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Note

Relationship of chemical shift to glycosidic conformation in the solid-state ^{13}C NMR spectra of (1 → 4)-linked glucose polymers and oligomers: anomeric and related effects

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In the ^{13}C NMR spectra of oligo- and poly-saccharides, the chemical shifts of the carbon atoms on either side of the glycosidic linkage vary over a range of up to 12 ppm, depending on the conformation of the linkage [1–5]. This effect operates both in solution and in the solid state, but is most readily observed in the CP-MAS (cross polarisation/magic-angle spinning) spectra of solids where the conformations are fixed and the chemical shifts are not motionally averaged. It has obvious potential for the determination of structures, and has already proved useful for this purpose in studies of gels, where crystallographic data are unavailable [4,6], and of cellulose, where crystallographic methods alone have not determined how the chains are packed [7,8].

However, the absence of a theoretical explanation of the effect has meant that its predictive power has been limited. Empirical relationships between chemical shift and glycosidic conformation have been established for groups of related carbohydrates [3,4,9], but those for α-(1 → 4)-D-glucans [4] fail to work for β-(1 → 4)-linked polymers [3,9–11] and vice versa. The semi-empirical γ-gauche effect, normally applied to alkanes, has been suggested [1] as an explanation. However, it is inapplicable to α-linked polysaccharides and incorrectly predicts similar changes in chemical shift at both ends of the γ relationship. In carbohydrates, there is little effect of glycosidic conformation on the chemical shifts of carbon atoms other than those involved in the glycosidic link.

These characteristics of the conformational effect in polysaccharides suggest that it may originate in the orientation of the lone pairs on the glycosidic oxygen atom relative to the configurations on either side of the glycosidic link. This relationship is involved in some manifestations of the anomeric effect, which has been extensively reviewed [12,13]. The origin of the anomeric effect is controversial

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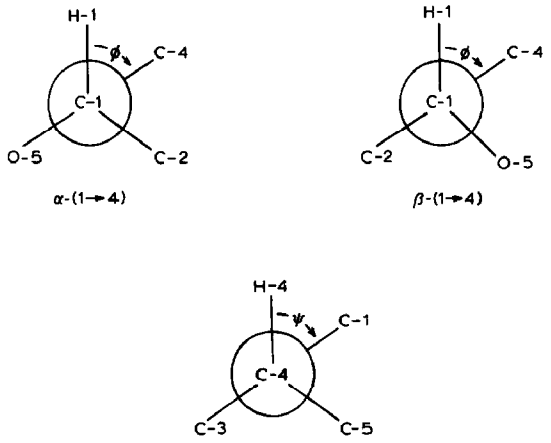

Fig. 1. Definitions of ϕ and ψ used here. O-4′ is at the rear in each case.

[12]. It has been explained in terms of interactions between dipoles or between nonbonding and antibonding orbitals [13], although both explanations present problems [14]. The second explanation is preferred here as it generally gives superior predictions of molecular geometry, although it is inferior for predicting equilibria between conformational isomers [12,13].

In this model, the classical anomeric effect is considered to arise from the donation of electron density from a lone-pair orbital of the ring oxygen into an antibonding σ^* orbital of the O-4′–C-1 bond when these orbitals are aligned in the α anomer. In straightforward cases, this explains the following observations: the O-4′–C-1 bond is lengthened, the C-1–O-5 bond is shortened, and the O-5–C-1–O-4′ bond angle opens as the C-1–O-5 bond takes on more π character [15]. It may be predicted that electron donation from O-5 into the σ^* (C-1–O-4′) orbital will increase the mean electron density in the immediate vicinity of C-1 and hence move its ^{13}C resonance to higher shielding.

In that classical form, the anomeric effect is independent of the conformation at the glycosidic linkage. However the anomeric effect in a broader sense can operate in any O–C–X system, where X is at least to some extent electron-withdrawing. Thus, it applies equally to the O-5–C-1–O-4′ system in the reverse direction, whenever the glycosidic torsion angle ϕ (defined here as H-1–C-1–O-4′–C-4′; Fig. 1) permits one of the lone-pair orbitals on O-4′ to donate electron density to the σ^* orbital of the C-5–O-5 bond (the exo-anomeric effect [12]). In an α-(1 → 4)-linked polysaccharide with a suitable value of ϕ ($-60°$ or 180°), the anomeric and exo-anomeric effects reinforce one another, because each makes the 'X' atom more electron-withdrawing for the other.

There is also evidence [16] that what will be called here the pseudo-anomeric effect is present in some O–C–C systems, with electrons donated into a suitably oriented σ^* orbital of the C–C bond. In (1 → 4)-linked polysaccharides, the C–C bond can be either C-3′–C-4′ or C-5′–C-4′ at suitable values of the other glycosidic torsion angle ψ (defined as H-4′–C-4′–O-4′–C-1).

