ChemComm

Furanosic forms of sugars: conformational equilibrium of methyl β-D-ribofuranoside†

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The investigation of an isolated ribofuranose unit in the gas phase reveals the intrinsic conformational landscape of the biologically active sugar form. We report the rotational spectra of two conformers of methyl β-D-ribofuranoside in a supersonic jet expansion. Both conformers adopt a near twisted ($^3T_2$) ring conformation with the methoxy and hydroxymethyl substituents involved in various intramolecular hydrogen bonds.

Sugars are flexible polymorphic species, exhibiting complex constitutional, configurational and conformational isomerism. The intramolecular reaction between carbonyl (typically reducing terminus) and hydroxyl groups gives rise to cyclic hemiacetal/ketals, particularly stable for five- or six-membered ring forms (furanose or pyranose, respectively, Scheme 1). Large amplitude motions, like ring puckering, inversion or pseudorotation, combine with the internal rotation of the hydroxyl groups to produce a rich conformational landscape, even in the most elementary monosaccharide units. A recent microwave spectroscopy study on ribose proved that this aldopentose is a pyranose (1) in the gas-phase, with six coexisting low-energy ($\Delta E < 6$ kJ mol^{-1}) conformers differing in ring conformation ($^1C_4$ or $^4C_1$) and epimerization (α/β).[1] Other rotational[2] and vibrational[3] studies of five- or six-carbon monosaccharides also confirmed the pyranose preference of free molecules, which had also been previously observed in the crystal[4,5] and liquid phases.[6] However, the preferred ribopyranose form starkly contrasts with the biological use of five-membered β-ribofuranose rings (2).

Ribofuranoside rings play different biochemical functions. In most species they appear as informational molecules and catalysts
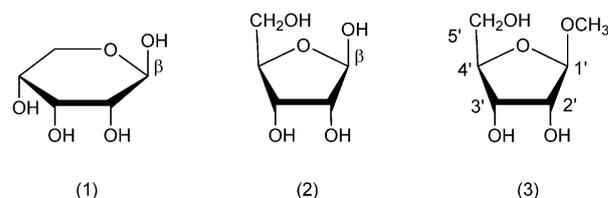
Scheme 1 The pyranose (1) and furanose (2) constitutional isomers of ribose, together with methyl-β-D-ribofuranoside (3), in Haworth projection.

(RNA), as substrates (ATP or sugar-diphospho-nucleosides), or as cofactors (NAD(P) or NAD(P)H).[7] Remarkably, their roles are often critical: DNA analogues in which the furanose rings are exchanged by pyranoses produce double helices with much stronger base pairing, but are unsuitable to replace DNA.[8] The biochemical functionality in ribose-based biomolecules probably relies on multiple related factors and functional optimization does not necessarily correlate with simple properties in the ground state. Changes in the furanose conformation can also critically modulate the ability of nucleosides to act as substrates with enzymes associated with disease processes, *e.g.* HIV-1 reverse transcriptase.[9] Furanosides also appear as part of oligosaccharides in plants and microbial organisms like bacteria, fungi and parasites, although not in humans or in mammals.[10]

Ultimately, the evolutionary preference for furanoses in RNA and other biomolecules may have a chemical origin, associated perhaps with the greater or differing flexibility of the five-membered ring. Saturated five-membered rings are structurally unique, as the small differences in energy between twisted and envelope forms give rise to pseudorotation, a quasi-monodimensional large-amplitude-motion in which the puckering rotates around the ring.[11] The furanose ring-puckered species thus may interconvert without passing through the planar species, making it difficult to specify conformational properties and pseudorotation pathways. The first pseudorotation model for nucleosides was developed by Altona and Sundaralingam (AS).[12] Cremer and Pople (CP) later solved the puckering problem using vibrational analysis and developed systematic curvilinear CP coordinates,[13] generally applicable to

