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Insights into Furanose Solution Conformations: Beyond the Two-State Model

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Abstract

A two-state model is commonly used for interpreting ring conformations of furanoses based on NMR scalar ^3J -coupling constants, with the ring populating relatively narrow distributions in the North and the South of the pseudorotation itinerary. The validity of this simple approach has been questioned, and is examined here in detail employing molecular dynamics (MD) simulations with a new GLYCAM force field parameter set for furanoses. Theoretical ^3J -coupling constants derived from unrestrained MD simulations with the new furanose-specific parameters agreed with the experimental coupling constants to within 1 Hz on average. The results confirm that a two state model is a reasonable description for the ring conformation in the majority of methyl furanosides. However, in the case of methyl α -D-arabinofuranoside the ring populates a continuum of states from North to South via the eastern side of the pseudorotational itinerary. Two key properties are responsible for these differences. Firstly, East and West regions in β - and α -anomers, respectively, are destabilized by the absence of the anomeric effect. And, secondly, East or West conformations can be further destabilized by repulsive interactions among vicinal hydroxyl groups and ring oxygen atoms when the vicinal hydroxyl groups are in *syn*-configurations (such as in ribose and lyxose) more so than when in *anti* (arabinose, xylose).

Keywords

Pseudorotation; Anomeric effect; Potential energy surface; Molecular dynamics simulation; GLYCAM; AMBER

Introduction

Furanoses are essential components in the backbone of nucleic acids (RNA and DNA) and are also frequent components of complex polysaccharides found in organisms ranging from bacteria to protozoa, fungi and plants (Taha et al. 2013). Their conformational properties affect the structure and recognition of the polymers in which they are found. In marked contrast to six-membered sugar rings (pyranoses), the five-membered forms (furanoses) exhibit a high level of internal ring flexing (Bartenev et al. 1987; Levitt and Warshel 1978; Seo et al. 2008). As a result, furanoses can interconvert between multiple ring conformations (Houseknecht et al. 2003a), whereas pyranoses are usually found in single, energy-favorable chair conformation (Angyal 1984). This feature of furanoses greatly complicates the

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development and interpretation of structure-function relationships for these small but crucial molecules.

The conformational analysis of ring conformations of carbohydrates molecules in solution relies heavily on deconvoluting NMR scalar ^3J -coupling constants into ring puckering geometries and populations (De Leeuw and Altona 1983; Padrta and Sklenar 2002; Sun et al. 2004). In the case of furanoses, interpreting their conformational populations from ^3J -coupling constants typically requires assumptions regarding to the number of states that are present. In the two-state model introduced by Altona et al. (1972) to interpret the ring conformations of the ribofuranosyl ring in nucleic acids, it is assumed that the rings populate only two states (Altona and Sundaral.M 1972; Altona and Sundaralingam 1973), referred to as North and South, with respect to their location on the pseudorotational itinerary (Fig. 1). However, the validity of a two-state model has been questioned when applied to other furanoses, particularly in the case of arabinofuranose (Hendrickx et al. 2010; Seo et al. 2008; Taha et al. 2010; Taha et al. 2011). An alternative approach that has shown promise, is to perform MD simulations of the furanose (Hatcher et al. 2009), and then back-calculate the ^3J -values directly from the MD data (Hendrickx et al. 2010; Raman et al. 2010; Taha et al. 2010). This approach eliminates the need for assumptions regarding states, but requires an accurate force field, adequate conformational sampling, and a reliable method for deriving scalar couplings from the MD trajectory. In general, the calculation of NMR ^3J -values from MD data can be performed in one of two ways, most commonly by employing a Karplus-type relationship to convert torsion angles between coupled spins into ^3J -values (Altona et al. 1994; Bose et al. 1998; Cloran et al. 1999; Haasnoot et al. 1980; Houseknecht et al. 2003b; Stenutz et al. 2002; Taha et al. 2010; Taha et al. 2011; Zhao et al. 2007), or by computing the ^3J -values quantum mechanically (QM) for each state (Gonzalez-Outeirino et al. 2006). The latter approach eliminates the approximations implicit in Karplus-relationships, but is far more computationally demanding, and has not yet been applied to furanoses, although it has shown benefits when applied to analyses of pyranose conformational properties (Gonzalez-Outeirino et al. 2006).