The magnitude of the change in chemical shift induced by the classical anomeric effect may be estimated from the solution-state spectra [17] of the simple model compound 2-methoxytetrahydropyran, in which the ring carries no substituents, other than on the anomeric carbon (C-2), to complicate the interpretation. In this compound, the anomeric effect operates almost in isolation in solution, because free rotation about the C-2–OMe bond largely dissipates the contribution from the exo-anomeric effect. In the isomer with the methoxyl equatorial, the anomeric effect is not possible and the C-2 resonance is at 103 ppm. In the isomer with the methoxyl axial, the classical anomeric effect displaces the C-2 resonance to 97.7 ppm, a difference of 5.3 ppm [17]. In agreement with this observation, chemical shifts observed for C-1 are normally at higher shielding in the α than in the β anomers of carbohydrates, both in the solid state (see Tables 1 and 2) and in solution [18], and the magnitude of the difference varies from zero to ca. 8 ppm, depending on the other factors affecting the C-1 chemical shift.

It may be assumed that the exo-anomeric effect is comparable in magnitude with the classical anomeric effect: for example, the bond angles around the anomeric carbon in α-glycosidic carbohydrates showing both effects are approximately symmetrical. Thus, when both the anomeric and the exo-anomeric effect operate together, it may be predicted that their combined effect will be large enough to explain the conformation-dependent variation in (C-1) chemical shift that is observed. The pseudo-anomeric effect is likely to be weaker, since the recipient carbon atom is less electron-withdrawing than the ring or bridging oxygen. It seems likely that a stereoelectronic effect similar to the pseudo-anomeric effect controls the dependence of the C-6 chemical shift in hexopyranosides on the torsion angle χ describing rotation around the C-6–C-5 bond. The observed variation in C-6 chemical shift is up to ca. 5 ppm [4,5], but depends on hydrogen bonding as well as conformation [5].

An anomaly in this description of the anomeric effect arises from the fact that although the two lone pairs on an oxygen atom are commonly represented as equivalent and sp^3-hybridised, they differ in energy. Using the formally equivalent representation as one σ and one π orbital, the π orbital is of higher energy and is thus favoured as an electron donor. So the anomeric effect should be maximised when the π orbital is parallel to the σ^*(C–O) orbital, but this is not what is found experimentally; there is some effect over a range of C–O torsion angles, but the maximum corresponds to the alignment of an sp^3-hybridised orbital with σ^*(C–O). It has been argued [13] that this simply means a small amount of electron density donated from the less favoured σ orbital along with a larger amount from the π orbital. The electron-density data of Longchambon et al. [19] confirm that the lone pairs on both the ring and the bridging oxygen are approximately tetrahedrally oriented and demonstrate that their shape is distorted by the anomeric effect, although the published data are not detailed enough to confirm the identity of the orbital receiving the electron density.

The question of the symmetry of the lone pairs is probably academic except when they are both involved in interactions with other orbitals. In this case, the geometry can correspond to (sp^3,sp^3) or (σ, π), but not a mixture, and while both

sp^3 orbitals can be electron donors to antibonding orbitals, the σ orbital is only likely to participate in delocalisation interactions with lone pairs on other oxygen atoms. Where lone pairs on the two oxygen atoms in a O–C–O system are aligned so that they can interact directly with one another through space, delocalisation tends to stabilise that conformation (with the associated orbital geometry on the oxygen atoms) and to reinforce any anomeric-type effects that it permits [12,20].

This note considers how chemical shifts can be predicted from glycosidic conformations, using the anomeric effect. Since the effect also makes testable predictions about bond lengths and angles, and since these can be useful in deducing how it operates, the discussion will be limited to (1 → 4)-linked glucans and related carbohydrates for which high-quality crystallographic data are available. These include the β-(1 → 4)-linked examples examined by Horii et al. [3] and a number of cyclodextrins and other α-linked glucose derivatives [4,5,21]. Carbohydrates crystalline enough to give good diffraction data normally also have well-resolved ^{13}C NMR spectra in the solid state, and are not normally contaminated with sufficient paramagnetic ions to affect ^{13}C chemical shifts in the solid state. Hydrogen bonding might be expected to complicate the interpretation, but the α-linked polymers considered here have no hydrogen bonds involving O-4′ and O-5 except for a weak H_2O–O-5 bond in amylose-V, and all the β-linked polymers have an O-3′–O-5 hydrogen bond which can be incorporated in the analysis.

