Conformational Dependence of ^{13}C Nuclear Magnetic Resonance Chemical Shifts in Oligosaccharides

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It is shown that ^{13}C n.m.r. chemical shifts of the anomeric and aglycone carbon atoms can be correlated with one of the glycoside torsion angles (ψ) which are involved in determining the conformation of oligosaccharides. The correlation is shown to be valid for oligosaccharides having an α-D-glucopyranosyl or α-D-galactopyranosyl unit at the non-reducing end of the molecule. Calculation of the minimum-energy conformation of the oligosaccharides is made on the basis of hard-spheres *exo*-anomeric (HSEA) effect calculations.

Recently the study of oligosaccharide conformations in solution has been intensified and a need has arisen for experimental parameters which can yield information about the conformation of glycosidic linkages defined by the torsion angles φ and ψ [φ: H(1)–C(1)–O(1)–C(4) and ψ C(1)–O(1)–C(4)–H(4) see Figure 1]. The sign of these bond angles is given according to IUPAC recommendations.[1]

The most detailed information about the preferred conformation of oligosaccharides in solution has been obtained from n.m.r. spectroscopic data. Particularly, proton chemical shifts and coupling constants, proton–proton nuclear Overhauser enhancements, proton–proton spin lattice relaxation times, and ^{13}C–^1H long-range coupling constants across the glycosidic linkage have provided valuable data for the study of the preferred conformation of oligosaccharides in solution.

However, more than ten years ago Colson *et al.* discussed the possibility of conformational dependence of the ^{13}C chemical shift,[2] a parameter which is very easily determined even in complex molecules. Lemieux and Koto[3] simultaneously reported the dependence of ^{13}C chemical shifts on conformation in a study of substituted cyclohexyl glycosides. Similar results were reported for natural glycosides with chiral aglycones by Seo *et al.*[4] For such glycosides ^{13}C n.m.r. data can be used to determine the relative stereochemistry of the aglycone. Similar results for allylic and benzylic glycosides were later reported by the same group.[5] Using these observations Kochetkov *et al.* have shown[6] that it is possible to determine the relative configuration of the monosaccharide units in an oligosaccharide by means of ^{13}C chemical-shift values.

In this paper we report that the ^{13}C chemical shifts for both the glycoside and the aglycone carbon in oligosaccharides can be directly correlated with one of the torsion angles (ψ) which are involved in the determination of the conformation of the glycoside linkage.

Results and Discussion

We have examined the literature for ^{13}C chemical shifts for the anomeric and aglycone carbon atoms of different glycosides or disaccharides which have an α-D-glucopyranosyl unit at the non-reducing end of the compound and the data are presented in Table 1. It is seen that the shifts of the anomeric carbon atoms range from δ 96.0 p.p.m. for (S)-2-methylcyclohexyl α-D-glucopyranoside to δ 102.3 p.p.m. for the corresponding (R)-isomer with almost all values within 1 p.p.m. between these extremes. The shifts can be represented as the glycosylation shifts *i.e.* by subtraction of the ^{13}C n.m.r. chemical shift for α-D-glucopyranose. The same range of glycosylation shifts is also observed for the aglycone carbon [^{13}C chemical shift for the glycoside (disaccharide) minus the ^{13}C chemical shift for the corresponding alcohol–monosaccharide]. We have then cal-

culated the preferred conformations for all these glycosides using the HSEA method,[7] and the results are presented in Table 1.

Kochetkov *et al.*[6] have suggested that the ^{13}C chemical shifts of disaccharides could be correlated with the distance between the two protons on the glycoside and aglycone carbon atoms, and analysis of the ^{13}C chemical shifts and this distance, taken from Table 1, indicates that there is a good correlation between
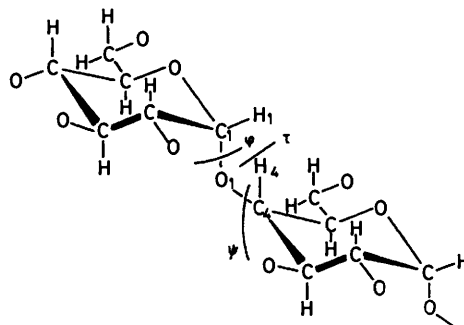

Figure 1. Definition of the torsion angles φ and ψ which determine the conformation of the glycoside linkage[1]

