

What can ^{13}C - ^1H and ^{13}C - ^{13}C J -couplings tell us?

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Introduction

A knowledge of the conformation of oligo- and polysaccharides is a prerequisite to understanding their role in cell recognition and immunology. The overall conformation of oligosaccharide chains is determined by the glycosidic torsion angles φ_{H} (H1-C1-On-Cn) and ψ_{H} (C1-On-Cn-Hn). In aldohexopyranoses linked in position 6 the ω torsion angle (O5-C5-C6-O6) is also of importance. The conformation of the ω torsion is best described as an equilibrium between the three staggered rotamers (scheme 1). The proportions of the rotamers can be determined from the $^3J_{\text{H5,H6}}$ [1] or $^3J_{\text{C4,H6}}$ [2] coupling constants. However the results vary substantially depending on the assumed values for the coupling constants in the three rotameric states. Conformational analysis of the C5-C6 linkage by ^1H -NMR has been reviewed by Bock and Duus [1].

The use of additional coupling constants in the analysis can increase the accuracy of the calculated rotamer populations and provides a control on the consistency of the calculation. In order to identify J -values which may be useful we investigated the dependence of several homo- and hetero-nuclear couplings on the ω - and C5-C6-O6-HO6 torsions. The results of these calculations were then used to interpret the coupling constants in four methyl glycosides; α - and β -D-galactopyranoside and α - and β -D-glucopyranoside.

Figure 1: $^3J_{\text{HH}}$ calculated by DFT (circles) and by an empirical equation [4].

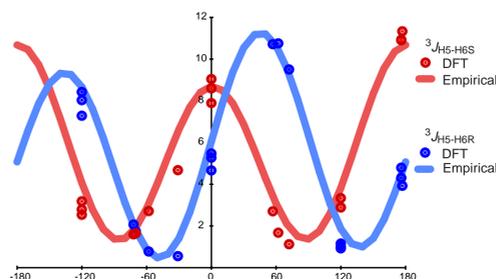


Figure 2: $^2J_{\text{CH}}$ calculated by DFT.

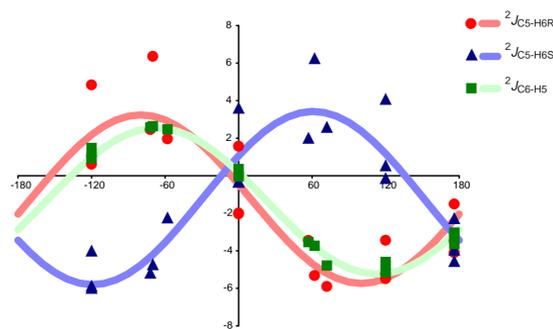


Figure 3: RMS deviation between calculated and experimental $^3J_{\text{HH}}$ (a) $^3J_{\text{CH}}$ (b) and $^2J_{\text{CH}}$ (c) values respectively in methyl α -D-galactopyranoside. An overlay of the 1Hz contours is shown in d.

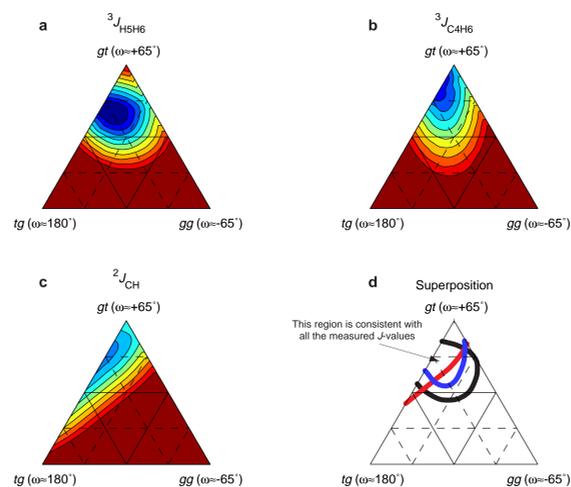


Figure 4: RMS deviation between calculated and experimental $^3J_{\text{HH}}$ (a) $^3J_{\text{CH}}$ (b) and $^2J_{\text{CH}}$ (c) values respectively in methyl α -D-glucopyranoside. An overlay of the 1Hz contours is shown in d.