α-(1 → 4)-Glucans.—All α-linked hexopyranose polymers are capable of showing the classical anomeric effect, and the α-linked (1 → 4)-glucans included in Table 1 had their C-1 resonance at higher shielding than the corresponding β-glucans, with little overlap (Tables 1 and 2). The upfield displacement of the C-1

Table 1
Crystallographic 22–25 and ^{13}C NMR [4,5] data for α-(1 → 4)-D-glucans

	α-Cyclodextrin hexahydrate [22,23]			Amylose-V_6 [24]	Amylose A [25]
	Residues 4 → 5 linkage	Residues 5 → 6 linkage	Mean of other linkages		
φ (°)	−31.8	−29.6	−13.6	−14.4	−25
ψ (°)	−3.7	+50.70	−8.1	−7.50	−32
δ(C-1) (ppm)	102 −103.8	98.1	102.9	103.5	99–101
δ(C-4′) (ppm)	80.6– 83.1	77.7	82.1	83.2	76
Bond lengths (pm)					
O-5–C-1	142.6	141.8	140.9	141.3	
C-1–O-4′	140.8	142.8	140.9	141.8	
O-4′–C-4′	143.3	144.9	143.4	143.0	
C-3′–C-4′	151.8	151.9	152.4	152.7	
C-5′–C-4′	153.5	155.3	152.8	152.9	
Bond angles (°)					
O-5–C-1–O-4′	111.9	110.4	110.7	111.1	
C-1–O-4′–C-4′	116.2	117.2	118.5	118.6	
C-3′–C-4′–O-4′	108.4	103.7	105.3	106.0	
C-5′–C-4′–O-4′	109.2	112.7	109.2	107.4	

Table 2
Crystallographic [26–30] and ^{13}C NMR [3] data for β-(1 → 4)-linked oligosaccharides and glucans

	β-Cellobiose [30]	Methyl β-cellobioside methanolate [29]	α-Lactose monohydrate [27]	β-Lactose [25]	Cellulose I (model) [26] [a]
ϕ (°)	+43.7	+28.9	+27.4	+49.3	+23.0
ψ (°)	−12.3	−40.7	−23.0	−11.3	−23.7
δ(C-1) (ppm)	104.9	106.5	107.9	103.6	106.3
δ(C-4′) (ppm)	84.9	85.5	88.0	82.0	90.6
Bond lengths (pm)					
O-5–C-1	142.5	143.2	142.7	141.3	
C-1–O-4′	139.7	139.0	138.9	140.2	
O-4′–C-4′	144.6	143.7	143.7	145.1	
C-3′–C-4′	153.0	153.3	153.3	153.6	
C-5′–C-4′	152.7	152.6	152.5	152.3	
Bond angles (°)					
O-5–C-1–O-4′	107.4	107.6	107.0	107.6	
C-1–O-4′O-4′	116.1	115.8	117.1	116.5	
C-3′–C-4′–O-4′	109.0	111.5	110.6	107.8	
C-5′–C-4′–O-4′	106.4	106.7	107.0	107.4	

[a] For cellulose I, chemical shifts are means of multiplets, and detailed bond lengths and angles are not given because the structure used [26] was not fully refined.

resonance was accentuated when the electron density on O-4′ was depleted by the other effects mentioned below.

The exo-anomeric effect is maximal at $\phi = -60°$ approximately. As ϕ approaches this value, a displacement of the C-1 resonance to higher shielding, and lengthening of the the C-1–O-5 bond, are predicted. This is observed in the linkage between residues 5 and 6 of α-cyclodextrin hexahydrate compared with the other residues (Table 1). By enhancing the classical anomeric effect, it also lengthens the C-1–O-4′ bond, but the O-5–C-1–O-4′ bond angle is not opened because the n–σ^* interactions are both in the O-5–C-1–O-4′ plane and no tendency towards sp^2 hybridisation at C-1 is possible. Probably the effect is similar in the A- and B-amyloses, with ϕ = ca. − 25° and the C-1 resonance at ca. 100 ppm, but details of bond lengths and angles are not available.

On the other side of the glycosidic linkage, a lone pair on O-4′ can, in principle, donate electrons into the σ^* antibonding orbital of the C-4′–C-5′ bond at ψ = ca. + 60° or into σ^* (C-4′–C-3′) at ψ = ca. − 60° (the pseudo-anomeric effect as defined above). The linkage between residues 5 and 6 of α-cyclodextrin hexahydrate has $\psi = +51°$, giving a long C-4′–C-5′ bond, a wide C-5′–C-4′–C-4′ bond angle, and a C-4′ resonance at higher shielding than for any of the other residues in the molecule (Table 1; Fig. 2). The same lone pair on O-4′ is involved in the exo-anomeric effect and the depletion of the electron density at O-4′ may be expected to provide further reinforcement of the anomeric effect. In line with this prediction, the C-1 resonance is displaced to higher shielding and the C-1–O-4′ bond lengthened more than in residue 4 of the same molecule, even though both