Previously, the GLYCAM06 force field (Kirschner et al. 2008) was created to be applicable broadly to a range of carbohydrates, including pyranoses and furanoses, however, MD simulations of furanoses have not resulted in consistently acceptable reproduction of solution NMR properties (Seo et al. 2008). Therefore, developing a reliable force field for furanoses that is capable of describing their conformational properties in solution, is essential for both theoretical and experimental studies of these molecules. As might be expected, the fluxional properties of furanosyl rings leads to unique challenges in developing a force field for these molecules, requiring long MD simulation times to ensure converged conformational sampling. In addition, the five-membered ring has more strain than found in pyranoses, as evidenced by the compression of bond angles, and this feature places unique demands on the derivation of the torsion terms relevant to the ring.

To address the question of the apparent discrepancy between MD-derived and NMR-derived ring populations in furanoses (Seo et al. 2008), we begin by first developing a set of furanose-specific parameters that are compatible with the GLYCAM06 force field (Kirschner et al. 2008), which are able to reproduce the quantum-computed relative energies

of the ring as it traverses the pseudorotational itinerary. Having derived a suitable set of parameters, MD simulations were performed in explicit water for both α and β anomers of the four methyl D-aldofuranosides: methyl D-arabinofuranoside (**1**), methyl D-lyxofuranoside (**2**), methyl D-ribofuranoside (**3**), methyl D-xylofuranoside (**4**), as well as methyl 2-deoxy- β -D-ribofuranoside (**5 β**) (Fig. 2). ^3J -coupling constants derived from the MD simulations of the examined furanosides with this new set of parameters were found to be in agreement with NMR ^3J -values to within an average error of less than 0.9 Hz. More importantly, ring conformations from the MD simulations have shown the two-state model is a reasonable description of the ring properties in the majority of the furanosides, but not in the case of the arabino configuration (**1 α**). Further analysis indicated that, for the two-state model to be applicable, barriers in the East and West must exist to confine the conformations to low-energy regions in the northern and southern quadrants of the pseudorotational surface. The relative stability of northern and southern conformations has been rationalized in terms of the numbers of stabilizing gauche effects between vicinal oxygen atoms (Callam and Lowary 2001; D'Souza et al. 2000; Plavec et al. 1993; Thibaudeau and Chattopadhyaya 1997) as well as the presence of the anomeric effect (Callam and Lowary 2001; Cosse-Barbi and Dubois 1987; Cossé-Barbi et al. 1989; D'Souza et al. 2000; Ellervik and Magnusson 1994; Houseknecht et al. 2003a; Plavec et al. 1994; Plavec et al. 1996; Plavec et al. 1993; Thibaudeau and Chattopadhyaya 1997; Thibaudeau et al. 1994). Here, we find that whether or not there is a barrier in the eastern or western regions depends on the absence of an anomeric effect, as well as the presence of unfavorable *syn*-orientations of vicinal hydroxyl groups. In the unique case of **1 α** , there is no barrier in the eastern quadrant due to the absence of any conformation in which hydroxyl groups can adopt *syn*-orientations, enabling the furanose to populate a continuum of states from North to South.

The present analysis provides an independent method for interpreting furanose conformational properties that does not rely on experimental NMR data as constraints, nor on assumptions of preferred states. In addition, QM calculations have been employed here to quantify for the first time the strength of the anomeric and exo-anomeric effects in furanosides.

Results & Discussion

Parameters

New atom type—In order to develop the parameters specific for furanoses, which are orthogonal with the current parameters for pyranoses, two new atom types were introduced: “*Cf*”, representing ring carbon atoms, and “*Of*”, representing the ring oxygen atom (Fig. S1). The “upper case lower case” naming of atom types in AMBER is reserved for use with carbohydrates in the GLYCAM force field. Van der Waals terms for *Cf* and *Of* were assumed to be the same as for the corresponding atoms in pyranoses (Kirschner et al. 2008). Ensemble-averaged partial atomic charges for the new atom types, as well as all other atoms (Table S1 and Table S2), were computed according to the standard GLCYAM protocol (Kirschner et al. 2008), discussed in METHODS.

Bond length and bond angle parameters

The average bond lengths for C-C and C-O bonds in furanose ring are nearly identical with those in pyranose ring (Ishii et al. 2014; Ishii et al. 2015; Longchambon et al. 1976; McDonald and Beevers 1952; Ohanessian et al. 1977; Ohanessian and Gillierpandraud 1976). Therefore, the bond length parameters for furanose ring were imported from GLYCAM06, and simulations with these parameters demonstrated good agreement with the corresponding crystallographic values (Table S3 and Table S4).