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any cycloalkane. Both models define furanoside puckering in terms of amplitude (q) and phase coordinates (φ_{AS} and φ_{CP} phase conventions are shifted by a constant angle). Noticeably, a first survey of crystal structures revealed that furanoside conformations in nucleosides display a bimodal distribution,[14] with two preferred puckering regions around $\varphi_{CP} = 288°$ ($\varphi_{AS} = 18°$, envelope $^3E \equiv C_3'$-*endo*)[15] $\varphi_{CP} = 72°$ ($\varphi_{AS} = 162°$, envelope $^2E \equiv C_2'$-*endo*). This two-state model has been instrumental for puckering determination in solution using NMR vicinal spin–spin coupling parameters.[16] More recent structural investigations on furanose glycosides showed a similar clustering in the conformational space, but they were heavily dependent on the pentose configuration.[10] In particular, β-ribofuranosides are located around the puckering phases of $\varphi_{CP} = 252°$ (envelope E_2) in the solid state. Recurrently, the question thus arises: does the furanose conformation in the crystal or solution reflect the intrinsic ring puckering properties, or is it imposed by the environment?

We answer this question by exploring the molecular structure of the five-membered ring unit of an isolated monosaccharide in the gas-phase. We analyzed methyl β-D-ribofuranoside (3) using a combination of chemical synthesis, microwave spectroscopy, supersonic jet expansion techniques, ultrafast laser vaporization and computational methods. The target pentose was specifically synthesized (see the ESI†) to lock the molecule as a five-membered ring, preventing the formation of the pyranose form dominant in the gas phase. Our main objectives include the observation of conformational preferences of furanose forms in C5 and C6 sugars, the determination of the number of coexisting species of the free molecule, and the comparison with the structural data in condensed phases. The conformational landscape was explored at different levels, including ring-puckering preferences, dynamics of the hydroxyl and hydroxymethyl groups, intramolecular hydrogen bonding and internal rotation of the methyl group. Our results give a valuable perspective on the intrinsic structural preferences of this biologically important aldopentofuranose, clarifying previous X-ray and neutron diffraction,[17] NMR[18] and *ab initio* data.[19]

The computational study included a comprehensive conformational search combining molecular mechanics (MMFFs) and special search algorithms based on stochastic and vibrational mode analysis,[20] together with *ab initio* (MP2) and density-functional-theory (M06-2X, B3LYP) molecular orbital reoptimizations, which refined the initial estimations. The final conformational energies and rotational constants are in Tables S1 and S2 (ESI†), while Table 1 gives the puckering properties, dipole moments and Gibbs energies for the six conformers with energies within 5 kJ mol^{-1}.

The calculated conformational stabilities were checked against the experimental microwave spectrum in the 6–14 GHz frequency region. The molecular jet was probed using a Fourier-transform microwave (FT-MW) spectrometer equipped with an UV ultrafast laser vaporization system[1] (experimental details in the ESI†). Two different sets of rotational transitions were independently assigned (Fig. 1). The first set was composed exclusively of R-branch ($J + 1 \leftarrow J$) μ_c-type transitions, with angular momentum quantum numbers spanning values $J = 2$–6. The second set included only R-branch μ_b-type rotational transitions ($J = 2$–7).

Table 1 Prediction of conformational energies for methyl β-D-ribofuranoside

Species[a]	ΔG^b/kJ mol^{-1}	q^c/Å	φ/deg	$\mu_a{}^d$/D	μ_b/D	μ_c/D
1 ($^3T_2$)	0.0/0.0/0.0	0.385	264.7	0.0	−0.3	−1.2
2 ($^3T_2$)	0.8/1.2/0.9	0.397	260.7	−0.9	−3.5	−0.9
3 ($^3T_2$)	2.9/0.4/2.2	0.397	266.4	−1.6	−3.5	1.7
4 (E_2)	3.5/4.6/2.9	0.400	252.3	1.3	−4.1	0.7
5 (^2E)	3.7/2.0/1.5	0.382	62.7	−2.1	−0.3	−0.5
6 (E_2)	4.9/5.0/3.6	0.391	247.8	−0.9	−1.4	2.9