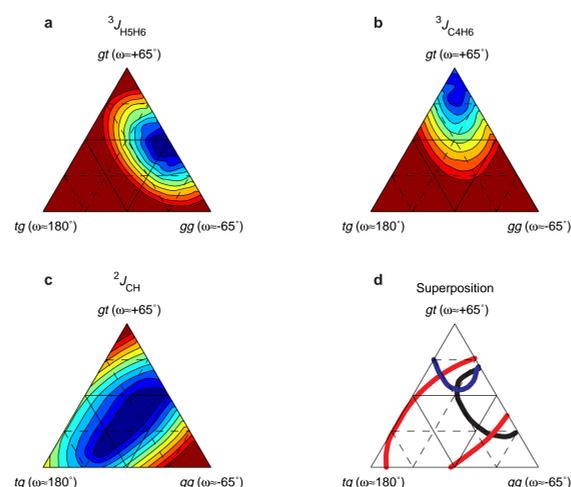


Table 2: Experimental $^2J_{\text{HH}}$ and $^1J_{\text{CC}}$ for the four methyl glycopyranosides

	α -D-galacto	β -D-galacto	α -D-gluco	β -D-gluco
$^2J_{\text{H6R,H6S}}$	-11.65	-11.74	-12.31	-12.34
$^1J_{\text{C5,C6}}$	44.7	44.8	43.3	43.3

Interpretation of the experimental results

Hydroxymethyl orientation

The root-mean-square deviations (RMS) between experimental and calculated coupling constants for the nine stable conformers were calculated (figure 3&4). The lowest RMS values, *i. e.* from the best fitting C6-O6 rotamer, are displayed as functions of the populations in the three staggered C5-C6 rotamers. Areas with good agreement between calculated and experimental J -values are indicated with blue color in figures 3a-c (methyl α -D-galactopyranoside) and 4a-c (methyl α -D-glucopyranoside). Plotting the contours for the $^3J_{\text{HH}}$, $^3J_{\text{CH}}$ and $^2J_{\text{CH}}$ values in the same graph (figure 3d/4d) shows that conformer ratios with low RMS for all seven couplings can be identified for the galacto- (*gt*:*tg*:75:25) and glucopyranoside (*gt*:*gg*:50:50). The absence of the *gg* and *tg* rotamers in the galacto- and gluco-compounds respectively is explained by the unfavourable 1,3-syn-axial interaction between O4 and O6 [7]. In the galactosides the *gt* rotamer is preferred over the *tg* rotamer since the former is stabilized by the *gauche*-effect [8]. The differences in coupling constants between anomers suggest a slight increase of the *tg* rotamer in the β -D-galacto- and of the *gt* rotamer in the β -D-glucopyranosides compared to the corresponding α -pyranosides.

Hydroxyl orientation

Using the $^2J_{\text{HH}}$ and $^1J_{\text{CC}}$ values, and the ratios of the C5-C6 conformers determined above, an estimate of the C6-O6 rotamers can be made. For both galacto and gluco compounds the preferred C5-C6-O6-HO6 is *gauche*. The slightly higher values of $^2J_{\text{HH}}$ and $^1J_{\text{CC}}$ in the galacto compounds may be due to a small amount of the *trans* rotamer.

Conclusion

The homo- and hetero-nuclear coupling constants related to the C5-C6 torsion angle have been investigated by *ab initio* methods. Seven coupling constants, $^3J_{\text{HH}}$ (2), $^3J_{\text{CH}}$ (2) and $^2J_{\text{CH}}$ (3), were found to depend mainly on the rotameric distribution around the C5-C6 bond. Two coupling constants, $^1J_{\text{CC}}$ and $^2J_{\text{HH}}$, depend on the C6-O6 torsion and may be used to estimate the 6-hydroxyl conformation.

