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DEPENDENCE OF ^{13}C CHEMICAL SHIFTS ON THE SPATIAL INTERACTION OF PROTONS, AND ITS APPLICATION IN STRUCTURAL AND CONFORMATIONAL STUDIES OF OLIGO- AND POLY-SACCHARIDES

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ABSTRACT

Regularities in the variation of chemical shifts and the glycosidation effects in the ^{13}C-n.m.r. spectra of disaccharides were found to depend on the configuration at the anomeric centre of the glycosidating pyranose, and the absolute configuration of both pyranoses moieties. These empirical regularities are explained in terms of the spatial proton–proton interactions within the statistically averaged, or preferred, conformation near the glycosidic linkage. The applicability of these effects for the determination of the anomeric and absolute configuration and the sequence of pyranose residues in oligo- and poly-saccharides is discussed. The conformational properties of glycosidic linkages in disaccharides and disaccharide fragments of oligo- and poly-saccharides are compared on the basis of ^{13}C-n.m.r. data.

INTRODUCTION

The alkyl ethers of alkyl glycosides have been used in ^{13}C-n.m.r. studies as model compounds for oligo- and poly-saccharides[1]. The α- and β-effects associated with methylation, which have been employed frequently to calculate the spectra of the residues in oligo- and poly-saccharides, have resulted in misinterpretations[2,3]. The spectra of some *O*-isopropyl derivatives of monosaccharides resemble more closely those of the corresponding monosaccharide residues in polysaccharides than do the spectra of the *O*-methyl derivatives[4,5]. However, this correspondence is usually far from satisfactory. The differences in α- and β-effects in the ^{13}C-n.m.r. spectra associated with alkylation and glycosidation prompted an attempt to analyse the factors that determine the magnitude of these effects, particularly the spatial interactions of protons.

RESULTS AND DISCUSSION

The influence of the interaction of protons at the 1,4-positions (γ-gauche) on the chemical shifts of carbon signals has been thoroughly studied[6], and results in an upfield shift of the signals of both carbon atoms. In contrast, the steric interaction of protons at the 1,5-positions (δ-interaction) causes[7,8] a downfield shift of the signals of the corresponding carbon atoms. These effects are widely used in the analysis of the ^{13}C-n.m.r. spectra of carbohydrates.

Beierbeck and Saunders[9] proposed the interaction of protons at the 1,3-positions (β-interaction) as a factor affecting the ^{13}C chemical shifts. Their opinion, substantiated by an analysis of the ^{13}C-n.m.r. spectra of polycyclic compounds, was that such an interaction leads to a downfield shift of the carbon signals. This effect has been neglected in previous analyses of the ^{13}C chemical shifts in carbohydrates (with the exception of ref. 10) and we now exemplify its value by considering a series of alkyl glucosides.

The data in Table I show that, for the series of alkyl D-glucopyranosides where the aglycon is Me (**1**), Et (**2**), ^{i}Pr (**5**), and ^{t}Bu (**7**), the C-1 signal is progressively shifted upfield. Koch *et al.*[11] explained this phenomenon in terms of the interaction of protons at 1,4-positions (γ-interaction). However, comparison of the data for the whole series of compounds in Table I shows that the number of protons at C-2′ of the aglycon does not affect the value of the glycosidation effect (*cf.*, for example, **2** and **4**, or **7** and **8**). However, there is a regular variation of the alkylation effects[12] in relation to the number of protons at C-1′ (3 protons, 7.6–7.8; 2 protons, 5.7–6.1; 1 proton, 4.5–4.6; no protons, 0.6–0.8 p.p.m.). From the standpoint of the concept of Beierbeck and Saunders[9], this sequence can be attributed to the decrease in the lifetime (or in the statistical weight) of the conformers that involve interaction of the protons at the 1,3-carbons.

Allowance for these interactions is necessary when interpreting substitution effects in the ^{13}C-n.m.r. spectra of carbohydrates. From this viewpoint, for instance, the abnormally large effects of methylation of the non-anomeric hydroxyl groups in carbohydrates, which are comparable in magnitude to those of nitrate and sulphate groups, become comprehensible. The small effect (2–4 p.p.m.) of methylation in methyl ketopyranosides[13], also associated with the anomeric carbon atoms in sucrose (0.1 p.p.m. for glucose C-1, 2.2 p.p.m. for fructose C-2), reflects the absence of the proton at the anomeric carbon atom of ketoses and the elimination of the β-interaction.

At first glance, the data in Table I indicate that the chemical shifts of the glucopyranose C-1 signals are independent of the γ-interaction of protons, and that the alkylation effects are due to β-interactions, which is the viewpoint of Beierbeck and Saunders[9]. A similar approach was used to account for the substitution effect on the chemical shifts of the signals of the 1,5-carbons, namely, that replacement of a proton at one of the carbon atoms eliminates the γ-interaction, and, as a result, the signal of the other carbon atom is shifted downfield[14]. In this case, consideration of the δ-interaction as an independent factor can be dispensed with. On the basis of our data on the influence of substitution in carbohydrates on their ^{13}C-n.m.r. spectra, we propose the alternation rule[15,16] that the spatial interactions of the protons at 1,3- and 1,5-positions (β- and δ-interactions, respectively) cause downfield shifts of the signals of the corresponding carbon atoms, whereas that of protons in 1,4-positions (γ-interaction) causes upfield shifts. All such interactions must be con-