[a] Conformational notation in ref. 15. [b] MP2/M06-2X/B3LYP calculations with a 6-311++G(d,p) basis set; relative Gibbs free energies at 298 K. [c] Cremer–Pople puckering parameters defined in ref. 13. [d] Electric dipole moment components (μ_a, μ_b, μ_c).
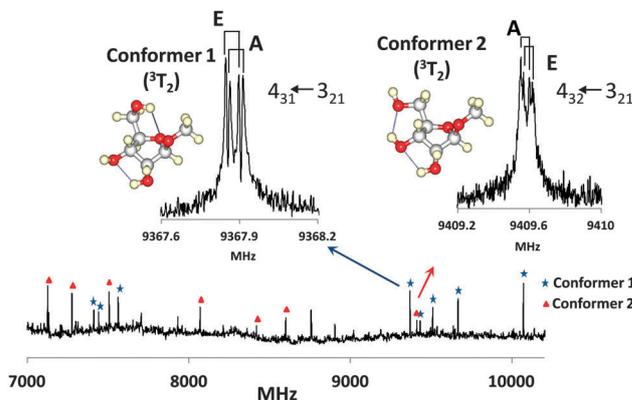
Fig. 1 A section of the experimental spectrum of methyl β-D-ribofuranoside in the region 7–10 GHz, with two typical rotational transitions split by internal rotation of the methyl group (each transition additionally split by Doppler effect).

Both datasets were mutually exclusive and they clearly corresponded to two different carrier species. Their similarity in rotational constants suggested that they are indeed isomers of the same molecule. Some of the observed lines showed small hyperfine effects for the two species, splitting some individual transitions into two close components separated by less than 20 kHz.

The hyperfine effects were attributed to the internal rotation of the O-1 methyl group in the ribofuranoside, detectable in the rotational spectra for small or moderate potential energy barriers.[21] The experimental observations were reproduced to experimental accuracy using a semirigid-rotor Watson Hamiltonian (S-reduction).[22] The internal rotation effects were analyzed using Wood's internal-axis-method (IAM).[23] Table 2 presents the results of least-squares fits of the experimental transitions of both conformers, which yielded accurate values of the rotational constants, the quartic centrifugal distortion constants and the internal rotation barrier height. The full set of transitions is collected in Tables S3 and S4 (ESI†). No lines attributable to other conformers were observed in the spectrum.

The conformational assignment of the two isomers of Fig. 2 relied on multiple arguments. An initial comparison of the rotational constants in Table 2 showed good agreement between the two lowest-lying conformations (relative errors below 1–2%). The prediction of (harmonic) centrifugal distortion constants was also consistent in magnitude and sign with the proposed

Table 2 Experimental parameters for the two detected conformations of methyl β-D-ribofuranoside, and comparison with the MP2 predictions

	Conf. 1		Conf. 2	
	Experiment	Theory	Experiment	Theory
A/MHz	$1307.1463(55)^a$	1295.21	1443.5094(14)	1438.72
B/MHz	1095.47128(50)	1101.10	924.98477(53)	943.70
C/MHz	717.8046(49)	718.90	661.97710(32)	674.86
D_J/kHz	0.953(81)		0.2752(48)	
D_{JK}/kHz	−0.339(88)		−1.264(27)	
D_K/kHz			1.702(97)	
d_1/Hz	0.723(66)		−0.1004(31)	
d_2/Hz	−0.277(24)			
$V_3{}^b$/kJ mol^{-1}	7.304(13)	7.90	7.503(64)	8.23
σ^c/kHz	4.7		3.0	
N^d	46		32	