In earlier bond angle parameter development for pyranoses in GLYCAM06 (Kirschner et al. 2008), acyclic molecules were distorted from their equilibrium geometries in order to determine the force constants and equilibrium angle values. Similarly, bond lengths were stretched or compressed to determine appropriate bond parameters. However, to determine these values for furanoses, an alternative procedure was adopted, in which the ring was distorted by twisting the relevant torsion angles over a range of ± 50 degrees. QM energy profiles for valence bond and angle parameter fitting were generated by optimizing tetrahydrofuran (THF) structures with ring torsion angles changing from -50° to 50° in 5° increments. Due to the symmetry of THF, only three energy profiles were necessary, namely for the C1-C2-C3-C4, C2-C3-C4-O4 and C3-C4-O4-C1 torsion angles. Parameters for the *Cf-Cf-Cf*, *Cf-Cf-Of* and *Cf-Of-Cf* angles were derived simultaneously by minimizing the differences between QM and molecular mechanics (MM) ring distortion energies. To assess the performance of the new parameters in reproducing the QM energy profiles, the errors between QM and MM energies were computed over the entire range of the distortion curves ($\langle \text{Error} \rangle_{\text{all}}$), as well as for the low energy conformations ($\langle \text{Error} \rangle_{\text{low}}$). The new parameters reproduced the gas-phase relative energies for THF markedly better than GLYCAM06 (Fig. S2). The $\langle \text{Error} \rangle_{\text{all}}$ for the new parameters for the three energy profiles in Fig. S2 is 0.1 kcal/mol, and in contrast to GLYCAM06, the new parameters better reproduced the QM energies in high energy regions.

Average values for the C-C-C, C-C-O, and C-O-C angles were computed from 300 ns MD simulations and compared to relevant crystallographic values (Table S3 and Table S4). Although both C-C-O and C-O-C angles showed larger differences from the crystallographic values, they are within the standard deviation.

Torsion angle parameters

Torsion terms associated with the five-membered ring were optimized based on their ability to reproduce the QM pseudorotational energies for discrete conformations of the ring generated at 1° increments in the ring phase angle (P). In this procedure several contributing torsion terms could be simultaneously optimized. Simultaneous parameter fitting has been applied recently in the generation of a set of force field parameters for protein modeling (Cerutti et al. 2014). Model structures were selected that nevertheless enabled the fitting of as few simultaneous torsion terms as possible, in order to minimize the number of new torsion terms, maximize parameter transferability, and provide insight into the underlying structural preferences (Table S5). All other torsion terms were generated directly by fitting to internal rotational energies. In order to reduce energy variations originating from interactions involving exocyclic moieties, the conformation of each of the exocyclic moieties

was restrained throughout the MM and QM pseudorotation energy minimizations (Table S5).

To assess the performance of the torsion angle parameters, the average errors between QM and MM energies were computed for the entire energy curves, $\langle |\text{Error}| \rangle_{\text{pseudo}}$, as well as for the minima, $\langle |\text{Error}| \rangle_{\text{minima}}$. The parameters resulting from the development protocol discussed below are presented in Table S6.

THF

The torsion terms (*Cf-Cf-Cf-Cf*, *Cf-Cf-Cf-Of* and *Cf-Cf-Of-Cf*) derived simultaneously from fitting to the pseudorotational energies for THF (**6**) are fundamental to all furanoses. The agreement between the MM and QM energies (Fig. 3) for the pseudorotational energy of THF computed with the optimized torsion parameters was within 0.1 kcal/mol for both $\langle |\text{Error}| \rangle_{\text{pseudo}}$ and $\langle |\text{Error}| \rangle_{\text{minima}}$. It is notable that the energy minima for the THF pseudorotation was observed to lie in the East/West quadrants, rather than North/South, as typically observed for furanoses. This observation suggests that the exocyclic groups in furanoses alter the conformational preferences of the five-membered ring, although the barriers to interconversion are less than approximately 0.5 kcal/mol. As can be seen in Fig. 3, the energy minima for the five-membered ring in THF occur at $P = 90$ and 270° , corresponding to East and West pseudorotational quadrants, respectively. In these conformations the C1-C2-C3-C4 torsion angle is 0° . However, in this conformation hydroxyl groups at C2 and C3 can be eclipsed (as in lyxose and ribose), disfavoring East/West conformations.