TABLE I

ALKYLATION EFFECTS[a] IN THE ^{13}C-N M R SPECTRA OF ALKYL D-GLUCOPYRANOSIDES

Compound	*Aglycon*	*Alkylation effect for C-1 (p.p.m.)*	
		α Anomer	*β Anomer*
1	Methyl	7.6	7.8
2	Ethyl	5.8	6.0
3	Allyl	—	6.1
4	Benzyl	5.7	6.0
5	Isopropyl	—	4.6
6	Cyclohexyl[b]	4.5	4.6
7	*tert*-Butyl	—	0.8
8	Trityl	—	0.6

[a]Difference in the chemical shift of the C-1 signal for the alkyl D-glucopyranoside and the corresponding pyranose. [b]Data of Lemieux and Koto[12].

sidered in the analysis of ^{13}C-n.m.r. spectra when structural alterations are made. This concept accords with the theory of Grant and Cheney[6], the main equation of which, as is well known, involves the angular-dependence term determining the signs of the effects of the proton–proton interactions.

The above rule could possibly be extended to include the interaction of the protons associated with more-distant carbons. Thus, in the ^{13}C-n.m.r. spectra of 3,4,6-tri-*O*-acetyl-1,2-*O*-alkylidene-α-D-glucopyranoses (ethylidene or 1-cyano-ethylidene), there was an upfield shift (2–3 p.p.m.) of the signals of glucopyranose C-5 and the alkylidene Me carbon atom for the *endo*-Me isomers compared to the *exo*-Me isomers (Fig. 1). Analysis of the ^{1}H-n.m.r. spectra confirmed the proximity of H-5 and the Me protons in the *endo*-Me isomers. Thus, the upfield shift of the C-5 and Me signals in the *endo*-Me isomers can be explained in terms of the interaction of the protons in the 1,6-positions. In the ^{13}C-n.m.r. spectra of 2-*O*-α-, 3-*O*-α-, 3-*O*-β-, and 4-*O*-α-L-rhamnopyranosyl-D-galactopyranoses[17], the unusual upfield-shifts (16.18 p.p.m.) of the C-6′ signal of the rhamnopyranosyl groups and the C-6 signals of the galactopyranose residues (60.9 and 61.0 p.p.m.) in the spectra of 4-*O*-α-L-rhamnopyranosyl-α- and -β-D-galactopyranose, are noteworthy. The chemical shifts of the signals of these carbon atoms in the other disaccharides do not exceed the limits of 17.3–17.5 and 61.6–61.9 p.p.m., respectively. Molecular models show that, for a certain configuration around the glycosidic linkage in 4-*O*-α-L-rhamnopyranosyl-D-galactopyranose (and in this compound only), spatial interaction of the protons at C-6 and C-6′ is feasible. Since there are no other reasons to explain the simultaneous upfield-shift of the C-6 and C-6′ signals than the spatial interaction of the protons at these 1,8-positions, this fact can be viewed as additional evidence for the alternation rule, which would predict the observed upfield-shifts.

Determination of the contribution of a particular proton–proton interaction to the chemical shift of a particular carbon atom can be a complicated task. Gener-

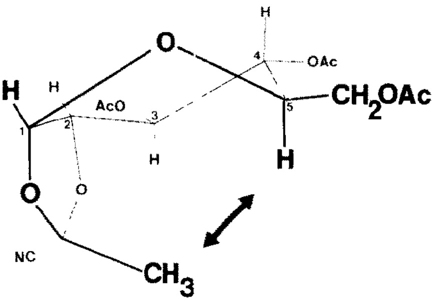


Fig. 1. Remote H–H-interaction within 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-cyanoethylidene)-α-D-glucopyranose.

ally, the changes in the chemical shifts of carbon signals result from the creation and/or removal of various proton–proton interactions in addition to such other factors as inductive interactions and the interaction of constant and fluctuating dipoles. For conformationally flexible systems, the statistical weight (lifetime) of the conformer associated with a particular proton–proton interaction needs to be determined. On the other hand, it is the effect of proton–proton interactions which enables the conformational properties of the glycosidic linkage to be investigated by ^{13}C-n.m.r. spectroscopy.

Lemieux and Koto[12] were the first to emphasise the dependence of ^{13}C chemical shifts on the conformation of glycosidic linkages when studying cyclohexyl, 2- and 6-methylcyclohexyl, and 2,6-dimethylcyclohexyl α- and β-D-glucopyranosides. A similar dependence was established for natural glycosides with chiral aglycons. For such glycosides, ^{13}C-n.m.r. spectra can be used[18–20] to determine the absolute configuration of both pyranoses and the aglycon.

An analysis of the chemical shifts of the signals in the ^{13}C-n.m.r. spectra of di-, oligo-, and poly-saccharides established that the large amplitude for the glycosidation effects, which is determined by the conformation around the glycosidic linkage, is also typical of some disaccharide moieties[10,15]. The latter involve (1→3)-linked units (frequently encountered in natural oligo- and poly-saccharides), in which a glycopyranose residue (Hex*p*1) is bonded at an equatorial hydroxyl group of a second residue (Hex*p*2) in which the substituents at the β-carbons (C-2 and C-4) have different orientations, *i.e.*, equatorial at C-2 and axial at C-4 or vice versa.