[a] Uncertainties in units of the last digit. [b] Internal rotation barrier. The geometrical parameters I_α (=3.195 μÅ²) and $<(i,a)$, $<(i,b)$, $<(i,c)$ (=23.1°, 81.3°, 68.8°, 51.4°, 43.9° and 72.3°, respectively, for conformers 1 and 2) have been fixed to the *ab initio* values. [c] Standard deviation of the fit. [d] Number of transitions.
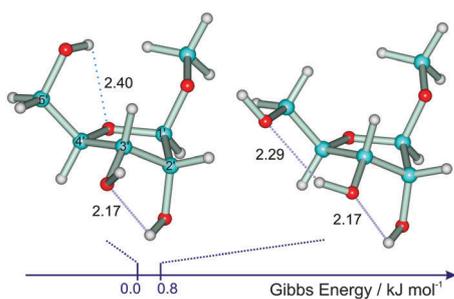
Fig. 2 Intramolecular hydrogen bonding distances (Å) and relative Gibbs energy (MP2) for the two observed conformers of methyl β-D-ribofuranoside.

Fig. 3 (a) Upper panel: Ring-puckering predictions (1–12) for methyl β-D-ribofuranoside in the gas phase (experimental conformers with green solid circles). (b) Lower panel: A survey of 30 fragment structures of furanose glycosides in the Cambridge Structural Database[10] (blue crosses), together with 2 neutron diffraction structures of methyl β-D-ribofuranoside[17] (red circles).
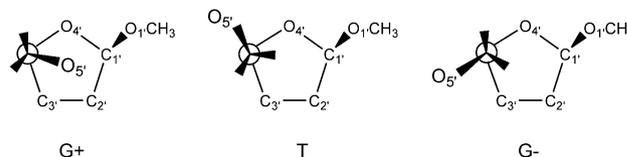
Scheme 2 Notation for staggered orientations around the $C_{4'}$–$C_{5'}$ bond.

assignment. Finally, the methyl group V_3 barrier increase for conformer 2 (V_3 = 7.3 *vs.* 7.5 kJ mol^{-1}) is in agreement with the *ab initio* predictions (7.9 *vs.* 8.2 kJ mol^{-1}). This evidence fully confirms the conformational assignments in Table 2 and Fig. 2 (interactive 3D model in Fig. S1 and S2, ESI†).

The ring-puckering properties were analyzed quantitatively, using the CP coordinates[13] in Fig. 3. The two detected isomers have similar puckering phases (φ_{CP} = 264.7° and 260.7°, respectively), intermediate between a twisted-$^3T_2$ and an envelope-E_2 ring conformation. Puckering amplitudes are also very similar (q = 0.385 and 0.397 Å, respectively). The hydroxymethyl side chain adopts the gauche staggered orientation of Scheme 2, either G$^+$ ($\tau_1(O_{5'}-C_{5'}-C_{4'}-C_{3'})$ = +56.2°) for the global minimum or G$^-$ (τ_1 = −58.1°) for the second conformer. Conversely, the methyl group is always *trans* with respect to $C_{2'}$ (T: $\tau_2(C_M-O_{1'}-C_{1'}-C_{2'})$ = 174.4° and 178.9°, respectively), so the observed conformations are denoted $^3T_2$ G$^+$T (global minimum) and $^3T_2$ G$^-$T.

The two ring hydroxyl groups, on opposite sides of the methoxy and hydroxymethyl substituents, are finally arranged to reach the highest possible number of internal hydrogen bonds. The global minimum exhibits two unconnected hydrogen bonds: a weak contact $O_{5'}$–H···$O_{4'}$ with the hydroxymethyl group (MP2: r(H···$O_{4'}$) = 2.4 Å) and a $O_{2'}$–H···$O_{3'}$ link (MP2: r(H···$O_{3'}$) = 2.17 Å). The second

conformer displays a network of two successive $O_{2'}$–H···$O_{3'}$–H···$O_{5'}$ hydrogen bonds involving three hydroxyl groups (MP2: r(H···$O_{3'}$) = 2.17 Å, r(H···$O_{5'}$) = 2.29 Å).

