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A ^{13}C-N.M.R. STUDY OF SOME *Shigella flexneri* O-POLYSACCHARIDES*

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ABSTRACT

^{13}C-N.m.r. data for some *Shigella flexneri* O-polysaccharides are reported. It is concluded that the chemical shifts observed for the linear *Shigella flexneri* type Y O-polysaccharide are similar to those calculated by adding substituent shifts, obtained from disaccharides with similar stereochemistry around the glycosidic linkage, to the chemical shifts for the monomers. The influence of substitution with α-D-glucopyranosyl and/or *O*-acetyl groups in different positions of this polysaccharide on the chemical shifts has been investigated. Possible interactions between sugar residues involved in the branching have been investigated by energy minimisations (GESA) of disaccharide and of trisaccharide elements.

INTRODUCTION

The O-polysaccharides from different types of *Shigella flexneri* are composed of oligosaccharide repeating-units containing the element **1**, substituted with α-D-glucopyranosyl and/or *O*-acetyl groups in different positions[1] (Fig. 1).

→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→

1

Substituent shifts introduced by the α-D-glucopyranosyl and *O*-acetyl groups are determined by comparison of the chemical shifts of the signals from the different polysaccharides with those from the type Y O-polysaccharide, in which **1** is unsubstituted. In a previous ^{1}H-n.m.r. study[2] of these polysaccharides, substituent shifts for signals from protons were obtained and we now report a similar ^{13}C-n.m.r. study.

In our studies of the n.m.r. characteristics of the glycosidic bond, several (1→2)-[3], (1→3)-[3,4], (1→4)-[5], and (1→6)-linked[6] disaccharides have been investigated. The substituent shifts determined in these studies are used in a

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computer program, CASPER, which predicts n.m.r. spectra of oligo- and polysaccharides[7]. Special problems arise when branching points are involved and we are therefore investigating substituent shifts for "branched" trisaccharides. An alternative way of obtaining such information is to investigate branched polysaccharides, and the *Sh. flexneri* O-polysaccharides are suitable models.

RESULTS AND DISCUSSION

The chemical shifts of the signals from a glycosyl residue in an oligo- or polysaccharide depend primarily upon the chemical shifts of this residue, substitution shifts, and shifts caused by different inter-residue interactions over the glycosidic linkages to the closest neighbours. As a first approximation, these substituent shifts are additive and, from data obtained from model disaccharides, the expected chemical shift displacements for ^{13}C resonances in spectra of polysaccharides can be estimated. However, branched structures may cause problems, especially when vicinal hydroxyl groups are substituted. Additional interactions between the two sugar residues linked to the disubstituted sugar may then change the conformations around the glycosidic linkages involved and thereby the chemical shifts. This type of branching occurs in some of the *Sh. flexneri* O-polysaccharides and causes deviations from pure additivity of substituent shifts, as will be discussed below.

The structures of the *Sh. flexneri* O-polysaccharides studied are given in Fig. 1. The sugar residues will be designated RhaI, RhaII, RhaIII, GlcNAc, and Glc in the Tables and in the text. The ^{13}C-n.m.r. chemical shift data for the O-polysaccharides and relevant monomers are given in Table I. The substituent shifts, relative to those of the corresponding monomers, are given in Table II and additional substituent shifts caused by Glc or *O*-acetyl groups, relative to the

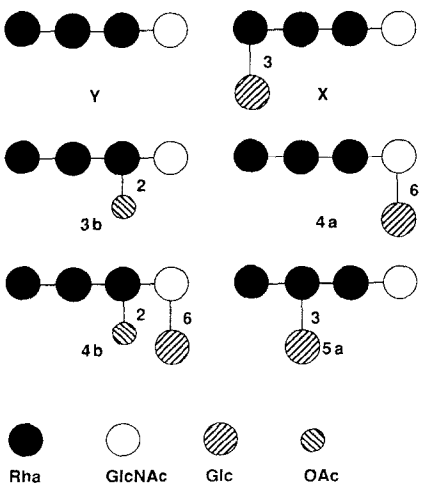


Fig. 1. Structures of the *Sh. flexneri* O-polysaccharides investigated.