Table II contains the chemical shifts of the signals for C-1 for Hex*p*1 in Hex*p*1-(1→3)-Hex*p*2 and in some related oligo- and poly-saccharides. Analysis of the tabulated data reveals that the configuration of the glycosidic centre of Hex*p*2 (*cf.* **25** and **26**), the orientation of HO-4 of Hex*p*1 (*cf.* **28** and **29**), and the nature of the 5-substituent (*cf.* **28** and **30**, or **35** and **36**) do not affect appreciably the chemical shift of the C-1 signal, and henceforth these factors will not be considered. In contrast, there are significant changes in chemical shifts of the C-1 signal of Hex*p*1 when the anomeric configuration of Hex*p*1 or the absolute configuration of

TABLE II

CHEMICAL SHIFTS (WITH REFERENCE TO Me_4Si) FOR C-1 OF Hex*p*1 IN Hex*p*1-(1→3)-Hex*p*2 DISACCHARIDE UNITS

No.	*Disaccharide unit*	*Chemical shift (p.p.m.)*	*Ref.*	*No.*	*Disaccharide unit*	*Chemical shift (p.p.m.)*	*Ref.*
9	α-D-Gal*p*-(1→3)-α,β-D-Gal*p*	96.6	21	**23**	→4)-α-D-Glc*p*A-(1→3)-β-D-Man*p*	100.5	30
10	α-D-Gal*p*-(1→3)-β-D-Gal*p*NAc	96.0	22	**24**	α-D-Glc*p*A-(1→3)-α,β-L-Rha*p*	94.4	31
11	α-D-Glc*p*-(1→3)-β-D-Gal*p*	96.55	23	**25**	α-D-Glc*p*-(1→3)-α-L-Rha*p*	96.4	32
12	→4)-α-L-Gal*p*-(1→3)-α,β-D-Gal*p*	101.9	21	**26**	α-D-Glc*p*-(1→3)-β-L-Rha*p*	96.1	32
13	α-D-Glc*p*-(1→4)-β-D-Fru*p*[a]	101.6	24	**27**	β-D-Glc*p*-(1→3)-β-D-Man*p*	100.2	28
14	β-D-Gal*p*-(1→3)-α,β-D-Gal*p*	105.2	25	**28**	β-D-Glc*p*-(1→3)-α,β-L-Rha*p*	105.0	33
15	β-D-Glc*p*A-(1→3)-α,β-D-Gal*p*	104.5	26	**29**	β-D-Gal*p*-(1→3)-α,β-L-Rha*p*	105.5	33
16	β-D-Glc*p*-(1→3)-α-L-Fuc*p*NAc	100.3	27	**30**	→4)-β-D-Glc*p*A-(1→3)-β-L-Rha*p*	104.8	34
17	→3)-α-D-Man*p*-(1→3)-α,β-D-Gal*p*	97.1	28	**31**	→3)-α-D-Man*p*-(1→3)-β-D-Man*p*	103.8	35
18	α-L-Rha*p*-(1→3)-α,β-D-Gal*p*	103.6	29	**32**	α-L-Rha*p*-(1→3)-α-D-Man*p*	98.3	36
19	β-L-Rha*p*-(1→3)-α,β-D-Gal*p*	98.1	29	**33**	α-D-Rha*p*-(1→3)-α-D-Rha*p*	103.4	37
20	β-D-Man*p*-(1→3)-α,β-D-Gal*p*	102.3	10	**34**	α-L-Rha*p*-(1→3)-α-L-Rha*p*	103.1	38
21	α-D-Glc*p*-(1→3)-α,β-D-Man*p*	101.4	28	**35**	→4)-β-D-Man*p*-(1→3)-β-D-Man*p*	98.7	39
22	→3)-α-D-Gal*p*-(1→3)-α-D-Man*p*	101.4	26	**36**	→2)-β-D-Rha*p*-(1→3)-α-D-Rha*p*	98.1	37

[a] β-D-Fru*p* is homomorphic to α-L-Gal*p*.

one of the residues is inverted, and if, for Hex*p*2, Man is replaced by Gal.

*Dependence of the chemical shift of the C-1 signal of Hex*p1 *on the chirality of C-5.* — Comparison of the chemical shifts of the C-1 signals for **9** and **12** shows that a D→L change at C-5 of either unit changes the chemical shift by ~5 p.p.m. (*cf.* **11** and **12**, or **27** and **28**). As would be expected, inversion of the configuration of both C-5 and C-5′ produces no change (*cf.* **33** and **34**).

*Dependence of the chemical shift of the C-1 signal of Hex*p1 *on the orientation of HO-2 and HO-4 of Hex*p2. — Comparison of the data for **9** and **22** shows that replacement of Hex*p*2 containing an axial 4-substituent with a unit containing an axial 2-substituent causes a change in the chemical shift of the C-1 signal by ~5 p.p.m. (*cf.* **15** and **27**, **17** and **31**, **18** and **32**). Since this dependence is of the same amplitude as the dependence of the chemical shift of the C-1 signal on the chirality of C-5, but is opposite in sign, the simultaneous variation of the two factors causes no change (*cf.* **17** and **32**, or **18** and **31**).