Models for mono- and dihydroxy substituents in furanoses

The ring conformation of furanoses is greatly affected by the configurations of the hydroxyl groups (Church et al. 1997; Houseknecht et al. 2003b; Kline and Serianni 1990; Serianni and Barker 1984; Taha et al. 2010; Taha et al. 2011), and presumably arises from a combination of steric and electrostatic interactions. These interactions may be between hydroxyl groups, as well as between the ring atoms and the hydroxyl groups. To reproduce the effect of these interactions on the ring pseudorotational energies, torsion terms were developed for the *Oh/Of-Cf-Cf-Oh*, and *Cf/Hc-Cf-Cf-Oh* sequences, employing the model compounds *cis*- (**7**) and *trans*-tetrahydrofuran-3,4-diol (**8**) and tetrahydrofuran-3-ol (**9**). As shown in Fig. S3, the new parameters reproduced the shapes of the QM pseudorotational energy curves for both molecules. In the case of **9**, the errors in the fits were similarly small. With the introduction of the vicinal hydroxyl groups, the minima now occur in the North/South regime, as expected in general for furanoses.

Models for pentofuranoses

To extend the model to pentofuranoses, a hydroxymethyl group was introduced into THF, which corresponds to the C4-substituent in pentofuranoses, employing 2-(hydroxymethyl)tetrahydrofuran (**10**) as the model compound. This molecule enabled us to develop the *Cf-Cf-Cf-Cg* and *Cf-Of-Cf-Cg* torsion terms (pertinent to the C2-C3-C4-C5 and C1-O4-C4-C5 atomic sequences). The fitting to the pseudorotational energies for **10** was performed for each of the three rotamers of the hydroxymethyl substituent (Fig. S4). While

the average errors were all less than 0.5 kcal/mol, not all low-energy states were well reproduced, however in the worst case the error was less than 1 kcal/mol. Similar agreements were obtained for *cis*- and *trans*-2-(hydroxymethyl)tetrahydrofuran-3-ol (**11** and **12**, respectively), which tested the ability of the parameters to reproduce the relative energies of the C4-C5 rotamers in the presence of a vicinal hydroxyl group at C3 (Fig. S5 and Fig. S6).

Models for methyl furanosides

The simplest model for the effect of anomeric configuration on five-membered ring conformational energy is (S)-2-methoxytetrahydrofuran (**13**), and, by analogy to the preceding section, the effect of interactions between the anomeric substituent and the adjacent hydroxyl group at C2 can be inferred from an analysis of *cis*- and *trans*-2-methoxytetrahydrofuran-3-ol (**14** and **15**, respectively). Torsion terms derived from fitting to **13** (*Cf-Of-Cf-Os* and *Cf-Cf-Cf-Os*), implicitly include any contributions from the anomeric effect (Callam and Lowary 2001; Cosse-Barbi and Dubois 1987; Cossé-Barbi et al. 1989; D'Souza et al. 2000; Ellervik and Magnusson 1994; Plavec et al. 1993; Thibaudeau and Chattopadhyaya 1997). In GLYCAM06, both the *endo*- and *exo*-anomeric torsion terms were parameterized by fitting to small acyclic molecular fragments, leading to the same set of terms for both applications. Here the torsion terms were derived for intact five-membered rings, with furanose-specific atom types for the ring atoms (Cf and Of), leading to unique terms for the *endo*- (*Cf-Of-Cf-Os*, and *Cf-Cf-Cf-Os*) and *exo*-anomeric (*Of-Cf-Os-Cf/Cg* and *Cf-Cf-Os-Cf/Cg*) sequences.

As shown in Fig. S7, the torsion angle terms for *endo*-anomeric sequence reproduced the lowest energy conformation, as well as the overall shape of pseudorotational energy curve. It should be noted that the new parameters lead to a local energy minimum at $P = 99^\circ$, while a similar minimum appears at $P = 71^\circ$ in the QM energy curve. The torsion angle parameters derived from **14** and **15** gave rise to slightly larger errors, reflecting in part the challenge of fitting highly asymmetric energy curves using a sum of symmetric cosine functions. The torsion angle terms for *exo*-anomeric sequence reproduced the rotational energy profiles of **13** in both northern ($P = 0^\circ$) and southern ($P = 180^\circ$) ring conformations (Fig. S8). Features of the *endo*- and *exo*-anomeric effects in furanoses are discussed in the following sections.

