

Interglycosidic Torsion Angle Estimation by ^{13}C - ^1H Coupling Constant MeasurementsClaude Morat^a, François R. Taravel^b

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Introduction

The relative orientation of contiguous carbohydrate units defining an oligosaccharide structure is customarily described by a set of torsion angles around each glycosidic linkage. For example, for α - or β - (1,4) linkage, two torsion angles, ϕ and ψ , have to be considered, where ϕ represents the dihedral angle in the fragment $\text{H1}'\text{-C1}'\text{-O-C4}$, and ψ the dihedral angle in the fragment $\text{C1}'\text{-O-C4-H4}$. The case of α - or β -(1,6) linkage is more complex as a third torsion angle ω around the C5-C6 bond should be defined.

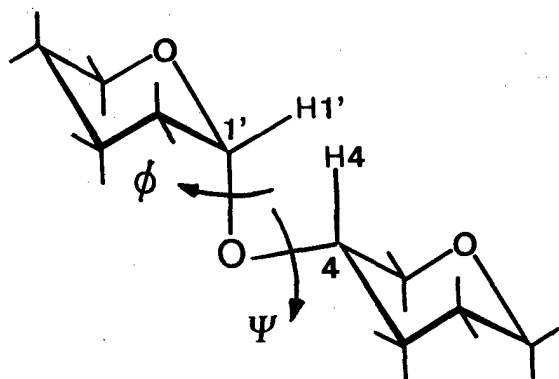


Figure 1. Example of an α -(1,4) linkage.

One way to estimate these torsion angles is to measure the three bond coupling constants (3J) between carbon 13 and proton in the pathways defined above. These couplings show a pronounced relationship with the torsion angles as was first noticed by Lemieux *et al.* (1) and Schwarcz *et al.* (2) in the carbohydrate field. Consequently, much effort has been invested in establishing such a Karplus-type equation. Recently Tvaroska *et al.* (3) have derived an equation of the general formula :

$$3J = A \cos^2 \theta - B \cos \theta + C$$

where θ represents the dihedral angle between proton and carbon 13. The coefficients A, B and C depend on the nature of the four atoms in the pathways as well as on the electronegativity and the relative orientation of the other substituents in the molecule (4).

For carbohydrates, several studies of ^{13}C -enriched and selectively deuterated compounds have pioneered the route to provide sufficient data necessary to characterize an accurate Karplus-type curve (5-10). Now, however, by use of the selective heteronuclear 2D-J experiment proposed by Bax and Freeman (11), and then developed by several laboratories, it is possible to determine these long range coupling constants in natural abundance compounds (12-17).

Methods

All samples were dissolved in deuterated chloroform and the solutions were degassed by bubbling with argon before sealing in 10 mm diameter tubes.

^{13}C (75 and 100 MHz) spectra were recorded on Bruker AM 300 and AM 400 spectrometers, each equipped with a multinuclear probe and an Aspect 3000 computer. The selective 180° proton pulse was obtained by use of a Dante sequence (18) including 100 minipulses. To minimize pulse imperfections, a composite 180° ^{13}C pulse was used ($90^\circ_x 180^\circ_y 90^\circ_x$) and EXORCYCLE phase cycling was implemented for ^{13}C pulses and receiver. After Fourier transformation in the F2 dimension, only the slices corresponding to ^{13}C resonances were processed in the F1 dimension. In order to avoid possible errors in coupling constant measurements, no apodization function was used prior to Fourier transformation.

Results and Discussion

By using this 2D-NMR method, long-range coupling constants of mono- and disaccharides have been determined and published (15-16,18). We now report our results on a series of oligosaccharides with a degree of polymerization ranging from 6 to 30, showing here that the method is also applicable to compounds with relatively short relaxation times (T_2). The values determined are listed in the Table for the acetylated cyclomalto-dextrins 1a,b,c and for cellulose and amylose triacetate 2 et 3 respectively.