*Dependence of the chemical shift of the C-1 signal on the anomeric configuration of Hex*p1. — The inversion of the anomeric configuration of Hex*p*1 residues in which HO-2 and/or HO-3 is axial, or their pyranosides with an achiral aglycon, has an insignificant effect[40] on the chemical shift of the signal for C-1. However, for the disaccharide moieties considered, the amplitude of the change in the chemical shifts of the C-1 signal of Hex*p*1 (Man or Rha) is up to 5 p.p.m., depending on the anomeric configuration (*cf.* **17** and **20**, **18** and **19**, **33** and **36**, **31** and **36**). For Glc*p* or Gal*p* or their pyranosides with an achiral aglycon, the C-1 signal is shifted upfield by ~4 p.p.m. on changing from β to α, due to the change in the orientation of the OH(OR)-group with respect to the remaining substituents and the ring oxygen[39]. When Hex*p*1 is Glc or Gal, the change in the chemical shift of the C-1 signal on inversion of the anomeric configuration is the algebraic sum of the contributions associated with the change in the orientation of the OR-group at C-1 with respect to the substituents in Hex*p*1 (the configurational component), and the change in the orientation near the glycosidic linkage in Hex*p*1 and Hex*p*2 (the conformational component). Thus, the chemical shifts of the C-1 signals when Hex*p*1 is Gal (**9** and **14**) differ by 8.6 p.p.m., which is ~5 p.p.m. greater than the difference in the chemical shifts of the C-1 signals for α- and β-D-Gal. In the example considered, the configurational and conformational components have the same sign. In contrast, the difference in the chemical shifts of the C-1 signals for α- and β-D-Glc*p* linked to D-Man*p* in **21** and **27** is small (1 p.p.m.), the C-1 signal for the β anomer being shifted upfield to an extent greater than that for the α anomer. In this instance, the configurational and conformational components have opposite signs, and, since the modulus of the former is somewhat less than that of the latter, an extraordinary inversion of the C-1 signal positions occurs with the anomers of Glc*p* and Gal*p*.

The examples presented show that the sign of the conformational component depends on the configuration at C-2 and C-4 in Hex*p*2. Comparison of the data for **21** and **27**, or **25** and **28**, indicates a similar dependence on the absolute configuration of the pyranose residues.

TABLE III

DEPENDENCE OF THE CHEMICAL SHIFT OF THE C-1 SIGNAL OF Hex*p* IN Hex*p*1-(1→3)-Hex*p*2 ON THE POSITION OF THE AXIAL SUBSTITUENT IN Hex*p*2 AND THE ABSOLUTE CONFIGURATION OF BOTH UNITS

Hexp1	*Chemical shift of C-1 signal of Hexp1 (p.p.m.)*	
	D-D,*a*4; L-L,*a*4; D-L,*a*2; L-D,*a*2;[a]	D-D,*a*2; L-L,*a*2 D-L,*a*4; L-D,*a*4;[a]
α-Glc (Gal)	*94.5–97*	100.5–102
β-Glc (Gal)	104–105.5	*100–101*
α-Man	*97–98.5*	103–104
β-Man	102–103	*98–99*

[a] D-D *etc.* refer to the absolute configuration of Hex*p*1 and Hex*p*2, respectively; *a*2 and *a*4 refer to 2- and 4-substituents.

Thus, for the (1→3)-linked disaccharide units considered, the conformational component associated with the chemical shift of the C-1 signal of Hex*p*1 has a nearly constant variation (5 p.p.m.) of amplitude depending on the three configurational factors, namely, the absolute configuration, the position of the axial hydroxyl group (2 or 4) in Hex*p*2, and the anomeric configuration of Hex*p*1. The changes in the chemical shift of the C-1 signal of Hex*p*1 on variation of one of the structural factors are shown in Table III. The data demonstrate the possibility of determining any structural factor (provided the remaining factors are known) for the (1→3)-linked disaccharide units, using the value of the chemical shift of the C-1 signal. The ranges of chemical shifts italicised in Table III are characteristic of (1→3)-linked disaccharides having Gal*p* or Man*p* as Hex*p*2 and therefore enable their detection when analysing the region for C-1 resonances in the ^{13}C-n.m.r. spectra of oligo- and poly-saccharides. It should be emphasised that the chemical shift of the C-1 signal of Hex*p*1 can be affected by the substitution of HO-2, the replacement of HO-2 by any other group, and the substitution of HO-2 and HO-4 in Hex*p*2 (*e.g.*, in branched trisaccharide units). In order to eliminate the influence of some of the additional factors and the configurational contribution on changing the anomeric configuration of Hex*p*1, it is more convenient to consider the effects of glycosidation rather than the values of the chemical shifts.

It is common practice to designate the α-, β-, and more remote effects of glycosidation as the difference of the chemical shifts of the signals of the corresponding carbon atoms in the free pyranose and the disaccharide unit. The α-effect refers to the carbon atoms involved in the glycosidic linkages, *i.e.*, C-1 of Hex*p*1 and C-3 of Hex*p*2 in Table III; the β-effect refers to C-2 of Hex*p*1 and C-2 and C-4 of Hex*p*2.

Table IV presents the α- and β-effects of glycosidation for Hex*p*1-(1→3)-Hex*p*2, and Table V contains the ranges of the glycosidation effects. These empirical regularities can be rationalised qualitatively on the basis of (*a*) changes in the three decisive structural factors; (*b*) changes in conformation near the glycosidic linkage caused by the changes in (*a*); and (*c*) analysis of the acquired or lost proton–proton interactions within the preferred (or statistically averaged) conformation, taking into account the alternating effects.

