-2–O-2 and C-3–O-3 bond lengths. Thus, though Me-ribose is predicted to favour conformations in which O-1 is axial, in accordance with experimental data,[20] the method used does not reproduce the shortening of the C-1–O-1 bond due to the anomeric effect.

The overall agreement between the bond lengths and angles calculated for Me-ribose and data derived from *X*-ray analyses appears, however, satisfactory.

Furanose ring conformation. The *N* and *S* domains of stable furanose ring conformations are characterized by a wide spectrum of *P* values (*P* ranges from −38 to 29° in the *N* region, and from 151 to 227° in the *S* region) corresponding to different conformations of the exocyclic hydroxy groups. The free enthalpy difference between stable states varies up to 34.7 kJ mol^{-1} depending on the orientation of the 2-, 3-, and 5-OH groups. The global minimum energy conformer is characterized by $P = 190.1°$, $\tau_m = 28.1°$, γ^+, β^+, δ^t, and ε^- (Figure 8).

In the *N* domain the first three stable states for all possible conformations about the C-4–C-5 bond are associated with (δ^-, ε^+), (δ^-, ε^t), and (δ^-, ε^-) rotamers. Symmetrically, in the *S* region the first three stable states are described by (δ^t, ε^-), (δ^+, ε^-), and (δ^-, ε^-) conformations independently of the conformation of the CH_2OH group.

The calculated values of *P* for Me-ribose differ from those usually found in ribose rings in nucleosides and nucleotides in the solid state.[17] Ribose rings in most nucleosides and nucleotides adopt a C-3′-*endo* (*P* = 0—18°) or a C-2′-*endo* (*P* = 144—180°) geometry. A notable exception is the C-4′-*endo* type of puckering observed in 2′,3′-cyclic nucleotides.[21] The conformational preference of the ribose ring in different molecules may thus arise from the particular nature of the substituents attached to the ring atoms.

In ribose rings found in most nucleosides and nucleotides, for which the centre of the crystallographic range of *N* states occurs at *P* = 7—11°, and the centre of the *S* domain at *P* = 157—164°,[22] the C-1′–N and C-4′–C-5′ bonds point outside from the ring. In the C-1-*endo*-C-2-*exo* and C-4-*endo*-C-3-*exo* ring conformations in Me-ribose (Figure 3) the C-1–O-1 and C-4–O-5 exocyclic bonds are more bent towards each other. The preference of ring conformations in Me-ribose, which are different from those commonly observed in ribonucleosides, may arise from the particular substituents bound to C-1 and C-4.

The results obtained in this study suggest that substituents attached to the ring atoms may also have a 'through-bond' influence on the conformation of ribose. In nucleosides C-2′ and C-3′ are displaced from the C-1′–O-4′–C-4′ plane. In 2′,3′- and 3′,5′-cyclic nucleotides the additional cyclization results in puckered states involving the C-4′, C-1′, or O-4′ atoms. In the system studied here the O-1 substituent may also modify the environment of C-1 in such a way that other parts of the ring than in nucleosides become more flexible.

In four cases for γ^+ orientation of the CH_2OH group and *S*-type ring conformation an intramolecular hydrogen bond O-5-H · · · O-4 has been found which could stabilize these forms with respect to the conformers in which such an interaction does not occur.

Our calculations indicate that in the isolated molecule the ribose ring favours almost exclusively *S*-type conformations (Table 2). According to the results obtained with the aid of several quantum-chemical methods,[4–7] the ribose ring conformational populations are approximately equal in the *N* and *S* domains. It is important, however, to bear in mind that in these calculations the conformation of the exocyclic hydroxy groups was not taken into account and that the model molecules were substituted at C-1 and C-4 with different atomic groups (NH_2 or nitrogen base, and H, CH_3 or CH_2OH respectively). Solid-state conformations of the exocyclic bonds were used in these studies, leading thus to a more or less accurate reproduction of solid-state statistical distribution, while the overwhelming preference of the *S* domain in the molecule studied in this work is mainly due to intramolecular electrostatic interactions. Experimental data for ribose obtained in the gas phase would be the best test for the results obtained here, but they are unfortunately unavailable.