Endo-anomeric effect

In carbohydrates, the anomeric effect relates to the preference of the endocyclic C-O-C-O torsion angle to adopt the *gauche* orientation over the *anti* conformation (Cossé-Barbi et al. 1989; Jeffrey et al. 1978; Juaristi and Cuevas 1992), and has been estimated from quantum mechanical calculations for pyranoses (Lii et al. 2003; Senderowitz et al. 1996) and related acyclic fragments (Jeffrey et al. 1978; Lii et al. 2005) to be 1–2 kcal/mol. Its presence in furanoses has been invoked as contributing to ring conformational preferences (Callam and Lowary 2001; Cosse-Barbi and Dubois 1987; Cossé-Barbi et al. 1989; D'Souza et al. 2000; Ellervik and Magnusson 1994; Houseknecht et al. 2003a; Plavec et al. 1994; Plavec et al. 1996; Plavec et al. 1993; Thibaudeau and Chattopadhyaya 1997; Thibaudeau et al. 1994), and is consistent with the observation that electronegative substituents at C1 in furanoses prefers a pseudoaxial over a pseudoequatorial configuration,^{29–34} but it has not been

quantified computationally. We address this here by noting that, over the course of the pseudorotational itinerary for **13**, the endocyclic C4-O4-C1-O torsion angle spans a range of 80 to 160°, or pseudo-*gauche* to pseudo-*anti*.

Thus the strength of the *endo*-anomeric effect in furanoses can be estimated to be 3.2 kcal/mol at the B3LYP/6-31G* level. In contrast, methoxycyclopentane (**16**) shows no such effect (Fig. 4). The *endo*-anomeric effect stabilizes eastern ring conformations (C4-O4-C1-O angle is less than 100°) but not western conformations (C4-O4-C1-O angle is greater than 140°). Therefore, western conformations in α -anomers (or eastern conformation in β -anomers) display high potential energies.

Exo-anomeric effect

The preference of the exocyclic C1-O bond in pyranoses to adopt *gauche* orientations is a direct corollary to the *endo*-anomeric effect, and this *exo*-anomeric effect has been extensively studied in pyranoses and acyclic molecules (Jeffrey et al. 1978; Kirschner et al. 2008; Lemieux and Koto 1974; Tvaroska and Bleha 1989).

To determine the strength of the *exo*-anomeric effect in furanoses, the rotational energies of the O4-C1-O-CH₃ torsion angle in **13** were computed for both North ($P = 0^\circ$) and South ($P = 180^\circ$) conformations. The *exo*-anomeric energy stabilizes the *gauche* orientations of the C1-O bond in **13**, relative to the cyclopentane analog **16**, by approximately 4 kcal/mol, which is comparable to that reported for pyranoses (Kirschner et al. 2008) (Fig. 5). This contribution is particularly important for defining the 3D structure of polymers of furanoses, as it reduces the conformational freedom of the C1-O bond to effectively a single state.

Assessments of solution populations

The performance of the new parameters was assessed by comparing NMR ^3J -coupling constants for conformations observed in MD simulations (300 ns) of furanosides with their experimental values. Based on monitoring the P-values (Fig. S9) convergence in ring interconversion was obtained within this timescale. By employing quantum mechanical ^3J -coupling constants corresponding to the states observed in the MD simulation, it was possible to avoid employing empirical Karplus equations (Altona et al. 1994; Bose et al. 1998; Cloran et al. 1999; Haasnoot et al. 1980; Houseknecht et al. 2003b; Stenutz et al. 2002; Taha et al. 2010; Taha et al. 2011; Zhao et al. 2007) or invoking approximations associated with the two-state model. GLYCAM06 was developed for use with partial atomic charges derived from electrostatic fitting to QM values computed at the HF/6-31G* level, however, we wished to examine what impact might arise from fitting to potentials computed at a higher level of theory. Recently, it has been shown that dipole moments computed at the B3LYP/cc-pVTZ level have approximately one half of the average error of those computed at the HF/6-31G* level (Hickey and Rowley 2014), and so we compared data for monosaccharide charge sets computed at both levels.