The influence of the change in the absolute configuration of Hex*p*1 and Hex*p*2 and the anomeric configuration of Hex*p*1 on the conformational properties of the glycosidic linkages is readily explained. Fig. 2 shows that the set of structural factors β,D-D (where β refers to Hex*p*1, and D-D to Hex*p*1 and Hex*p*2, respectively) characterises a particular orientation of C-2 and C-4 in Hex*p*2 with respect to C-2′ and O-5′ in Hex*p*1. Simultaneous alteration of the two structural factors (β,L-L, α,D-L, α,L-D) does not affect the situation. However, a change of one or three factors (α,D-D, α,L-L, β,D-L, β,L-D) results in a spatial reorientation of the specified atoms. Fig. 2 shows the identical conformations for each situation (ϕ ±60°, ψ is small). The values of ϕ and ψ depend on the chirality at C-2 and C-4 in Hex*p*2, since bulky axial and equatorial substituents have different energy barriers to rotation around the O–C-3 bond in Hex*p*2. Indeed, different enhancements (due to the n.O.e. for H-3 and H-4 in Hex*p*2) have been observed[10] on saturating the H-1 resonance of Hex*p*1 in **18** and **19**. For the disaccharide **18**, the enhancement is 0.6% for H-4 and 7.0% for H-3 in Hex*p*2, and, for **19**, 6.5% and 6.4%, respectively. Such variation in the n.O.e. shows that, in the statistically averaged conformation of **18**, H-1 of Hex*p*1 and H-3 of Hex*p*2 are in closer proximity than in **19**. In contrast, H-1 of Hex*p*1 and H-4 of Hex*p*2 are in closer proximity in **19** than in **18**.

The differences in the effects of glycosidation in **18** and **19** can be explained as follows. The α-effect of glycosidation for C-3 (Table IV) is reduced by 1.3 p.p.m. due to the increase in the distance between H-1 of Hex*p*1 and H-3 of Hex*p*2, and,

TABLE IV

EFFECTS OF GLYCOSIDATION[a] IN THE ^{13}C-N M R SPECTRA OF THE (1→3)-LINKED DISACCHARIDE UNITS[b]

No.	*Hexp1*	*Determining configurational factors*[c]	*α-Effect for C-1 of Hexp1*	*α-Effect for C-3 of Hexp2*	*β-Effect for C-2 of Hexp2*	*β-Effect for C-4 of Hexp2*	*Ref.*
9	Gal	α,D-D,*a*4	+3.0	+6.0	−1.4	−4.3	21
12	Gal	α,L-D,*a*4	+8.3	+8.5	−0.7	−0.6	21
18	Rha	α,L-D,*a*4	+8.6	+7.9	+0.5	−0.6	29
14	Gal	β,D-D,*a*4	+7.9	+9.7	−1.3	−0.1	27
20	Man	β,D-D,*a*4	+7.7	+9.3	−0.8	−0.6	10
19	Rha	β,L-D,*a*4	+3.5	+6.6	−1.7	−3.1	29
23	Glc	α,D-D,*a*2	+7.0	+7.8	+0.2	−0.5	30
37	Glc	β,D-D,*a*2	+4.2	+7.5	−2.2	−1.5	43
35	Man	β,D-D,*a*2	+4.1	+7.0	−2.9	−1.0	39
28	Glc	β,D-L,*a*2	+8.0	+9.5	−0.2	−0.9	33
34	Rha	α,L-L,*a*2	+8.3	+7.6	−0.5	−0.4	38

[a] Difference (p.p.m.) in the chemical shifts for the corresponding carbon atoms in Hex*p* involved in the disaccharide unit and free Hex*p*: +, downfield shift; −, upfield shift. [b] See Table II and ref. 43. [c] α or β refers to Hex*p*1, D-D *etc.* refer to the absolute configuration of Hex*p*1 and Hex*p*2, respectively, and *a*2 and *a*4 refer to 2- and 4-substituents.

TABLE V

RANGES OF α- AND β-EFFECTS OF GLYCOSIDATION IN Hex*p*1-(1→3)-Hex*p*2 UNITS[a]

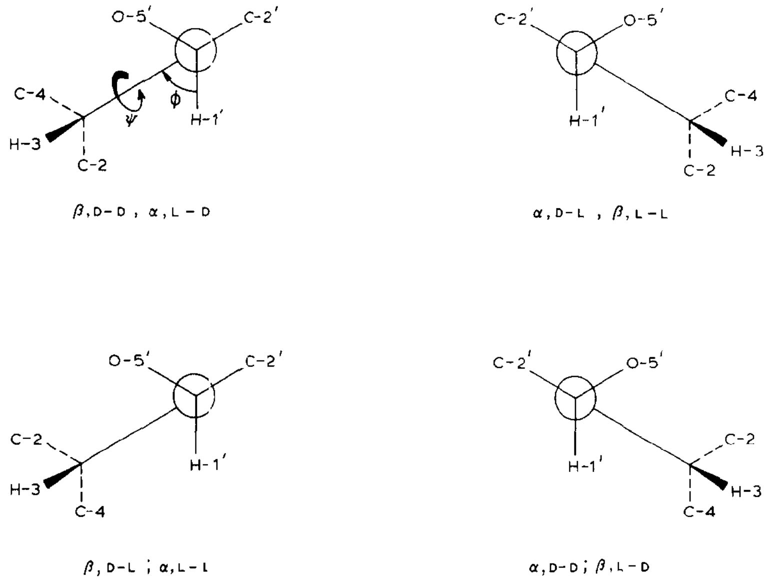
Determining configurational factors[b]	*α-Effects for C-1 of Hexp1*	*α-Effects for C-3 of Hexp2*	*β-Effects for carbon atoms bearing an axial substituent (Hexp2)*
α,D-D,*a*4; α,L-L,*a*4; β,D-L,*a*4; β,L-D,*a*4; β,D-D,*a*2; β,L-L,*a*2; α,D-L,*a*2; α,L-D-,*a*2.	3–4 p.p.m.	5–7.5 p.p.m. (α, 5–6.5 p.p.m.; β, 7–7.5 p.p.m.)[c]	2–5 p.p.m. upfield shift
β,D-D,*a*4; β,L-L,*a*4; α,D-L,*a*4; α,L-D,*a*4; α,D-D,*a*2; α,L-L,*a*2; β,D-L,*a*2; β,L-D,*a*2.	7–9 p.p.m.	8–10 p.p.m. (α, 8–8.5 p.p.m.; β, 9–10 p.p.m.)[c]	≯1.5 p.p.m. up- or down-field shift

