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Carbohydrate Research 338 (2003) 955–962
CARBOHYDRATE RESEARCH
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NMR and modelling studies of disaccharide conformation☆

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Received 25 February 2002; received in revised form 30 January 2003; accepted 10 February 2003

Abstract

Long-range heteronuclear coupling constants were measured across the glycosidic linkages for a series of eight α- or β-linked disaccharides in aqueous solution. Multiple ^{13}C site-selective excitation experiments using ^{1}H decoupling in conjunction with pulsed field gradient-enhanced spectroscopy were used to determine $^{3}J_{C,H}$ values. These were subsequently compared with the respective couplings calculated, using a Karplus relationship, from molecular dynamics simulations with the explicit inclusion of water.

Keywords: Disaccharides; Conformation; ^{1}H NMR; ^{13}C NMR; Long-range ^{13}C–^{1}H coupling constants; Molecular dynamics

1. Introduction

This paper compares experimentally-derived disaccharide glycosidic dihedral angles with those obtained by molecular modelling. The objective was to determine whether or not a relatively simple modelling approach would be adequate to provide results comparable to those obtained by NMR couplings.

In the conformational analysis of oligosaccharides in solution, NMR techniques are valuable in obtaining information about three-dimensional structures free of crystal lattice constraints.[1–3] NMR spectroscopy based on measurements of vicinal $^{3}J_{H,H}$ and long-range heteronuclear couplings are used to gain information on both the intra-residue and the inter-residue conformation(s) (i.e., the dihedral angles between constituent monosaccharides) of an oligosaccharide. *J*-Couplings are weighted averages over an ensemble of conformers or rotamers. Another possible influence on the solution conformation of an oligosaccharide is hydrogen-bonding, which can be studied experimentally by measuring the linewidth (lw) temperature coefficient, and $^{3}J_{HO,C,H}$ couplings of the hydroxyl resonances in low temperature spectra.[4–6]

Nuclear Overhauser enhancement spectroscopy (NOESY) has been used to obtain inter-glycosidic spatial constraints to help define the linkage conformations of carbohydrates. Until recently, much of the conformational data on complex carbohydrates has come from NOE experiments. [2,7] The measurable NOE'S across a glycosidic linkage are typically limited to a few, so such studies on linear oligosaccharides typically produce too small a number of distance constraints to model adequately the ϕ_H (H-1–C-1–O-1–C′-x) and φ_H (C-1–O-1–C′-x–H′-x) torsion angles around the glycosidic bonds. It is therefore useful to obtain as many other (CH, OH, NH) nOe's as possible.[4,8] Long-range heteronuclear scalar coupling constants through the glycosidic bonds also provide spatial constraints for the torsion angles ϕ and φ around a linkage when interpreted by a Karplus-type relationship.[2] Two such parameterisations for the glycosidic linkage of saccharides show only minor differences and either can be used to relate measured $^{3}J_{C,H}$ values to the averaged torsional angles.[9,10] Several NMR pulse sequences have been developed for the sensitive and accurate measurement of $^{3}J_{C,H}$ values of carbohydrates including the 2-D proton flip (i.e., heteronuclear *J*-resolved spec-

☆ Presented, in part, at the XXI International Carbohydrate Symposium, Cairns, Australia, July 7–12, 2002.

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0008-6215/03/$ - see front matter
doi:10.1016/S0008-6215(03)00069-7

troscopy)[11] 2-D INEPT[12] and 2-D ^{1}H-detected techniques with chemical shift filtering and/or selective excitation of the atoms of interest.[7] A recent development has been the use of 1-D single or multiple ^{13}C site-excitation experiments using shaped pulses to determine $^3J_{C,H}$ values.[3,13,14] The detected signals show opposite phase for the homonuclear and heteronuclear couplings i.e., the ^{1}H–^{1}H couplings and ^{1}H–^{13}C couplings are observed in-phase and in anti-phase, respectively.