The computational results were used to interpret the coupling constants in four glucosides. In glucosides with axial O4 the preferred rotamers where *gt* and *tg* (75:25), in those with equatorial O4 *gt* and *gg* (50:50). The use of $^3J_{\text{HH}}$, $^3J_{\text{CH}}$ and $^2J_{\text{CH}}$ -values gave similar results. The preferred orientation of the 6-hydroxyl is *gauche* to C5.

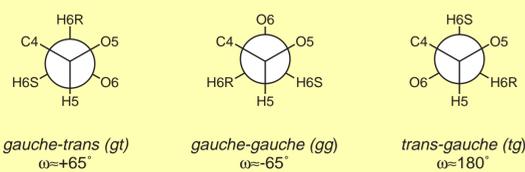
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Scheme 1: C5-C6 rotamers.



Calculations

2-hydroxymethyloxane was used as a model for aldohexopyranoses (carbohydrate numbering is employed in the following discussion). The conformations of staggered and eclipsed rotamers of the C5-C6 linkage and each of the staggered rotamers of the C6-O6 linkage (total of $6 \times 3 = 18$ structures) for the model compound were calculated using the DFT formalism [3]. All homo- and heteronuclear coupling constants involving H6R, H6S or C6, except $^3J_{\text{C4,C6}}$ and $^3J_{\text{C1,C6}}$, were calculated.

The C6-O6 torsion influences the calculated coupling constants both directly by changing the coupling magnitude for a given value of the ω torsion and indirectly by changing the value of ω in the staggered rotamers. Only the $^3J_{\text{H5,H6}}$, $^3J_{\text{C4,H6}}$ and $^2J_{\text{C6,H5}}$ coupling constants are free of direct influences from the C6-O6 torsion.

Of the examined couplings the $^3J_{\text{HH}}$ (figure 1), $^3J_{\text{CH}}$, $^2J_{\text{CH}}$ (figure 2) and $^2J_{\text{CC}}$ values are most easily related to the ω torsion whilst 1J values seem to be sensitive to a number of different influences. The $^2J_{\text{HH}}$ and $^1J_{\text{CC}}$ values are strongly correlated with the orientation of the hydroxyl group. The calculated $^3J_{\text{HH}}$ values are in excellent agreement with experimental values in carbohydrates and those predicted by a frequently used empirical equation [4] (figure 1).

Experimental coupling constants

Four methyl glycopyranosides; methyl α - and β -D-galactopyranoside and methyl α - and β -D-glucopyranoside; were prepared selectively ^{13}C -labeled in position 4, 5 or 6 (total of 12 compounds)[5]. The ^{13}C - ^{13}C coupling constants were measured directly from the splittings in the 1-D ^{13}C -NMR spectra whereas the ^1H - ^1H and ^{13}C - ^1H coupling constants were determined by spin simulation of the 1-D ^1H -NMR spectra [6] (table 1&2). The sign of $^2J_{\text{CH}}$ is known to vary but could in most cases be determined in the simulation. No $^2J_{\text{C4,C6}}$ couplings were observed ($J \leq 0.5$ Hz).

Table 1: Experimental $^3J_{\text{HH}}$, $^3J_{\text{CH}}$ and $^2J_{\text{CH}}$ for the four methyl glycopyranosides

	α -D-galacto	β -D-galacto	α -D-gluco	β -D-gluco
$^3J_{\text{H5,H6R}}$	8.13	7.89	5.55	6.13
$^3J_{\text{H5,H6S}}$	4.17	4.36	2.29	2.22
$^3J_{\text{C4,H6R}}$	1.8	1.9	1.2	1.1
$^3J_{\text{C4,H6S}}$	1.7	1.7	2.3	2.4
$^2J_{\text{C6,H5}}$	-5.2	-5.5	-1.5	(-)2.1
$^2J_{\text{C5,H6R}}$	-5.0	-5.0	-1.9	-2.4
$^2J_{\text{C5,H6S}}$	+0.8	± 0.2	-1.4	-1.1