In conclusion, the combination of rotational data and *ab initio* calculations provides a direct comparison between β-ribofuranoside ring conformations in the gas phase and crystal structures. The isolated molecule preferably occupies three regions of the conformational space, represented by the $^3T_2$–E_2, ^2E and ^4E structures in Fig. 3 (*ab initio* structures in Tables S5–S7, ESI†).

The three most stable structures (<3 kJ mol^{-1}) share a twisted $^3T_2$ and envelope E_2 character, but most of the following conformations up to *ca.* 8 kJ mol^{-1} move toward the neighboring envelope E_2. The higher-energy forms of the isolated molecule also comprise envelopes ^2E and ^4E, which appear at relative energies above 3.7 and 7.3 kJ mol^{-1} (MP2), respectively, and become the most abundant in the upper range of the analyzed energy window. The two observed conformations for the isolated molecule simply represent alternative arrangements of the sugar hydroxymethyl side-chain (the third conformer being equivalent to the global minimum with reversed $O_{3'}$–H···$O_{2'}$ hydrogen bond orientation). In previous rotational studies it was argued that

intramolecular hydrogen bonding is the primarily stabilizing effect in isolated monosaccharides.[1,2] In methyl β-D-riboſuranoside the hydrogen bond pattern seems to favor conformer 2, predicted only 0.8 kJ mol^{-1} (MP2) to 1.2 kJ mol^{-1} (M06-2X) above the global minimum. However, the predicted stability could also reflect other contributions, like hyperconjugative effects, previously suggested in tetrahydrofuran[24] and other monosaccharides. Unfortunately, the small energy difference between both conformations cannot be verified experimentally, as the lack of common selection rules precludes the estimation of the intensity ratios. Noticeably, the neutron diffraction experiments of methyl β-D-riboſuranoside also identified two conformations with E_2 ring-puckering ($\varphi_{CP} = 258.6°$, 249.6°), relatively close to the $^3T_2$–E_2 structures observed here.[17] The two observed crystal structures mostly differ in the orientation of the ring substituents, which are both *trans* oriented to favor intermolecular hydrogen bonding, concomitant with this condensed phase. No intramolecular hydrogen bond is apparent in the crystal structures.

The comparison between the conformational behavior of an isolated unit of methyl β-D-riboſuranoside and that of the molecule embedded in a crystal matrix reveals features of the factors affecting the molecular structure. The ring puckering characteristics of the riboſuranose unit are very similar in the gas and crystal phases, and clearly suggest that the puckering forces associated with the minimization of the ring strain originate from the ring configuration and number and position of substituents, and not from crystal forces. This argument is reinforced by the comparison of the puckering characteristics of a set of 30 structural fragments in the Cambridge Structural Database (CSD), in Fig. 3, similarly centered around E_2. Furthermore, crystal data show that a change in the substituents' stereochemistry radically alters the ring puckering.[10] This view is confirmed by previous NMR studies, emphasizing the dominant influence of the anomeric substituent on the ring conformation in the liquid phase.[25] Natural Bond Orbital calculations in Table S8 (ESI†) are consistent with electronic hyperconjugation originated by the methoxy and ring oxygen atoms (*endo/exo* anomeric effects), but its contribution is relatively similar for the four lowest lying conformers. The molecular stability would thus primarily result from a combination of orbital effects reducing the ring strain, complemented with the effects of intramolecular hydrogen bonding. As a consequence, the presence of the heterocyclic bases found in nucleosides surely influences the conformational properties of the flexible ring, thereby determining its final biological properties.

The results for methyl β-D-riboſuranoside highlight the value of current crystal structures but also call for additional gas-phase experiments on related furanosides, nucleosides and nucleotides, both to assess the theoretical models and to compare with conventional data from condensed media. Considering the vital role of riboſuranosides in all life forms, it is remarkable that their structural properties free of any environmental effects have been unknown until now. These new results provide empirical data that may help to provide information about structural, predictive and enzymological RNA (and DNA) biology. In this sense, the emergence of new techniques in rotational spectroscopy is helping to provide a global view of the inherent structural properties of these biomolecular building blocks.

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