N-Type ring conformers in Me-ribose have a greater amplitude of pucker than *S*-type forms (see Figure 3 and Table 3).

If one defines the mean free enthalpy difference $\Delta\tilde{G}_{NS}$ between *N* and *S* domains as the difference between the mean free enthalpy $\tilde{G}_N$ of *N* states and the mean free enthalpy $\tilde{G}_S$ of *S* states, one obtains equation (5). The mean free enthalpy

$$\Delta\tilde{G}_{NS} = -RT \ln (\sigma_N/\sigma_S) \qquad (5)$$

difference between *N* and *S* forms in the isolated molecule is 6.4 kJ mol^{-1} at room temperature (RT = 2.5 kJ mol^{-1}). The value obtained in this work is of an order of magnitude greater than the energy differences between *N* and *S* states in ribose models calculated by Sato *et al.*,[6] Levitt and Warshel,[4] or Olson.[6] It is again the result of the considerable stabilization of several *S* forms with γ^+ conformation by an intramolecular hydrogen bond.

The overwhelming preference of *S*-type forms in Me-ribose indicates that the frequently alluded to symmetry of the *N* and *S* stable ring conformations[4,6,7] may be precluded by the nature of exocyclic substituents.

In γ^t and γ^- rotamers an $N \rightleftarrows S$ interconversion occurred for particular orientations of the hydroxy groups. This suggests that besides the pseudorotation route through *P* = 90° or the interconversion through the planar state[23] the pseudorotation pathway through *P* = 270° is accessible. It necessitates the γ^t or γ^- conformation and a slight flattening of the ring. This is

supported by crystallographic data for 2′,3′-cyclic ribonucleotides and 2,2′-cycloarabinonucleosides in which a γ^t or γ^- conformation is associated with a pentose pucker close to the O-4′-*exo* state.

Conformation about the C-4–C-5 *bond.* The γ^+ region is the most highly populated. Stable conformers with a γ^t or γ^- orientation occur closer to the O-4-*exo* ($P = 270°$) state than conformers in which the orientation about the C-4–C-5 bond is γ^+. This result seems to support the hypothesis that interconversion by pseudorotation through the O-4-*exo* conformation necessitates the γ^t or γ^- conformation.

The Molecule in Solution.—Furanose ring conformation. The molecular conformations chosen as models of Me-ribose in solution are characterized by ring conformations defined by P from -25 to $-5°$ in the N region of the pseudorotation wheel, and P from 177 to 223° in the S region. Particular stable states of the pentose ring correspond to different conformations of the exocyclic hydroxy groups.

CFF calculations performed in this study indicate that in Me-ribose the sugar ring favours N-type conformations (Table 4), and the mean free enthalpy difference $\Delta\tilde{G}_{NS}$ is -3.2 kJ mol^{-1}. The favoured range of P values found is in agreement with the conformational preferences of the C-1-*endo* form proposed for methyl β-D-ribofuranoside on the basis of ^{13}C n.m.r. measurements.[20] It is also consistent with the estimated population of N-type conformers obtained from ^{1}H n.m.r. measurements[8] and agrees with the predominance of N forms in the furanose ring in ribo-nucleosides and -nucleotides in solution.[24]

Some comments on the interconversion by pseudorotation of the furanose ring and on the energy barrier to such a process may be made on the basis of the results obtained in this study. All typical conformations of the 2- and 3-OH groups were examined in the initial molecular geometries used. Most initial conformations about the C-2–O-2 and C-3–O-3 bonds were not modified in energy minimization. This suggests that a single path of interconversion by pseudorotation does not exist. There seem to be as many routes of interconversion in the multidimensional pseudorotational space as there are stable conformations of the exocyclic groups.