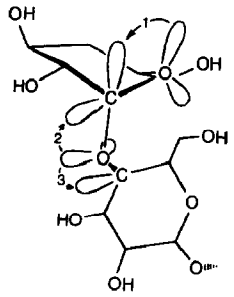

Fig. 2. Interactions between orbitals in the linkage between residues 5 and 6 of α-cyclodextrin hexahydrate. 1, Anomeric effect: donation of electron density from lone pair on O-5 into antibonding σ* orbital of C-1–O-4′ bond. 2, Exo-anomeric effect: donation from lone pair on O-4′ into σ*(C-1–O-5) orbital. 3, Pseudo-anomeric effect: donation from lone pair on O-4′ into σ*(C-5′–C-4′) orbital.

residues have almost the same value of ϕ. Thus, in some circumstances, the conformation on one side of the glycosidic linkage can influence the chemical shift of the carbon nucleus on the other side. The alternative pseudo-anomeric interaction of a lone pair on O-4′ with σ*(C-4′–C-5′) is approximated, although less closely, in A- and B-amylose ($\psi = -32°$). This interaction and the exo-anomeric interaction would involve opposite lobes of the lone pair with *p* symmetry on O-4′. This may be why it gives a larger effect on the chemical shift of C-4′ and a smaller effect on that of C-1′, but the details are uncertain without data on bond lengths.

β-(1 → 4)-Linked oligo- and poly-saccharides.—The classical anomeric effect is absent in β-linked glucans, but the exo-anomeric effect increases with ϕ towards ($\phi = +60°$) and is associated with a progressive displacement of the C-1 resonance to higher shielding as predicted (Table 2). The exo-anomeric effect is enhanced by the O-3′–O-5 hydrogen bond, which makes O-5 more electron-withdrawing. If the two lone pairs on O-4′ are considered as being sp^3-hybridised, the other can simultaneously become oriented to interact increasingly through space [18] with the axial lone pair on O-5 and possibly with one of the lone pairs on O-3′, with rotation of the C-3′–O-3′ bond being prevented by the hydrogen bond to O-5 (Fig. 3). This opportunity for delocalisation may encourage approximately sp^3-like hybridisation to be maintained on both O-5 and O-4′, so that little consistent

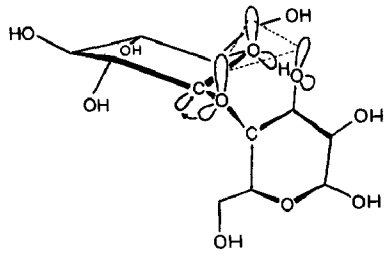

Fig. 3. Interactions between orbitals in the glycosidic region of β-cellobiose. Exo-anomeric effect as in Fig. 2; hydrogen bond involving O-3′ and equatorially oriented lone pair on O-5; delocalisation between axial lone pairs on O-5, O-4′, and O-3′.

change in the associated bond lengths and angles accompanies the change in chemical shift of C-1.

The chemical shift of C-4′ is more difficult to explain, because, except for methyl β-cellobioside, ψ shows little variation and, so far as it does vary, correlates with ϕ. The C-4′ chemical shifts predicted from ψ, based on the pseudo-anomeric effect, would vary much less than observed and in the wrong direction. It seems more likely that they are determined by ϕ as originally claimed by Horii et al. [3]. It is possible that the mechanism by which the effect is transmitted across the glycosidic linkage involves through-space delocalisation around the group of axially oriented lone pairs on O-5, O-4′, and O-3′ mentioned above, but the details are not clear. Because of the extent of delocalisation, this system may be beyond the scope of the analysis outlined here. In the β-(1 → 4)-D-mannans [10] and chitin [11], the relationship of chemical shift to conformation resembles that in the β-(1 → 4)-glucans, and it may be assumed that similar mechanisms operate.

Methyl β-cellobioside is an exception in the β-linked series, as its structure permits a pseudo-anomeric effect. With $\psi = -40°$, one of the lone pairs on O-4′ can donate electron density into the σ^*(C-4′–C-3′) orbital, opening the C-3′–C-4′–O-4′ bond angle and displacing the C-4′ resonance by ca. 4 ppm to higher shielding compared with other structures having similar values of ϕ.

The principles described above—chemical shift displacements to higher shielding due to the anomeric and exo-anomeric effects (C-1) and the pseudo-anomeric effect (C-4′ here), enhanced by through-space delocalisation of lone-pair electrons —are general enough to be applied to most oligo- or poly-saccharides. They predict the empirical rules devised by previous authors [3,4,9,21] in the systems for which these were designed. The fact that they also make reasonably accurate predictions about bond lengths and angles suggests that they have a genuine basis, and indeed that NMR in the solid state may have potential for elucidating the mechanism of the anomeric effect. They should make it possible to deduce conformational changes in ϕ and, in favourable cases, ψ directly from the chemical shift displacements when crystallographic data are not available, as in gels and intact biological systems.

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