The method used to extract the numerical value of the long-range coupling constant will depend on the complexity of the spectra of the carbohydrate in question, and on the experimental techniques used. It may be obtained readily by direct measurement of the in-phase or anti-phase peak-to-peak separation due to splitting of the NMR signal if multiplicity is low, spectral overlap is not extensive, the lw is small in comparison with the measured splitting (i.e., $^3J_{C,H} \cong >$ 1.5 times lw) and $^3J_{H,H}$ is not equal to $^3J_{C,H}$. When the multiplicity is complex or the lw exceeds the scalar couplings, a scaling-factor approach or the evaluation of an integral (e.g., *J*-doubling) must be used to extract the desired $^3J_{C,H}$ values.[3] Techniques for the measurement of other long-range couplings (e.g., $^3J_{C,C}$ (–C–O–C–C–) and $^2J_{C,C}$ (–C–O–C–)) in ^{13}C-enriched carbohydrates and the development of appropriate Karplus relationships are recent developments.[2,15]

This study comprises NMR and molecular dynamics (MD) investigations of the conformational behaviour of eight disaccharides in aqueous solution, and provides further experimental and modelling data to assist in such endeavours. Experimentally-determined long-range three-bond heteronuclear coupling constants have been converted, using a Karplus-type equation, to yield values for the glycosidic dihedral angles of each disaccharide. These were subsequently compared with the respective values calculated theoretically from molecular dynamics simulations with explicit inclusion of water as solvent (s-MD). The major emphasis in this paper is the comparison between the NMR-determined ϕ_H and φ_H dihedral angles, and those determined by s-MD. A more comprehensive analysis of the MD data will be presented in a later paper, in which the focus will be on hydration patterns and their relationship to intramolecular oxygen–oxygen distances.

2. Experimental

2.1. Disaccharide configurations

α-D-Glucopyranosyl α-D-glucopyranoside (α,α-trehalose)
4-*O*-α-D-Glucopyranosyl-D-glucose (Maltose)
4-*O*-β-D-Galactopyranosyl-D-glucose (Lactose)
4-*O*-β-D-Glucopyranosyl-D-glucose (Cellobiose)
6-*O*-β-D-Glucopyranosyl-D-glucose (Gentiobiose)
6-*O*-α-D-Galactopyranosyl-D-glucose (Melibiose)
3-*O*-α-D-Glucopyranosyl-D-glucose (Nigerose)
3-*O*-β-D-Glucopyranosyl-D-glucose (Laminarabiose)

2.2. Materials and reagents

The disaccharides cellobiose, melibiose and trehalose were purchased from British Drug House Ltd. Maltose and lactose were obtained from May and Baker Ltd (UK). Gentiobiose, laminarabiose and nigerose were supplied by Sigma–Aldrich Chemical Company (USA). The NMR reagent deuterium oxide (D_2O) was obtained from Cambridge Isotope Laboratories (MA, USA). All *J*-coupled spectra were obtained from 10 to 20 mmol solutions of a sample in D_2O. Each sample was dissolved in 0.6 mL D_2O, and freeze-dried. The procedure was repeated at least twice before the final sample was prepared to achieve maximum deuteration of hydroxyl groups.

2.3. General NMR protocols

NMR experiments were carried out using a Bruker-600 MHz spectrometer operating in the Fourier transform (FT) mode at 600.1 MHz for ^{1}H and 150.9 MHz for ^{13}C. A 5 mm TX1 probe was used for all the experiments, which were carried out at 298 K except for laminarabiose. These were performed at 293 K to observe the set of signals for one of the anomeric protons that occurred under the residual D_2O peak at ambient temperature. The proton spectra were referred to the residual HDO signal at δ 4.7 ppm. Standard Bruker software and pulse sequences were used for various 2-D experiments that were required to obtain the desired chemical shifts and homonuclear coupling data. All such experiments were carried out using a sweep width of 1653.5 Hz. The NMR data obtained were processed, analysed and plotted with Bruker NMR software on a personal Iris workstation (Silicon Graphics).