[a] Taking into account the data in ref. 15. Values given to the nearest 0.5 p.p.m. [b] See footnote *c* to Table IV. [c] The ranges in brackets are for α- and β-Hex*p*1 (see Discussion).

consequently, weakening of the interaction of the protons at the 1,3-positions. The decrease in the α-effect of glycosidation for C-1 of Hex*p*1 can be partly explained in a similar manner. However, a large enhancement of the variation of the latter effect includes, apparently, a contribution from the interaction of H-1 of Hex*p*1 and H-4 of Hex*p*2, present in **19** and resulting in an upfield shift of the signals of C-1 (Hex*p*1) and C-4 (Hex*p*2). Likewise, the difference in the β-effects of glycosidation for C-4 of Hex*p*2 in **18** and **19** is related to the interaction of the protons at 1,4-positions (H-1 of Hex*p*1 and H-4 of Hex*p*2) in **19**.

Using *a*2 and *a*4 to denote the axial hydroxyl groups at positions 2 and 4 in Hex*p*2, the disaccharide fragments in Tables IV and V can be classified into two groups according to the effects of glycosidation. The first group, which includes fragments characterised by the structural factors α,D-D,*a*4 and those differing therefrom by an even number of structural factors, is characterised by small α-effects of glycosidation for C-1 of Hex*p*1. A nearly constant value of these α-effects indicates that the conformational properties of the glycosidic linkages of the members of this group (including **19**) are qualitatively similar and are characterised by the conformation involving a near approach of H-1 of Hex*p*1 and the equatorial H-2 or H-4 of Hex*p*2. Accordingly, the small α-effects for C-1 of Hex*p*1 in this group parallel the large (in modulus) β-effects for the carbon atom carrying the axial hydroxyl group in Hex*p*2.

The second group consists of the disaccharide fragments with structural factors β,D-D,*a*4 and those differing therefrom by an even number of structural factors. For this group, which includes the disaccharide **18**, the α-effects of glycosidation are also similar in magnitude and, on average, are 5 p.p.m. greater than those for the first group. Clearly, the large α-effects are related to the interaction of H-1 of Hex*p*1 and H-3 of Hex*p*2 in the statistically averaged or preferred conformation. The close proximity of these protons implies that H-1 of Hex*p*1 and H-4



β,D-D, α,L-D α,D-L, β,L-L

β,D-L; α,L-L α,D-D; β,L-D

Fig. 2 Relative arrangement of atoms around a glycosidic linkage in (1→3)-linked disaccharide units having different anomeric configurations of Hex*p*1 and absolute configurations of Hex*p*1 and Hex*p*2.

(or H-2) of Hex*p*2 are remote, which accords with the small (in modulus) values of the β-effects of glycosidation.

Comparison of the α-effects of glycosidation for C-3 of Hex*p*2 in the different groups supports the suggestions on the conformational properties of glycosidic linkages, namely, that the small glycosidation effects for C-1 of Hex*p*1 within the first group parallel the small effects for C-3 of Hex*p*2. However, there is another contribution, other than conformational, to the effect of glycosidation for C-3 of Hex*p*2, since the values are larger when Hex*p*1 is β.

The difference in the values of the effects of glycosidation for the two groups of disaccharide units and the similar values within each group stem largely from the distinction in the chirality of C-2 and C-4 in Hex*p*2. For "aglycons" that are more symmetrical, *e.g.*, for the (1→4)-linked disaccharides [where Hex*p*2 is Glc or Man (Rha)], the amplitude of the change in the effects of glycosidation, as function of the anomeric configuration of Hex*p*1 and the absolute configuration of Hex*p*1 and Hex*p*2, becomes negligible[15]. Differences in the conformational properties of glycosidic linkages for the two groups of disaccharides, which differ by an odd number of structural factors, are largely determined by the relative size of the equatorial substituents (CH_2OH and OH, respectively) at C-5 and C-3 of Hex*p*2. The conformational calculations for maltose and cellobiose show that this difference in the size of the substituents gives rise to a short-lived conformer in which H-1 of Hex*p*1 interacts with H-3 of Hex*p*2. As a result, (1→4)-linked disaccharides can be subdivided into two groups similar to those for the (1→3)-linked com-