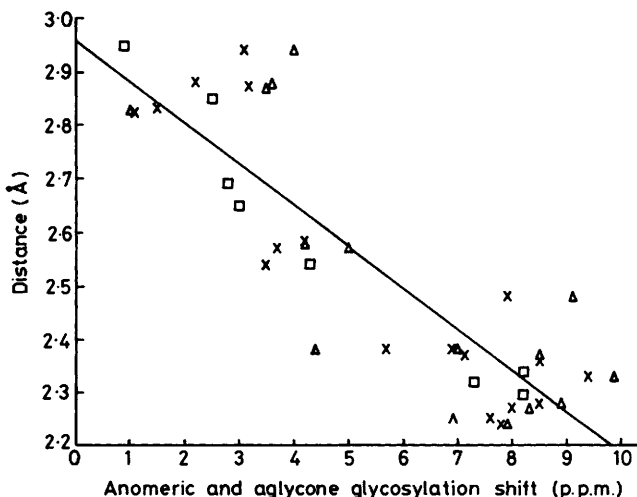Anomeric and aglycone glycosylation shift (p.p.m.)

Figure 2. Correlation of glycosylation shift for anomeric and aglycone carbon atoms with the distance between the protons attached to the glycoside and aglycone carbon atom, respectively. ×, Data from glycosidic carbon atoms of α-D-glucose; $\triangle$, those from corresponding aglycone carbon atoms; $\square$, those from glycoside carbon atoms of α-D-galactose. The linear regressional fit follows the equation: Distance = -0.0776 (p.p.m.) $+ 2.961$ with a correlation coefficient r of -0.917

Table 1. [13]C Chemical shifts [δ(p.p.m.) of glycoside and aglycone carbon atoms and minimum-energy conformation for α-D-glucopyranosyl disaccharides

	[13]C Anomeric	Glycosylation shift[a] anomeric	[13]C Aglycone	Glycosylation shift[a] aglycone	φ (±5°)	ψ(±5°)	Distance (±0.1 Å)	Ref.
α-D-glc 1-1 α-D-glc	94.0	1.1	94.0	1.1	-50	-50	2.82	12
α-D-glc 1-1 α-D-man	94.4	1.5	96.0	1.0	-50	-50	2.83	12
α-D-glc 1-4 α-D-glc(3-deoxy)	95.1	2.2	95.4	3.6	-57	-46	2.88	b
α-D-glc 1-(2-methyl)-(S)-cyclohexyl	96.0	3.1	79.6	4.0	-63	-45	2.94	3
α-D-glc 1-4 β-D-allo	96.1	3.2	71.2	3.5	-56	-42	2.87	b
α-D-glc 1-3 α-L-rham	96.4	3.5			-48	-26	2.54	6
α-D-glc 1-3 β-D-gal	96.6	3.7	78.8	5.0	-48	-32	2.57	c
α-D-glc 1-2 α-D-glc	97.1	4.2	76.7	4.2	-49	-32	2.58	c
α-D-glc 1-2 β-D-glc	98.6	5.7	79.5	4.4	-41	-15	2.38	c
α-D-glc 1-3 α-D-glc	99.8	6.9	80.8	7.0	-41	-20	2.38	c
α-D-glc 1-4 α-D-xyl	100.0	7.1	78.9	8.5	-47	-7	2.37	b
α-D-glc 1-4 α-D-glc(6-deoxy)	100.5	7.6	83.3	6.9	-22	-25	2.25	b
α-D-glc 1-4 α-D-glc	100.7	7.8	78.5	7.9	-21	-24	2.24	c
α-D-glc 1-4 α-L-rham	100.8	7.9	82.4	9.1	-32	-11	2.48	b
α-D-glc 1-4 α-D-man	100.9	8.0	76.3	8.3	-23	-27	2.27	b
α-D-glc 1-3 α-D-man	101.4	8.5			-48	-6	2.36	6
α-D-glc 1-4 α-D-gal	101.4	8.5	79.2	8.9	-39	-8	2.28	c
α-D-glc 1-(2-methyl)-(R)-cyclohexyl	102.3	9.4	85.5	9.9	-45	+2	2.33	3

[a] Chemical shifts for the corresponding monosaccharides are taken from K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 1984, **42**, 193. [b] Present work. [c] From ref. in note a.