2.4. Experimental determination of $^3J_{C,H}$ values

Heteronuclear interglycosidic coupling constants for the disaccharides were determined by a pulse sequence developed especially for this purpose by G. Ball. The measurements were performed using the procedure of Blechta and co-workers[13] for multiple ^{13}C site-selective excitation experiments with extensions that included proton decoupling after polarisation transfer and pulsed field gradient-enhanced spectroscopy.[14] A spectral width of typically 1650 Hz was sampled with 8050 data points. Zero filling and multiplication of the FID with an exponential weighting function was applied

prior to FT. The desired couplings were extracted via a *J*-multiplication procedure using eight or 16 functions in the frequency domain. The error of the determined values was estimated to be not greater than 0.2 Hz based on the digital resolution of the spectrum. The 1-D ^{1}H spectrum was also simulated using standard Bruker software to facilitate extraction of the coupling constants.

2.5. Calculation of heteronuclear coupling constants

The coupling constants $^3J_{H1,\ C'x}$ and $^3J_{C1,H'x}$ across the glycosidic linkage were calculated for the proton-based torsion angles $\phi^* = \phi_H$ = H-1–C-1–O-1–C′-x and $\varphi^* = \varphi_H$ = C-1–O-1–C′-x–H′-x, respectively, using the curve of Tvaroska and co-workers[10] viz., $J = 5.7\cos^2\phi^* - 0.6\cos\phi^* + 0.5$. Averages of $\cos^2\phi^*$ and $\cos\phi^*$ were used for the conformationally averaged calculations of $^3J_{C,H}$ from s-MD trajectories.

2.6. Computational procedures

The crystal conformation of each disaccharide was used as starting geometry for all the molecular dynamics simulations. With the obvious exception of trehalose, the β-anomer of each disaccharide was modelled.

Molecular dynamics simulations with the explicit inclusion of water as solvent (s-MD) were carried out on the disaccharides using the Amber-H force field of Homans[15] with the Insight II Molecular modelling program (Version 4.0.0) and molecular mechanics/dynamics package (Version 2.9) (Biosym Technologies, San Diego, CA, USA). Atomic types and partial atomic charges of Homans were used for the atoms involved in the glycosidic linkage, with a minor proportional adjustment of the charges for these atoms to maintain overall neutrality.[15] The SPC-type model of water was used.

2.7. Minimization

In vacuo minimizations of the disaccharides were initially carried out for 100 iterations using the steepest descents algorithm to shorten running time. Minimization was continued using the conjugate gradient algorithm until a maximum derivative (rms) of < 0.001 kcal/Å was achieved. The final energy-minimized conformations were used as the starting geometries for solvation. Minimizations with explicit inclusion of solvent were carried out by placing the solute in the centre of a $20 \times 20 \times 20$ Å^3 box containing 252 SPC water molecules and the imposition of periodic boundary conditions (PBC) with an orthogonal space group. A non-bonded cutoff criterion of 10.0 Å and a switching distance of 1.5 Å were used for all the runs. This step helped relax steric conflicts which might have been created in the generation of the box. Interactions between atoms more than 10 Å apart were truncated and switching functions applied on a group-by group basis (i.e., entire water molecules and electrostatically neutral groups in the solute) to smoothly turn off long-range interactions between 9 and 10 Å.

2.8. Molecular dynamics (MD)

Molecular dynamics with explicit inclusion of solvent were performed at constant pressure of 1 bar and loose coupling to a 300 K thermal bath with a coupling constant of 0.1 ps. Simulations were run with a time step of 0.1 ps and after a 10 ps equilibration period the data was collected and analyzed for a further 400 ps. Coordinates were saved every 1 ps for subsequent analysis. Equilibration was monitored empirically by observing the total energy, and all the systems were well equilibrated with no overall drift in the temperature or energy. For calculation of coupling constants, the average glycosidic angle values over the full s-MD runs were used. The final MD conformations, reminimized under conditions identical to those used for earlier minimizations, were also recorded.