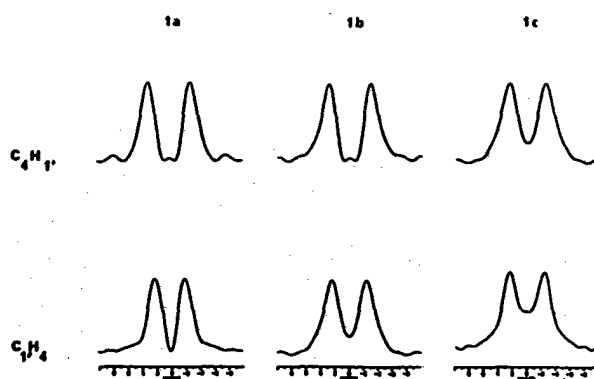
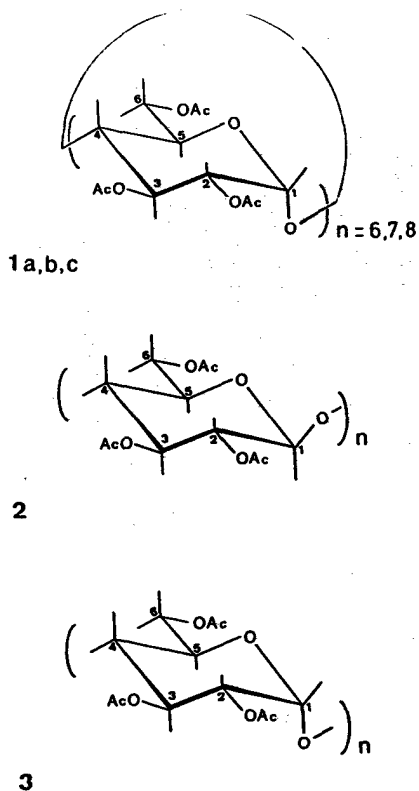


Figure 2. 2D F1-dimension slices of C-1' and C-4 corresponding to H-4 (bottom) and H-1'(top) selective inversion on compounds 1a,b,c.

The coupling constants measured (see Table) lead, via the A, B and C coefficients proposed by Tvaroska *et al.* (3), to torsion angles for the acetylated cyclomaltooligosaccharides in the region $0-35^\circ$. The results show a slight variation of the torsion angles in parallel to the increase of the annular aperture in going from the cyclic hexasaccharide 1a to the octasaccharide 1c.

The conformation of cellulose triacetate 2 in deuterated chloroform can be described by torsion angles close to zero. This result is consistent with the torsion angles found in the solid state ($\phi = 15^\circ$, $\psi = -22^\circ$) from X-ray and electron diffraction analysis (20). This leads to the presence in solution of chains with a two-fold axis.

The results for amylose triacetate 3 could indicate that in solution the molecule has a relatively high degree of freedom as the dihedral angles estimated differ from those published for the crystalline structure (21).

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An example of the resolution obtained is shown in Figure 2. The cyclomaltooligosaccharides are cyclic oligosaccharides consisting of six, seven, or eight α -(1,4)-linked glucose units respectively, while cellulose and amylose triacetate have a degree of polymerization of about 30 and are characteristic of β -(1,4) and α -(1,4) linkages respectively.

Linkage	$^3J_{C,H}$	Value(Hz) ^a	Estimated Torsion angle ^b
1a	α -(1,4) C-4,H-1'	5.6	0
	C-1',H-4	4.0	33
1b	α -(1,4) C-4,H-1'	5.5	0
	C-1',H-4	4.6	25
1c	α -(1,4) C-4,H-1'	4.8	22
	C-1',H-4	4.5	26
2	β -(1,4) C-4,H-1'	5.6	0
	C-1',H-4	5.7	0
3	α -(1,4) C-4,H-1'	2.9	45
	C-1',H-4	4.6	25

^a Coupling constants are given at ± 0.5 Hz. This leads to estimate the torsion angle to $\pm 10^\circ$.

^b As several solutions exist for a given coupling constant only the absolute value in the zero region is presented.

Table : Interglycosidic carbon-proton coupling constants for some oligosaccharides.

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