Table 2. [13]C Chemical shifts [δ(p.p.m.)] of glycoside carbon atoms and minimum-energy conformation for α-D-galactopyranosyl disaccharides

	[13]C Anomeric	Glycosylation[a] shift anomeric	φ (±5°)	ψ(±5°)	Distance (±0.1 Å)	Ref.
α-D-gal 1-3[α-L-fuc 1-2]β-D-gal	94.1	0.9	-64	-48	2.95	c
α-D-gal 1-2[β-D-gal 1-3]α-D-glc	95.7	2.5	-61	-43	2.85	c
α-D-gal 1-3 β-D-galNAc	96.0	2.8	-52	-35	2.69	6
α-D-gal 1-3 β-D-gal	96.2	3.0	-50	-34	2.65	c
α-D-gal 1-2 α-D-glc	97.5	4.3	-48	-30	2.54	c
α-D-gal 1-4 α-L-rham	100.5	7.3	-40	-15	2.32	c
α-D-gal 1-4 α-D-gal	101.4	8.2	-41	-11	2.30	c
α-D-gal 1-4 α-D-man	101.4	8.2	-48	-4	2.34	b

[a] Chemical shifts for the corresponding monosaccharides are taken from K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 1984, **42**, 193. [b] Present work. [c] From ref. in note a.

the two parameters as seen from Figure 2. However, as discussed by Thøgersen et al.[7] many combinations of φ and ψ will obey the distance requirement and a single conformer cannot be determined solely from this correlation.

We have therefore tried to correlate the glycosylation shifts with ψ because the exo-anomeric effect[3] shows a strong preference for φ_H in the range of 40—60° in α-D-glycosides[8] as also proposed by Lemieux and Koto.[3] The results of these correlations are shown in Figures 3a and b for the anomeric and aglycone glycosylation shifts, respectively. It is obvious that there are good correlations with correlation coefficients similar to the one found for the correlation with the proton–proton distances.

Table 2 shows similar values for α-D-galactosides and the data are included in the correlations in Figure 2 and 3, inasmuch as the changes in ^{13}C chemical shifts for the anomeric and aglycone carbon atoms with the configurational change of the OH group in the 4-position are very small.

These results indicate that it is possible to use ^{13}C chemical shifts to yield information about the preferred conformation around the glycoside linkage and particularly for the determination of ψ. This observation explains the observed upfield shifts or lack of glycosylation shifts as discussed by e.g. Lemieux[9] for the A and B blood group trisaccharides when a branched structure is synthesized. The upfield shift of the α-D-galactopyranose unit is thus caused by an interaction between α-D-galactopyranosyl in the 3-position and α-L-fucopyranosyl in the 2-position. Similarly, the dependence of ^{13}C chemical shifts on torsion angles discussed by Pavia et al.,[10] Liptak and Göndör,[11] and Bock et al.[12] is in agreement with the present observation.

Other results which are in accord with the above discussion have been published by Jane et al.[13] in a study of complexes of amylose in different solvents, and data published by Hisamatsu et al.[14] on cyclic β-1,2-glucans likewise indicate the conformational dependence of the ^{13}C chemical shifts. Finally, results from solid-state [13]C n.m.r. spectroscopic data of cyclomaltohexose and inclusion complexes have been explained in a similar manner.[15]