3. Results and discussion

3.1. The Karplus relationship

The interglycosidic long-range heteronuclear (H–C) scalar couplings were determineded, and related to the glycosidic dihedral angles by the use of an appropriate Karplus-type correlation curve for the C–O–C–H segment.[2] These angles are defined according to the glycosidic hydrogen: ϕ_H = H-1–C-1–O-1–C′x and φ_H = C-1–O-1–C′-x–H′-x. Only one curve is provided by Tvaroska and co-workers[10] (and Mulloy and co-workers[9]) for the calculation of both ϕ_H and φ_H, despite the different linkage pathways: H_1–C_1–O_1–C_x– and –C_1–O_1–C_x–H_x, respectively. This appears to be standard practice. The curve is a composite of 17 values derived from monosaccharide structures. No $^3J_{C,H}$ value used to construct the curve was derived from an actual glycosidic linkage, and only four are derived from an H_1–C_1–O_1–C_x pathway. With three exceptions (φ_H of the -(1→6)-linked melibiose and gentiobiose, and laminarabiose) the numerical values of the glycosidic dihedral angles are below 60°. This is the region of the curve where the data are of lower precision.[10]

The curves of both Tvaroska and co-workers[10] and Mulloy and co-workers[9] have few experimental data points in this region. The former authors admit that the dihedral angles of the compounds used for the 0–60° region were only estimated values, while the latter authors suggest that the spread of experimental data in

their curve may be due to the use of angles taken from crystal data to represent solution structures, though it is not clear whether or not this approach was used for the compounds which provided data for the 0–20° region of their curve.

While the above shortcomings contribute to the recent tendency for Karplus equations derived from measured coupling constants in rigid molecules to be replaced by direct density functional theory (DFT) quantum mechanics calculations of coupling constants, a recently-developed Karplus curve[17] derived by such DFT methods for the trans-glycosidic C–O–C–C pathway also suffers from a lack of data points in the region 0–20°. It predicts larger coupling constants in this region than those obtained using the existing curves.[9,10] Couplings calculated in more recent work by the same group[18] on four β-(1→4)-linked disaccharide mimics, were in good agreement with experimental results, confirming the general shape of the experimental Karplus curves previously developed.[17] While conceding that the small set of geometries and structures studied could partly explain the deviation from the experimental curves, the authors suggested that some experimental reassessment of the couplings for angles around zero and 180° may be warranted. Taking the above points into consideration, the results produced by use of the Tvaroska and co-workers curve are remarkably accurate.

3.2. Measurement of heteronuclear coupling constants

Scalar long-range $^{3}J_{C,H}$ couplings were measured for the disaccharides using selective excitation of anomeric and substitution carbons with a subsequent transfer of the magnetisation to the protons for detection. All the 3-bond C, H couplings occurred as anti-phase doublets that were further spilt by in-phase homonuclear (H, H) couplings. Experimentally, the use of gradients and homonuclear decoupling during the acquisition period helped to suppress artefacts, increase the s/n ratio and allow an easier interpretation of spectra by reducing the multiplicity. These features simplified the extraction of $^{1}H-^{13}C$ couplings using the *J*-multiplication method in which an integral is sought which also retains the correct proton multiplicity.[3] The values extracted by this procedure were in good agreement with those measured directly from the anti-phase separation in well-resolved proton-detected signals such as for trehalose.

The extraction of the desired $^{3}J_{C,H}$ value was relatively straightforward when the heteronuclear coupling to be measured was at least 1.5 times the observed linewidth provided that $^{3}J_{H,H}$ differed substantially from $^{3}J_{C,H}$. Otherwise, cancellations were observed in the spectrum. Proton multiplicity, which was present in most cases for the disaccharides, made the extraction of the $^{1}H-^{13}C$ coupling constants more complicated. A number of different multiplicities were present at the anomeric or linkage protons, a common one being the splitting of H-1 into a doublet by an approx 8 Hz homonuclear coupling to H-2. Two other less-frequently encountered problems were the spectral overlap of signals from ring atoms and a poor s/n ratio leading to a weak resolution of the signals for the linkage atoms (e.g. gentiobiose). Since all the disaccharides except D-trehalose were present as a mixture of α- and β-anomers in solution, the relevant couplings had to be extracted for both the major and minor anomers (where possible). This also complicated the overall spectroscopic analysis. The simulated ^{1}H spectrum was sometimes used to facilitate the measurement of $^{3}J_{C,H}$ values in such cases.