Both charge sets led to very similar distributions of solution conformations, as indicated by the resulting J -couplings, which differ by on average 0.1 Hz (Table 1 and Table S7). Thus the following discussion focuses only on the data generated with the B3LYP/cc-pVTZ

charges. The average error between the computed and experimental ^3J -coupling constants for **1–4** (α and β) and **5 β** was within 0.9 Hz. It is notable that, while the overall agreement with experiment is good, in the case of **2 α** , the theoretical value for the $^3J_{C1,H4}$ coupling constant (0.6 Hz) is significantly below that reported experimentally (3.7 Hz). However, this coupling constant is less than 0.5 Hz in all other α -anomers, suggesting a potential error in the experimental data. In the case of **5 β** there is also a significant difference between the theoretical (9.2 Hz) and experimental (~5.7 Hz) values for the $^3J_{H2R,H3}$ coupling constant, however, the experimental report noted uncertainty in that particular measurement (Church et al. 1997).

The rotamer distributions for the C4-C5 were also generated, and the average error between theoretical and experimental ^3J -values associated with this bond for **1** (α and β), **3** (α and β) and **5 β** was 0.4 Hz; while that for **2** (α and β) and **4** (α and β) was 1.4 Hz (Table 2 and Table S8). The origin of the larger average error in these latter cases is uncertain, but may relate to the fact that the parameters for the C4-C5 bond were imported from GLYCAM06 and not re-derived herein. The overall correlation (R^2) between the theoretical and experimental ^3J -values for coupling constants related to ring conformation was computed to be 0.76 (see Fig. S10).

Two-state equilibrium model

The ring conformation distributions of **1–4** (α and β) and **5 β** in explicit solvent MD simulations are shown in Fig. 6. The majority of the furanoses populated conformations predominantly in the northern and southern regions, and could therefore be described as approximately satisfying a two-state distribution, regardless of the partial charge model. A notable exception to this was seen for **1 α** , which populated a continuum of states from North to South via the eastern side of the pseudorotational itinerary. This is in contrast both to the other furanoses, and to earlier studies of **1 α** , performed with GLYCAM06 that indicated a preponderant population of states in the North (Seo et al. 2008). The overall agreement between the theoretical and experimental NMR data from the present simulations provides compelling support for the present simulation results.

By plotting conformational energies onto the pseudorotational surface (Fig. 7 and Fig. S11), it is possible to conveniently visualize the presence or absence of energy barriers between northern and southern states. In the case of a furanoside such as **3 β** , that prefers conformations in the North and South, both quantum- and classically-computed conformational energies confirm the presence of barriers in the East and West, resulting in a clear division of the conformational space into northern and southern quadrants (Fig. 7). In contrast, in the case of **1 α** , both quantum and classical mechanical methods indicate a barrier only in the West, enabling the ring to populate a continuous distribution of conformations from North to South via the East. Previous NMR (Angyal 1979; Serianni and Barker 1984; Taha et al. 2010), as well as QM (D'Souza et al. 2000; Gordon et al. 1999) studies, have also been shown to be consistent with conformations in the North, East, and South and it has been proposed that the anomeric effect (Cosse-Barbi and Dubois 1987; Cossé-Barbi et al. 1989; D'Souza et al. 2000; Ellervik and Magnusson 1994; Juaristi and Cuevas 1992; Plavec et al. 1993; Richards et al. 2013; Thibaudeau and Chattopadhyaya

1997) and the *gauche* effect (Callam and Lowary 2001; D'Souza et al. 2000; Houseknecht et al. 2003a; Plavec et al. 1994; Plavec et al. 1996; Plavec et al. 1993; Thibaudeau and Chattopadhyaya 1997; Thibaudeau et al. 1994) among the vicinal hydroxyl groups and the ring oxygen atom were the key influences on ring conformation in furanoses. In the case of **1a**, it has been argued that the anomeric and *gauche* effects both stabilize the South conformation (Callam and Lowary 2001; D'Souza et al. 2000; Gordon et al. 1999; Houseknecht et al. 2003a). From the analysis of the anomeric effect presented in Fig. 4, we can conclude that the barrier in the western quadrant in **1a** is due in part to the absence of stabilizing contributions from the anomeric effect. In the case of other furanoses, for example **3b**, in which there are *syn*-interactions between hydroxyl groups, an examination of the interaction energies between the hydroxyl groups and the ring oxygen atom (Fig. 8) leads to the conclusion that repulsions between these groups introduce a barrier in the eastern quadrant. Thus in furanoses other than **1a** (that has no *cis*-hydroxyl groups), either the lack of an anomeric effect, or the presence of repulsive oxygen-oxygen interactions results in barriers in both the East and West, effectively establishing a preference for the populations in the North and South.