TABLE VI

RANGES OF SOME DIAGNOSTIC α- OR β-EFFECTS OF GLYCOSIDATION IN Hex*p*1-(1→4)-Glc*p* (OR Man*p*)[a], Hex*p*1-(1→3)-Glc*p*[b], AND Hex*p*1-(1→2)-α-Man*p*[b]

Determining configurational factors[c]	*Hexp1-(1→4)-Glcp (or Manp)*	*Hexp-(1→3)-Glcp*		*Hexp-(1→2)-α-Manp*
	β-Effects for C-3 of Hexp2	*β-Effects for C-2 of Hexp2*	*β-Effects for C-4 of Hexp2*	*α-Effects for C-1 of Hexp1*
α,D-D; α,L-L; β,D-L; β,L-D.	−1– +1 p.p.m.	−1.1– −2.0 p.p.m.	−0.9– +0.6 p.p.m.	~8 p.p.m.
β,D-D; β,L-L; α,D-L; α,L-D.	< −1 p.p.m.	−0.9– +0.6 p.p.m.	−1.1– −2.0 p.p.m.	~5 p.p.m.

[a]Ref. 15. [b]Ref. 44. [c]See footnote *c* to Table IV.

pounds, but differing in a single spectral parameter, namely, the value of the β-effect for C-3 of Hex*p*2 (Table VI). Similarly, (1→3)-linked disaccharide units, when Hex*p*2 is Glc or Qui, constitute two groups that differ in the β-effect of glycosidation for both C-2 and C-4. Thus, it appears that, when the β-effect of glycosidation depends on the anomeric configuration of Hex*p*1, the dependence of the effect on the absolute configuration of both residues will be observed.

General consideration of the influence of chiral centres near the glycosidic linkage in Hex*p*2 enables a prediction about two other groups of disaccharides that differ significantly in the effects of glycosidation and involve a glycosidic linkage through an axial position of Hex*p*2 with the neighbouring carbons carrying axial and equatorial substituents. This assumption has been confirmed recently by us for disaccharide fragments Hex*p*1-(1→2)-α-Man*p* (or α-Rha) (Table VI).

When HO-6 is substituted, a difference in the β-effects of glycosidation for C-5 of Hex*p*2 is possible. However, no clear regularity in the variation of the α- and β-effects of glycosidation at an axial hydroxyl group in Hex*p*2 is likely to be found where both substituents at the β-carbons are equatorial, since no theoretically possible conformations permit an interaction of H-1 of Hex*p*1 with any proton at the β-positions in Hex*p*2.

Similar analysis of the dependence of the amplitude of the variation of glycosidation effects on the structure of the chiral alcohols occurring in the glycosides has been carried out[20]. The most marked changes in the chemical shifts were observed for cyclic aglycons where two β-carbon atoms differ in the number of substituents. Marked changes in the chemical shifts were also observed by Lemieux and Koto[12] for 2-methyl- or 6-methyl-cyclohexyl α- and β-D-glucopyranosides, which, apparently, were related only to the different chirality of the aglycons. This report[12] provides evidence of especial interest on the role of proton–proton interactions in determining the chemical shifts of the signals of carbon atoms near the glycosidic linkage, since it presents the calculated, preferred conformations for glycosides having cyclic aglycons. Analysis of the reported[12] ^{13}C-n.m.r. data shows that all the changes in the chemical shifts of carbon atoms for the series of glycosides can be interpreted unambiguously, provided the alternation rule suggested above for the effects of the proton–proton interactions in the preferred conformers is taken into consideration.

The general approach to the interpretation of the effects of glycosidation in the ^{13}C-n.m.r. spectra of carbohydrates, presented here, opens the way for the study of the conformational properties of glycosidic linkages in oligo- and poly-saccharides. Analysis of the currently available ^{13}C-n.m.r. data for carbohydrates shows that, as a rule, the chemical shifts of the signals of carbon atoms near the glycosidic linkage of a given disaccharide or corresponding disaccharide unit are almost constant in value, irrespective of the structure of the oligo- and poly-saccharide in which this unit is incorporated. In accord with our study and previous investigations[12,18–20], this fact means that the preferred, or statistically averaged, conformation of a disaccharide is preserved in oligo- and poly-saccharides containing that unit. However, there are other factors, unrelated to the structural features of the disaccharide units, which can affect the conformation near the glycosidic centre. These include macrocycle formation[1], gel–sol transitions of polysaccharides[41], and steric hindrance caused by the substitution at the geminal hydroxyl groups[42]. These effects are reflected in the chemical shifts, or in the effects of glycosidation, and, hence, they can be studied by ^{13}C-n.m.r. spectroscopy. The trend in the shift of the signal and the magnitude of the effect could allow the mode of the conformational change to be inferred on the basis of the dependence of the chemical shifts of the carbon signals on the non-bonded proton–proton interactions.