3.3. Interpretation of coupling data

The heteronuclear interglycosidic coupling constants of oligosaccharides were measured for the disaccharides and the experimental data were compared with those obtained from s-MD simulations in order to assess the 3-D structures. The trigonometric dependence of the correlation curve (i.e., $\cos^2\phi$ modulated) used to interpret the coupling data implies that more than one value of the dihedral angle is compatible with any given *J* value. This along with the fact that only time-averaged conformational information can be obtained from such an analysis makes the interpretation of $^{1}H-^{13}C$ data in terms of detailed unambiguous information about the conformation at the glycosidic linkage difficult.[3,16] In order to correlate the measured $^{3}J_{C,H}$ values to time-averaged 3-D structures, the obtained C,H couplings were compared with the *averaged* dihedral angle values obtained from the computer-generated MD trajectories.

The experimental and calculated dihedral angles plus the $^{3}J_{H,C}$ coupling constants obtained from several studies of the assessed disaccharides are summarised in Table 1.

3.4. Molecular dynamics

Unless otherwise specified, only MD results from simulations with explicit inclusion of water molecules are reported. Such simulations are now computationally feasible, and are most likely to yield results closer to reality than are alternative approaches to the treatment of solvent effects.[19–21]

The Amber-Homans force field was chosen as it was the one used in our previous studies on monosaccharide methyl glycosides with explicit inclusion of water molecules.[22] In that study the MD work was successful in predicting values for the rotamer populations for the exocyclic hydroxymethyl group in reasonable agreement with experimental results obtained by other

groups. Molecular dynamics simulations without explicit inclusion of water in general perform poorly in this respect. As was the case for the monosaccharides previously studied,[22] in the current study the hydroxymethyl groups of the non-reducing *gluco*-residues, initially set to the *tg* conformation, changed to the *gg* or *gt* conformation during the equilibration period, and remained distributed between them for the remainder of the simulations. Similar behaviour was observed for the hydroxymethyl groups of the reducing residues of the -(1→3)- and -(1→4)-linked disaccharides, and for both residues of trehalose. The proportion of time spent in the *gt* conformation was generally slightly greater than that spent in the *gg*, and the *tg* conformation was not revisited.

In the cases of the non-reducing *galacto*-residues in lactose and melibiose, the *tg* conformation was not revisited, despite the fact that considerable time is reportedly spent in this conformation by sugars having the *galacto*-configuration at C-4.[23] The same behaviour was observed in our monosaccharide studies[22] and, as suggested thererin, could be due to a parameter/forcefield bias. Recent quantum mechanical and NMR studies[23] confirm the conclusions reached by ourselves[22] and others[23,19] that the rotamer preference in aqueous solution is due to hydrogen-bonding and solvation effects. During the s-MD runs, the glycosidic dihedral angles ϕ and φ both adopted values close to the eventual average soon after equilibration, and remained fluctuating about them for the remainder of the simulation. The presence of water had a dampening effect on the ϕ and φ fluctuations, compared with those found during MD using a dielectric of 80 (data not reported). Homans has proposed that a transient network of solute–solvent hydrogen bonds hydrogen bonds may have this effect.[15]

In summary, no new, disaccharide-specific results were obtained from these studies of glycosidic dihedral angles or exocyclic hydroxymethyl rotamer populations.