A crude estimate of the highest energy barrier to pseudorotation may be obtained from the greatest free enthalpy difference between stable N and S forms. For models of Me-ribose in solution the greatest free enthalpy difference between N and S conformers with the same conformation about the C-4–C-5 bond is 14.3 kJ mol^{-1}. Hence the highest barrier to pseudorotation in Me-ribose in solution must be greater than 14.3 kJ mol^{-1}. This estimate is in good agreement with evaluations of the activation energy for internal motions associated with the ring carbon atoms in ribonucleosides (15—20 kJ mol^{-1}) obtained from n.m.r. measurements of ^{13}C longitudinal relaxation rates.[25]

Comparison of calculated and experimental coupling constants. Calculation of proton–proton coupling constants yields an acceptable agreement between calculated and experimental values of J_{12}, J_{23}, and J_{34} (Table 5). Some aspects of the results attained should nevertheless be commented upon. The root mean square deviation in the calculated couplings (0.8 Hz) is slightly higher than the values usually obtained by Altona and his co-workers in their reconstruction of molecular geometries from $^3J_{HH}$ coupling constants.[26,27] It nevertheless lies below the limiting value of 1 Hz considered by these workers to indicate serious errors in the model used. The results presented in this paper have been obtained by means of an energy-calculating program which was devised as a method of simulating geometrical and energetic properties of molecular systems. The aim of the CFF method does not consist in a simple geometrical reconstruction of a molecular model using vicinal coupling constants.

Moreover, in the prediction of stable states of models for Me-ribose in solution interactions with solvent molecules were not included in the calculations; solute–solvent interactions may modify the geometry and/or the conformational distribution of the solute molecules.

If one additionally takes into account that the root mean square deviation in the experimental coupling constants is substantial (0.4 Hz), the results presented here should be considered an excellent achievement of the CFF method in simulating the properties of the furanose ring in Me-ribose.

Conformation about the C-4–C-5 *bond.* The preferred conformation about the C-4–C-5 bond was found to be γ^-, followed by γ^t, while the population in the γ^+ region is practically negligible (Table 4). This result is at variance with ^{1}H n.m.r. measurements,[8] which indicate that the γ^t and γ^+ conformers are nearly equally favoured, and the γ^- region is less populated. The overwhelming preference of the γ^- rotamer shows that the conformers selected among all the stable forms of the isolated molecule do not adequately reproduce the solution conformational behaviour of the CH_2OH group in Me-ribose.

It is known that solute–solvent interactions influence the orientation of the CH_2OH group in ribose derivatives. In 3-methyl-2′,3′,-isopropylideneuridine,[28] for example, an increase in the polarity of the solvent causes a decrease in the γ^+ population. The introduction of solute–solvent terms into the force field used, or a study of the hydration of Me-ribose by means of molecular dynamics, should give way to a better prediction of the conformational distribution about the C-4–C-5 bond.

Conclusions.—Energy calculations performed on methyl β-D-ribofuranoside show that the conformational analysis of ribose cannot be restricted only to the ring atoms. Stable ring states in Me-ribose occur in the N and S regions of the pseudorotational wheel; with each region there are associated a number of local minima, corresponding to different orientations of the exocyclic OH groups and of the CH_2OH group. The manifold of local minima indicates that there exists no single route of interconversion by pseudorotation of the furanose ring. There exist as many paths of interconversion as there are stable conformations of the exocyclic groups.

For an isolated molecule the average ring conformation in the N and S domains has been found to be C-1-*endo*-C-2-*exo* and C-2-*endo*-C-3-*exo*, respectively. The amplitude of pucker τ_m is greater for N-type conformers. The calculated mean free enthalpy difference between N and S conformers is 6.4 kJ mol^{-1}. The most favoured conformation about the C-4–C-5 bond is γ^+.

For models of the molecule in solution the average N-type ribose ring has a C-2-*exo* geometry, while the average S-type ring has a C-3-*exo* geometry. N conformers have a greater amplitude of pucker than S conformers. The mean free enthalpy difference between N and S states is -3.2 kJ mol^{-1}. These results are in good agreement with experimental n.m.r. data. J_{12}, J_{23}, and J_{34} coupling constants calculated by means of a modified Karplus equation using dihedral angles derived from the final stable geometries differ on the average by 0.8 Hz from the measured values. This is a surprisingly good agreement, and it may be considered a successful test of the CFF method, which was not after all devised to reconstruct the geometry of five-membered rings from vicinal coupling constants.

The most favoured orientation of the CH_2OH group in models of the molecule in solution has been found to be γ^-, which is at variance with n.m.r. results. It is expected that the addition of solute–solvent interaction terms to the potential

energy would improve the simulation of solution conformations.

Acknowledgements

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