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS[a] (δ) FOR *Shigella flexneri* TYPE Y, X, 3b, 4a, 4b, and 5a O-POLYSACCHARIDES

Type	*→2)-α-L-Rhap-(1→2) (Rha I)*						*→2)-α-L-Rhap-(1→3) (Rha II)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Y	101.77	79.56	70.78	73.32	70.02	17.32[b]	101.56	78.92	70.98	73.19	69.91	17.55
X	102.00	75.10	74.92	71.78	70.33	17.29	101.45	79.22	70.90	73.21	69.90	17.58
3b	101.80	79.52	70.76	73.29	70.07	17.47	101.77	78.76	71.00	72.97	70.16	17.47[b]
4a	101.85	79.61	70.83	73.31	70.04	17.48	101.58	79.05	70.94	73.20	69.95	17.56
4b	101.84	79.54	70.81	73.30	70.07	17.49	101.84	78.88	70.99	72.99	70.14	17.49[b]
5a	101.58	79.32	70.93	73.34	70.11	17.30[b]	101.34	75.41	75.01	71.55	69.93	17.75

Type	*→3)-α-L-Rhap-(1→3) (Rha III)*						*→3)-β-D-GlcpNAc-(1→2) (GlcNAc)*							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*NAc*
Y	101.89	71.47	78.31	72.46	69.91	17.47[b]	102.92	56.41	82.36	69.47	76.71	61.75	174.89	23.19
X	101.95	71.47	78.22	72.49	69.88	17.65	102.37	56.24	82.53	69.48	76.89	61.80	174.58	23.44
3b	99.24	73.17	77.01	72.63	69.79	17.24[b]	102.88	56.39	82.95	69.40	76.76	61.73	175.15	23.30
4a	101.85	71.47	78.28	72.45	69.88	17.36	103.03	56.39	82.24	69.40	75.21	66.97	174.88	23.20
4b	99.11	73.16	77.02	72.62	69.83	17.29[b]	103.00	56.37	82.81	69.38	75.27	67.01	175.15	23.32
5a	101.88	71.46	78.19	72.50	69.87	17.50[b]	102.69	56.46	82.35	69.57	76.79	61.93	174.82	23.20

Type	*α-D-Glcp-(1→X) (Glc)*							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*OAc*
Y								
X	95.58	72.29	74.10	70.59	72.38	61.43		
3b							173.69	21.01
4a	99.07	72.45	74.11	70.66	72.84	61.65		
4b	99.20	72.44	74.10	70.67	72.85	61.65	173.70	21.01
5a	95.77	72.11	73.99	70.66	72.68	61.63		
α-L-Rhap[c]	94.84	71.81	71.00	73.19	69.12	17.67		
β-D-GlcpNAc	95.85	57.86	74.81	71.06	76.82	61.85		
α-D-Glcp	92.99	72.47	73.78	70.71	72.37	61.84		

[a]Chemical shifts were obtained from spectra recorded at 70°, using 1,4-dioxane as internal reference (δ 67.40). [b]These chemical shifts could be exchanged. [c]Chemical shifts of monomers were obtained from refs. 4 and 6. The spectrum of 2-acetamido-2-deoxy-β-D-glucopyranose was recorded at 70° and using assignments from ref. 13.

TABLE II

CHEMICAL SHIFT DIFFERENCES FROM MONOMERS FOR *Shigella flexneri* TYPE Y, X, 3b, 4a, 4b, and 5a O-POLYSACCHARIDES

Type	*→2)-α-L-Rhap-(1→2) (Rha I)*						*→2)-α-L-Rhap-(1→3) (Rha II)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Y	6.93	8.25	−0.22	0.13	0.90	−0.35[a]	6.72	7.61	−0.02	0	0.79	−0.12
X	7.16	3.79	3.92	−1.41	1.21	−0.38	6.61	7.91	−0.10	0.02	0.78	−0.09
3b	6.96	8.21	−0.24	0.10	0.95	−0.20	6.93	7.45	0	−0.22	1.04	−0.20[a]
4a	7.01	8.30	−0.17	0.12	0.92	−0.19	6.74	7.74	−0.06	0.01	0.83	−0.11
4b	7.00	8.23	−0.19	0.11	0.95	−0.18	7.00	7.57	−0.01	−0.20	1.02	−0.18[a]
5a	6.74	8.01	−0.07	0.15	0.99	−0.37[a]	6.50	4.10	4.01	−1.64	0.81	0.08

Type	*→3)-α-L-Rhap-(1→3) (Rha III)*						*→3)-β-D-GlcpNAc-(1→2) (GlcNAc)*							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*NAc*
Y	7.05	0.16	7.31	−0.73	0.79	−0.20[a]	7.07	−1.45	7.55	−1.59	−0.11	−0.10	−0.60	0.09
X	7.11	0.16	7.22	−0.70	0.76	−0.02	6.52	−1.62	7.72	−1.58	0.07	−0.05	−0.91	0.34
3b	4.40	1.86	6.01	−0.56	0.67	−0.43[a]	7.03	−1.47	8.14	−1.66	−0.06	−0.12	−0.34	0.02
4a	7.01	0.16	7.28	−0.74	0.76	−0.31	7.18	−1.47	7.43	−1.66	−1.61	5.12	−0.61	0.10
4b	4.27	1.85	6.02	−0.57	0.71	−0.38[a]	7.15	−1.49	8.00	−1.68	−1.55	5.16	−0.34	0.22
5a	7.04	0.15	7.19	−0.69	0.75	−0.17[a]	6.84	−1.40	7.54	−1.49	−0.03	0.08	−0.67	0.10

Type	*α-D-Glcp-(1→X) (Glc)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Y						
X	2.59	−0.18	0.32	−0.12	0.01	−0.41
3b						
4a	6.08	−0.02	0.33	−0.05	0.47	−0.19
4b	6.21	−0.03	0.32	−0.04	0.48	−0.19
5a	2.78	−0.36	0.21	−0.05	0.31	−0.21

[a]These chemical shift differences could be exchanged.

chemical shifts observed for the type Y O-polysaccharide, are given in Table III. Signals were assigned by 2D C–H shift-correlation spectroscopy, as assignment of the proton signals has already been performed[2].