Table 1
Experimental (NMR) and theoretical (MD) glycosidic dihedral angles (ϕ_H, φ_H; degrees) and $^3J_{H,C}$ coupling constants (Hz) for the disaccharides

Sample	Experimental [a]		Reference	Theoretical [b]		Reference
	ϕ_H; $^3J_{H,C}$	φ_H; $^3J_{H,C}$		ϕ_H; $^3J_{H,C}$	φ_H; $^3J_{H,C}$	
Maltose	−45.0; 3.0	−50; 2.5	24	−62.3	−49.4	28
	−28.8; 4.3	−26.8; 4.5	27	−49.0; 2.7	−36.0; 3.9	19
	−36.0; 3.5	−20.0; 3.9	25	−17.0; 5.1	−18.0; 5.1	20
	−26.8; 4.5	4.5	26			
	−31.5; 4.12 (0.2)	−24.5; 4.68 (0.1)	[c]	−25.3; 4.62	−13.9; 5.3	[c]
Trehalose	−42.2; 3.19 (0.05)		[c]	−38.0; 3.55		[c]
	−45.0; 3.0		30	−50.0 [d]; 2.4		30
	−41.0; 3.3		31			
	2.5		24	−54.0		21
Lactose	35.8; 3.8	−21.1; 4.9	32 [e]			
	34.3; 3.89 (0.1)	−21.2; 4.9 [f] (0.3)	[c]	50.2; 2.5	1.4; 5.6	[c]
Cellobiose	32.2; 4.08 (0.04)	−21.1; 4.75 (0.25)	[c]	44.1; 2.9	−0.24; 5.6	[c]
	1–2		43			
Gentiobiose	48.6; 2.68 [f] (0.08)	−129; 2.36 (0.06)	[c]	37.0; 3.7	−126.0; 2.1	[c]
Melibiose	−43.2; 3.1 (0.4)	−135; 2.9 (0.4)	[c]	−11.5; 5.4	−123; 1.9	[c]
Nigerose	−38.8; 3.49 (0.1)	−22.6; 4.58 [f] (0.05)	[c]	−35.6; 3.8	−9.6; 5.5	[c]
Laminarabiose	34.2; 3.9 (0.07)	−23.8; 4.72 (0.05)	[c]	28.5; 4.37	−61.2; 1.53	[c]

[a] Experimental ϕ_H, φ_H values calculated from the observed $^3J_{H,C}$ coupling constants of the major disaccahride anomer (β-D)using the Karplus equation derived by Tvaroska and coworkers.[10]

[b] Theoretical $^3J_{H,C}$ coupling constants calculated from the modelling-derived ϕ, ψ values using using the Karplus equation derived by Tvaroska and co-workers.[10]

[c] This paper.

[d] Dielectric constant = 80.

[e] Methyl-β-lactoside.

[f] Determined from the minor anomer (-α-D-) NMR data. nd. Not determined because of spectral overlap and multiplicity. Numbers in brackets beside $^3J_{H,C}$ values indicate ± errors.

3.5. Maltose

Earlier experimental work[24] on maltose probably yielded the least reliable $^3J_{H,C}$ coupling data. More recent work[25–27] and that reported here has provided values which converge to give close agreement (i.e., average values of about 4.1 ± 0.4 and 4.4 ± 0.4 Hz) for the coupling constants which relate to ϕ_H and φ_H, respectively.

It is interesting to note the improvements in the MD data for maltose over some 10 years. The average of earlier MD simulations[19,28] yielded significantly larger numerical values for ϕ_H ($-55.5°$) and φ_H ($-42.7°$) than the values reported in more recent work ($\phi_H = -17$ and $\varphi_H = -18.0°$) derived by using updated parameters in the empirical force field AMB99C.[20] Our MD-derived results ($\phi_H = -25.3$; $\varphi_H = -13.9°$) and our results calculated from the experimental $^3J_{H,C}$ coupling constants ($\phi_H = -31.5$; $\varphi_H = -24.5°$) fall within the lowest energy contour (1 kcal/mol) for maltose on a recently-reported energy surface which was calculated with a hybrid of HF/6-31G* and MM3 (96) energies.[29] Most (114 of 134) maltosyl linkage conformations extracted from the Protein Data Bank also fell within this contour,[29] so correspondence with these experimental values, derived from crystal structures of maltosyl carbohydrates bound to proteins, adds credence to our results.