REFERENCES

1 T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, (1973) 2425–2432.
2 G. K. Hamer and A. S. Perlin, *Carbohydr. Res.*, 49 (1976) 37–48.
3 S. M. Bociek, A. H. Darke, D. Welti, and D. A. Rees, *Eur. J. Biochem.*, 109 (1980) 447–456.
4 P. A. J. Gorin and M. Mazurek, *Carbohydr. Res.*, 48 (1976) 171–186.
5 V. N. Vasil'ev, I. Ya. Zakharova, and A. S. Shaskov, *Bioorg. Khim.*, 8 (1982) 120–125.
6 D. M. Grant and B. V. Cheney, *J. Am. Chem. Soc.*, 89 (1967) 5315–5318.
7 S. H. Grover, J. P. Guthrie, J. B. Stothers, and C. T. Tan, *J. Magn. Reson.*, 10 (1973) 227–230.
8 J. G. Batchelor, *J. Magn. Reson.*, 18 (1975) 212–214.
9 H. Beierbeck and J. K. Saunders, *Can. J. Chem.*, 54 (1976) 2985–2995.
10 A. S. Shashkov, *Bioorg. Khim.*, 9 (1983) 246–253.
11 K. F. Koch, J. A Roades, E. W. Hagaman, and E. Wenkert, *J. Am. Chem. Soc.*, 96 (1977) 3300–3305.
12 R. U. Lemieux and S. Koto, *Tetrahedron*, 30 (1974) 1933–1944.
13 P. C. M. Herve du Penhoat and A. S. Perlin, *Carbohydr. Res.*, 36 (1974) 111–120.
14 G. Engelhardt, H. Janke, and D. Leiden, *Org. Magn. Reson.*, 8 (1976) 655–657.
15 A. S. Shashkov, A. I. Usov, Yu. A. Knirel, B. A. Dmitriev, and N. K. Kochetkov, *Bioorg. Khim.*, 7 (1981) 1364–1371.
16 A. S. Shashkov, V. I. Grishkovets, and V. Ya. Chirva, *Bioorg. Khim.*, 8 (1982) 1393–1399.
17 A. Lipták, Z. Szurmai, P. Nánási, and A. Neszmélyi, *Carbohydr. Res.*, 99 (1982) 177–185.
18 R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, (1977) 175–178.
19 K. Tori, S. Seto, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, (1977) 179–182.

20 S. Seto, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, 100 (1978) 3331–3339.

21 A. I. Usov, V. V. Barbakadze, S. V. Jarotsky, and A. S. Shashkov, *Bioorg. Khim.*, 4 (1978) 1507–1512.

22 A. S. Shashkov, M. Sh. Zaretskaya, S. V. Jarotsky, I. B. Naumova, O. S. Chizhov, and Z. A. Shabarova, *Eur. J. Biochem.*, 102 (1979) 477–481.

23 N. K. Kochetkov, V. I. Torgov, N. N. Malysheva, A. S. Shashkov, and E. M. Klimov, *Tetrahedron*, 36 (1980) 1227–1230.

24 H. C. Jarrell, T. F. Conway, P. Moyna, and I. C. P. Smith, *Carbohydr. Res.*, 76 (1979) 45–57.

25 M. Messer, E. Trifonoff, W. Stern, J. G. Collins, and J. H. Bradbury, *Carbohydr. Res.*, 89 (1980) 327–334.

26 G. G. S. Dutton and J. Di Fabio, *Carbohydr. Res.*, 87 (1980) 129–139.

27 A. S. Shashkov, Yu. A. Knirel, N. A. Kocharova, B. A. Dmitriev, and N. K. Kochetkov, *Bioorg. Khim.*, 6 (1980) 1332–1337.

28 K. Okutani and G. G. S. Dutton, *Carbohydr. Res.*, 86 (1980) 259–271.

29 V. I. Torgov, V. N. Shibaev, A. S. Shashkov, and N. K. Kochetkov, *Bioorg. Khim.*, 6 (1980) 1860–1871.

30 A. S. Shashkov, N. Gullyev, A. F. Sviridov, S. E. Gorin, O. S. Chizhov, and N. K. Kochetkov, *Bioorg. Khim.*, 3 (1977) 1028–1033.

31 G. G. S. Dutton and T. E. Folkman, *Carbohydr. Res.*, 80 (1980) 147–161.

32 G. G. S. Dutton and A. V. Savage, *Carbohydr. Res.*, 83 (1980) 351–362.

33 P. Colson and R. R. King, *Carbohydr. Res.*, 47 (1976) 1–13.

34 A. S. Shashkov, V. A. L'vov, N. V. Tokhtamysheva, B. A. Dmitriev, and N. K. Kochetkov, *Bioorg. Khim.*, 7 (1981) 729–735.

35 P. A. J. Gorin, *Can. J. Chem.*, 51 (1973) 2375–2383.

36 P. A. J. Gorin, R. H. Haskins, L. R. Travassos, and L. Mendonca-Previato, *Carbohydr. Res.*, 55 (1977) 21–23.

37 Yu. A. Knirel, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, N. V. Kosyanchuk, and I. Ya. Zakharova, *Bioorg. Khim.*, 6 (1980) 1851–1859.

38 C. Laffite, A. M. Nguyen Phuoc Du, F. Winternitz, R. Wilde, and F. Pratviel-Sosa, *Carbohydr. Res.*, 67 (1978) 105–115.

39 P. A. J. Gorin, *Carbohydr. Res.*, 39 (1975) 3–10.

40 A. S. Shashkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1983) 1328–1336.

41 H. Saito, T. Ohki, and T. Sasaki, *Biochemistry*, 16 (1977) 908–914.

42 R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto, and V. S. Rao, *Can. J. Chem.*, 58 (1980) 631–653.

43 A. F. Sviridov, A. S. Shashkov, N. K. Kochetkov, I. V. Botvinko, and N. S. Egorov, *Bioorg. Khim.*, 8 (1982) 1242–1251.

44 Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, *Eur. J. Biochem.*, in press.