Conclusions

The present study confirms that the two-state model, commonly employed in interpreting ring conformations of furanoses in solution, is not applicable to all furanoses. Results from MD simulation indicated that **1a** exhibited a continuum distribution from North to South via East of pseudorotation, which agreed with the low energy pathway found in its pseudorotational potential energy surface, and gave rise to experimentally-consistent scalar coupling values for the ring protons. Nevertheless, the two-state model is a reasonable description for other furanoses, to the extent that their ring conformations broadly populate the northern and southern quadrants. The division (or not) of the populations into two states can be explained by the presence (or absence) energy penalties in East and West regions of the pseudorotation itinerary. These barriers are found to arise from the absence of a stabilizing anomeric effect and unfavorable interactions among vicinal hydroxyl groups and the ring oxygen atom.

Notably, the new force field parameters permit accurate simulations of furanoses to be performed (average difference between theoretical and experimental NMR ^3J -values was within 1 Hz), making the need to adopt an assumption about state preferences obsolete. This is likely to be particularly valuable when examining complex oligofuranosides that may contain additional chemical modifications, as are typical in bacterial (Besra et al. 1995; Bhamidi et al. 2008; Brennan and Nikaido 1995; Crick et al. 2001) or fungal pathogen (Beauvais et al. 2007; Costachel et al. 2005; Fontaine et al. 2000; Lamarre et al. 2009; Latge et al. 1994; Leitao et al. 2003) surfaces. The ability to characterize the conformational properties of such structures is an important component in understanding the mechanisms of disease infection (Carapito et al. 2009; Golgher et al. 1993), as well as immune response (Cooper and Petrovsky 2011; Predy et al. 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Pseudorotational itinerary of furanoses depicting different Envelope (E) and Twist (T) ring conformations with associated conformational phase angle (P) values in degrees.

Fig. 2.
Methyl D-furanosides examined in present study.

Fig. 3.

Pseudorotational energy curves for **6**. Solid lines: energies computed at the B3LYP/6-31G* level to be consistent with the GLYCAM06 parameters (Kirschner et al. 2008); dashed lines: energies computed with new parameters.

Fig. 4.

Upper: pseudorotational energy curves for **13** with each quadrant of pseudorotation color coded and **16** (grey) computed at the B3LYP/6-31G* level, with the orientation of the exocyclic O4-C1-O-CH₃ torsion angle restrained at 60°. Lower: the relative energy of **13** and **16** as functions of the C4-O4-C1-O and C4-C5-C1-O torsions, respectively.

Fig. 5. Rotational energy curves of O4-C1-O-CH₃ angle in **13** (black) and C5-C1-O-CH₃ angle in **16** (grey) computed at B3LYP/6-31G* level, while maintaining the ring conformation at $P=0$ (a) and 180° (b); rotational energy curves of O5-C1-O-CH₃ angle in (S)-2-methoxytetrahydropyran (**17**) (black) and C6-C1-O-CH₃ angle in methoxycyclohexane (**18**) (grey) computed at B3LYP/6-31G* level, while maintaining the ring conformation at $^1C_4$ (c) and $^4C_1$ (d). Regions stabilized by the *exo*-anomeric effect are indicated by vertical arrows.

Fig. 6. Ring conformation distribution for **1–4** (α and β) and **5** β from MD simulations (300 ns each), red/blue lines correspond to data from the HF/6-31G* and B3LYP/cc-pVTZ-derived partial charge models, respectively. a-d: α anomers of **1–4**, e-i: β anomers of **1–5**.

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Fig. 8. Pseudorotational energy curves for **7** (upper) and **8** (bottom) computed at the B3LYP/6-31G* level, with the orientation of the vicinal hydroxyl groups (C2-C3-O3-H3O and C3-C2-O2-H2O) restrained at 180°. Curves in each quadrant of pseudorotation are color coded.

Table 1

MD-derived^a, Karplus-equation-derived, and experimental ¹H-¹H and ¹³C-¹H coupling constants for **1–4** (α and β) and **5β**.