Thus the MD-derived values for the dihedral angles of maltose in aqueous solution have converged over the last 10 years toward the experimentally-derived values of approx ($-20°$, $-20°$) $\pm 10°$.

3.6. Trehalose

This disaccharide comprises two D-glucopyranose residues linked -α-(1→1)-α- and as such shows only one set of resonances for the equivalent rings. Omitting earlier results[24] the agreement between the $^3J_{C,H}$ values from the other groups reported in Table 1 is excellent.[30,31] The theoretical values for the dihedral angle show greater variation, though one[30] is based on data from a simulation not using explicit solvent molecules. Of all the theoretical dihedral angles, ours ($-38°$) is closest to that predicted ($-42.0°$) by the average (3.16 Hz) of the three $^3J_{C,H}$ couplings listed in Table 1. The crystal and solution conformations of trehalose are similar, and interpretations of MD results concluded that the molecule is relatively rigid.[21]

3.7. Lactose

Lactose is proposed to have one highly-favoured extended conformation in aqueous solution, with little rotation about the glycosidic bond.[18,32] The present experimental $^3J_{C,H}$ values yield results ($\phi_H = 34.3$; $\varphi_H = -21.2°$) which are in good agreement with the solution values reported in the literature ($\phi_H = 35.5$; $\varphi_H = -21.1°$) [32] while agreement with the reported crystal data ($\phi_H = 45.0$; $\varphi_H = -15.0°$) [33] indicates that the solution and crystal conformations occupy the same region of conformational space. The MD-derived ϕ_H (50.2°) is close to the crystal structure value, and lies within the lowest-energy contour on a hybrid energy surface for cellobiose, calculated using HF/6-31G* and MM3 (96) energies[29] as does the MD-derived value of 1.4 for φ_H, despite being numerically distant from the experimental value. The calculated coupling of 2.5 Hz for ϕ_H was considerably different from the measured value of 3.89, and the value of 5.6 Hz for φ_H was outside the Karplus curve range (giving a $\cos\theta$ of greater than 1) indicating that perhaps the modelling values are suspect.

3.8. Cellobiose

Cellobiose and lactose possess the same glycosidic linkage type, so are likely to have similar conformations across the glycosidic linkage. This is borne out in the similar (especially in our experimental) values of the lactose and cellobiose $^3J_{C,H}$ coupling constants (Table 1). As with lactose, our solution ($\phi_H = 32.2$; $\varphi_H = -21.1°$) and the crystal ($\phi_H = 42.3$; $\varphi_H = -17.9°$)[34] results indicate very similar conformations. The theoretical sMD-derived value for ϕ_H of 44.1°, though only in fair agreement with experimental solution value, lies within the lowest energy contour in the hybrid energy surface of French and co-workers[40] as does the φ_H value of -0.24. The coupling constant of 5.6 Hz calculated for φ_H was again outside the range predicted by the Karplus curve.

Notably, the both the experimental and modelling data for lactose and cellobiose were in close agreement, as would be expected of such similar structures.

3.9. Gentiobiose

No other $^3J_{C,H}$ coupling constants appear to have been determined or solution modelling been carried out for this -β-(1→6)-linked disaccharide. The differences between the crystal structure values ($\phi_H = 61.5$; $\varphi_H = -155.2°$)[35,36] and our experimental solution values ($\phi_H = 48.6$; $\varphi_H = -129°$) probably reflect the conformational freedom permitted in solution by the (1→6)-linkage. Our theoretical values ($\phi_H = 37$; $\varphi_H = -126.0°$) are in reasonable agreement with our experimental solution results.