We are therefore convinced that the dependence of the ^{13}C glycosylation chemical shifts on the preferred conformation of the glycoside linkage, and particularly with the ψ torsion angle as shown in Figures 3a and b, is a diagnostically useful correlation in the conformational analysis of oligosaccharides, particularly because it is easy to measure the ^{13}C chemical shifts. We have not made any attempt to measure the chemical shifts more accurately than the published values, which can change relatively by 0.1—1 p.p.m. due to temperature effects or
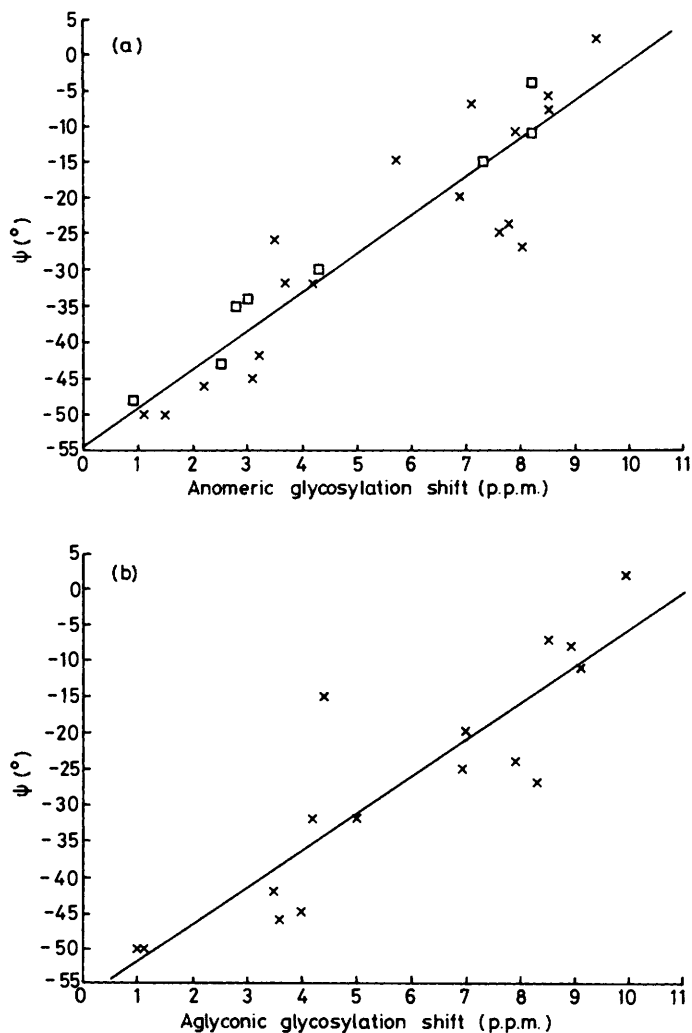
Figure 3. Correlation of glycosylation shifts for the anomeric (a) and the aglycone (b) carbon atoms with dihedral angle ψ. $\times$, Data from α-D-glucose; $\square$, those from α-D-galactose. The linear regressional fits follow the equations: $\psi = 5.296$ (p.p.m.) $- 54.24$ for the anomeric shifts (a) and $\psi = 5.133$ (p.p.m.) $- 56.93$ for the aglycone shifts (b) with correlation coefficients r of 0.916 and 0.891, respectively

by use of different reference substances. The data will not allow a determination of the preferred conformation with a high degree of accuracy but rather indicate, using the slope of the curve, that a change in an observed ^{13}C chemical shift of *ca.* 2 p.p.m. corresponds to an average change in ψ *ca.* 10°. It should be pointed out that the n.m.r. parameters obtained in solution, including ^{13}C n.m.r. chemical shifts, can only yield information about the average structural changes because the timescale of the n.m.r. experiment is so slow relative to the molecular reorientations that only integrated responses will be observed.

It is expected that a similar dependence exists for β-D-

glucosides, -galactosides, or -glycosides of *e.g.* mannose or rhamnose. We have chosen to illustrate the dependence between the ^{13}C n.m.r. glycosylation shifts and the angle ψ with α-glycosides of glucose and galactose because compounds derived from these monosaccharides are most abundant.

Experimental

^{13}C N.m.r. chemical shifts not found in the literature (Tables 1 and 2) were measured on a Bruker AM-500 spectrometer at 300 K in D_2O using dioxane (1%) as internal reference (δ 67.4 p.p.m.).

Hard-spheres *exo*-anomeric effect calculations were performed using potentials as described in ref. 7 but with a new version of the HSEA program.[16] The co-ordinates for the α-D-glucopyranose and α-D-galactopyranose units were taken from neutron diffraction studies[17,18] and the co-ordinates for the different aglycones were, for compounds where X-ray data were available, constructed from the heavy atoms and calculations of the proton co-ordinates using a C–H bond distance of 1.10 Å. The X-ray data for the (R)-[or (S)-]methylcyclohexyl α-D-glucopyranoside were kindly provided by Delbaere.[19] In compounds where X-ray structures were not available the co-ordinates were constructed by bond modification of the appropriate sugar structures using a C–H bond distance of 1.10 Å and tetrahedral geometry as discussed by Arnott and Scott.[20]

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