	³ J _{H1,H2}				³ J _{H2,H3}				³ J _{H3,H4}				³ J _{C1,H4}			
	MD/QM ^b	MD/Karplus ^{c,d}	Expt.	MD/QM	MD/Karplus ^d	Expt.	MD/QM	MD/Karplus ^d	Expt.	MD/QM	MD/Karplus ^d	Expt.	MD/QM	MD/Karplus ^f	Expt.	Expt.
1α	1.7	2.2	1.7 ^e	3.6	2.9	3.4 ^e	5.5	4.6	5.8 ^e	0.8	1.0	<0.5 ^{fg}				
1β	5.4	5.6	4.5 ^h	5.9	4.7	7.9 ^h	5.3	4.8	6.7 ^h	3.9	1.8	3.2 ^f				
2α	3.4	4.5	3.6 ⁱ	5.4	4.9	4.8 ⁱ	5.4	4.9	4.3 ⁱ	0.7	0.6	3.7 ^f				
2β	6.1	5.7	4.8 ^j	5.6	4.9	5.0 ^j	4.7	4.5	4.6 ^j	1.8	0.3	2.2 ^f				
3α	4.7	5.5	4.3 ^j	6.2	5.3	6.2 ^j	5.9	5.4	3.4 ^j	0.7	1.1	<0.5 ^{fg}				
3β	0.6	1.2	1.2 ^j	5.7	4.9	4.6 ^j	8.2	7.0	6.9 ^j	2.8	0.9	2.7 ^f				
4α	4.4	5.0	4.5 ^j	2.9	2.3	5.5 ^j	5.3	4.6	6.1 ^j	0.1	0.3	<0.5 ^{fg}				
4β	0.4	1.3	^j	1.5	1.3	1.7 ^j	4.6	4.7	5.1 ^j	1.4	0.4	2.4 ^f				
5β	1.7/6.2 ^k	2.3/6.5 ^k	2.6/5.4 ^{k,l}	7.9/9.2 ^k	7.7/6.9 ^k	6.7/5.7 ^{k,l}	5.2	5.2	4.2 ^j							

^aComputed with partial atomic charges fit to the B3LYP/cc-pVTZ QM potentials.

^bThe standard derivations of all MD derived ³J-values are within approximately 0.3 Hz.

^cThe standard derivations of all Karplus-equation-derived ³J-values are within approximately 0.1 Hz.

^dAltona et al. 1994.

^eTaha et al. 2010.

^fHouseknecht et al. 2003b.

^gA value of 0 Hz was employed in calculations of average error between the computed and experimental ³J-values.

^hTaha et al. 2011.

ⁱSerianni and Barker 1984.

^jKline and Serianni 1990.

^kReference are reported for couplings involving H2S and H2R (S/R).

Church et al. 1997.

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Table 2

MD-derived^a, Karplus-equation-derived, and experimental $^3J_{\text{H}^{\alpha}\text{H}^{\beta}}$ and $^3J_{\text{H}^{\alpha}\text{H}^{\gamma}}$ values for **1–4** (α and β) and **5**.

	$^3J_{\text{H}^{\alpha}\text{H}^{\beta}}$ coupling constants (Hz)				$^3J_{\text{H}^{\alpha}\text{H}^{\gamma}}$ coupling constants (Hz)			
	MD/QM ^b	MD/Karplus ^{c,d}	Expt.	MD/QM	MD/Karplus	Expt.	MD/QM	MD/Karplus
1 α	3.9	3.3	3.3 ^e	6.1	5.3	5.8 ^e		
1 β	4.0	3.4	3.4 ^f	6.5	5.8	6.7 ^f		
2 α	3.5	3.1	4.4 ^g	8.7	7.8	6.7 ^g		
2 β	3.1	3.0	4.5 ^g	9.2	8.1	7.6 ^g		
3 α	3.5	3.0	3.3 ^h	5.1	4.8	4.6 ^h		
3 β	3.5	3.1	3.1 ^h	6.8	6.0	6.6 ^h		
4 α	3.6	3.3	3.8 ^g	8.8	7.7	6.0 ^g		
4 β	3.3	3.1	4.4 ^g	9.1	7.9	7.6 ^g		
5 β	3.4	3.2	4.6 ⁱ	6.8	6.0	7.0 ⁱ		

^aComputed with partial atomic charges fit to the B3LYP/cc-pVTZ QM potentials.

^bThe standard derivations of all MD derived ^3J -values are within approximately 0.5 Hz.

^cThe standard derivations of all Karplus equation derived ^3J -values are within approximately 0.4 Hz.

^dAltona et al. 1994.

^eTaha et al. 2010.

^fTaha et al. 2011.

^gSerianni and Barker 1984.

^hKline and Serianni 1990.

ⁱChurch et al. 1997.