3.10. Melibiose

The crystal structure of melibiose has been determined by several groups, typical values[37] being: $\phi_H = -43.9$;

$\varphi_H = -173.9°$. One of our experimental values ($\phi_H = -43.2$) agrees well with this, but the other ($\varphi_H = -135°$) is well away from that of the crystal structure, perhaps not surprisingly for the solution conformation of a (1→6)-linkage freed from crystal lattice constraints. The theoretical φ_H angle calculated from our MD modelling ($-123°$) is reasonably close to the experimental value, but our theoretical ϕ_H angle ($-11.5°$) derived from MD is considerably different from $-43.2°$.

3.11. Nigerose

The crystal structure of methyl-α-nigeroside yields[38]: $\phi_H = -20.1$; $\varphi_H = -15.8°$ Our experimental solution values ($\phi_H = -38.8$; $\varphi_H = -22.6°$) and theoretical solution values ($\phi_H = -35.6$; $\varphi_H = -9.6°$) are not particularly close to those of the crystal. However, all the values lie well within the 'allowed' region on early conformational maps of nigerose constructed by Rao and coworkers[39,40] which predict that only about 5% of the ϕ_H, φ_H space is likely to be occupied. Comparison with the much more recent data of French and coworkers[41] show that our experimental and modelling data both yield values of ϕ_H and φ_H which lie within the lowest energy contour on a relaxed potential energy surface calculated for disaccharide analogues using the HF/6-31-G level of theory.

3.12. Laminarabiose

The laminarabiose crystal structure values ($\phi_H = 27.9$; $\varphi_H = -37.5°$)[42] are close to our experimental solution values derived from $^3J_{C,H}$ data ($\phi_H = 34.2$; $\varphi_H = -23.8°$).

Conformational maps of laminarabiose constructed by Rao and coworkers[39,40] indicate that only about 5% of the ϕ_H, φ_H space is likely to be occupied, so the correspondence of the crystal, experimental solution, and theoretical solution results for ϕ_H is consistent with this. While our MD-derived solution value of 28.5° for ϕ_H is in good agreement with experiment, the correspondence between the average φ_H value ($-61.2°$) [and the derived $^3J_{C,H}$ of 1.53 Hz] and the experimental results is less convincing, though our values together do place the conformation within the second-lowest energy contour on the relaxed potential energy surface calculated for disaccharide analogues mentioned above.[41] The trajectory over the 400 ps of MD fluctuated about the $-61.2°$ average, with no transitions to other regions. If one uses our minimised final MD conformation, the φ_H value becomes $-47.8°$ which is only about 10° from the crystal structure result.

4. Conclusions

4.1. NMR

Despite any shortcomings of the Karplus-type relationship used, the respective dihedral angles obtained were in nearly all cases in good agreement with those calculated using other experimental or modelling procedures.

4.2. Modelling

In two instances, lactose and cellobiose, the MD-derived dihedral angles yielded coupling constants (just) outside the range of the Karplus-type equation. Both these were for the φ_H dihedral angle of -(1→4)-β-D-linked monosaccharides. The modelling parameters, the Karplus parameters, or both could be at fault. As all the experimentally-determined $^3J_{C,H}$ values translated to reasonable dihedral angles, the modelling parameters would appear to be most suspect.

The correspondence of the great majority of experimental and modelling data presented here with those of the of previous work, and especially with recent work based on a combination of theoretical and experimental data[29,41] is encouraging, but not entirely compelling. It at least shows that a relatively simple modelling approach may yield results which compare well with the 'accepted wisdom'.

Though both the derivation of experimental couplings and theoretical modelling are providing converging results as more data using ever-increasing computational power are reported, larger databases, including ^{13}C NMR results from –C–O–C–C– and C–O–C– pathways, are required to provide tests for the utility of dihedral angles as conformational restraints in oligosaccharide studies.

Acknowledgements

This research was carried during the tenure of an Australian Postgraduate Award to P.D. The valuable advice and practical assistance of Dr J. Hook and Ms H. Stender, UNSW NMR Facility, is gratefully acknowledged.

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