The basic structure, Sh. flexneri *type Y.* — The ^{13}C-n.m.r. spectrum of this polysaccharide has been analysed by Bock *et al.*[8] and most of our assignments agree with theirs. ^{13}C-N.m.r. substituent shifts (Table IV) for three of the four disaccharide elements in this polysaccharide are available from authentic disaccharide glycosides, namely, those corresponding to α-L-Rha*p*-(1→2)-α-L-Rha*p*[9], α-L-Rha*p*-(1→3)-α-L-Rha*p*[9], and α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc[8]. Substituent shifts for the last element, β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*, were obtained from two derivatives (Table IV) as no direct comparison could be made. There was good agreement between the chemical shift observed for the type Y O-polysaccharide and those calculated using the disaccharide models, thus confirming that short-range interactions predominate.

We have shown that, by using disaccharides containing monosaccharide components other than those in the polysaccharide but with similar stereochemistry around the glycosidic linkage, good agreement between calculated and observed spectra may also be obtained. Such disaccharides, namely, glycosides of α-L-Fuc*p*-(1→3)-β-D-Glc*p*[4] and α-D-Fuc*p*-(1→3)-α-D-Man*p*[4], have been used as models for α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc and α-L-Rha*p*-(1→3)-α-L-Rha*p*, respectively (Table IV). The comparison shows that there are only small differences between calculated and observed values at the linkage positions and those next to the linkage in the aglycon. In the glycosyl group, however, a deviation is observed for the C-2 resonance, due to the different stereochemistry at this carbon.

Substitution in the 3- and 6-positions of β-D-*Glc*p*NAc,* Sh. flexneri *type 4a.* — In type 4a, the GlcNAc residue is substituted in the 6-position by a Glc group. The only residue in the basic structure for which significant substituent shifts are observed is GlcNAc. Thus, the induced shift values for the C-4 to C-6 resonances are close to the corresponding values in the model disaccharide methyl glycoside α-D-Glc*p*-(1→6)-β-D-Glc*p*[6]. The substituent shifts observed for the glycosyl group of the disaccharide and the Glc group in the type 4a polysaccharide are also similar, the largest difference (0.3 p.p.m.) being found for the C-1 signal. As only small shifts for the signals from the other residues in the polysaccharide are observed, it can be concluded that the Glc group only has significant interaction with the GlcNAc residue and that additivity of substituent shifts holds for 3,6-disubstitution. Differences in rotamer distribution around the C-5–C-6 bond in the polysaccharide could be the reason for the differences between the substituent shifts observed for the carbons close to the linkage.

Substitution in the 2- and 3-positions of α-L-*Rha*p, Sh. flexneri *types X and 5a.* — In type X, the RhaI residue in the linear structure is substituted in the 3-position by a Glc group (Fig. 1). As the RhaI residue is already substituted in the 2-position, by a GlcNAc residue, vicinal 2,3-disubstitution occurs, resulting in possible steric crowding and restricted rotation around the glycosidic linkages.

TABLE III

EFFECTS OF SUBSTITUTION FROM α-D-GLUCOPYRANOSYL AND/OR *O*-ACETYL GROUPS ON THE CHEMICAL SHIFTS OF THE *Shigella flexneri* TYPE Y O-POLYSACCHARIDE AS SHOWN BY CHANGES IN THE CHEMICAL SHIFT IN THE SUBSTITUTED RESIDUE AND OTHER LARGER CHANGES

Type	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	
X (RhaI)	0.23	−4.46	4.14	−1.54	0.31	−0.03	−0.55 (C-1, GlcNAc)
4a (GlcNAc)	0.38	−0.02	−0.12	−0.07	−1.50	5.22	
5a (RhaII)	−0.22	−3.51	4.03	−1.64	0.02	0.20	−0.19 (C-1, RhaI)
3b (RhaIII)	−2.65	1.70	−1.30	0.17	0.12	−0.23	0.59 (C-3, GlcNAc), 0.21 (C-1, RhaII)
4b (RhaIII *cf.* 4a)	−2.74	1.69	−1.26	0.17	−0.05	−0.07	0.57 (C-3, GlcNAc), 0.26 (C-1, RhaII)

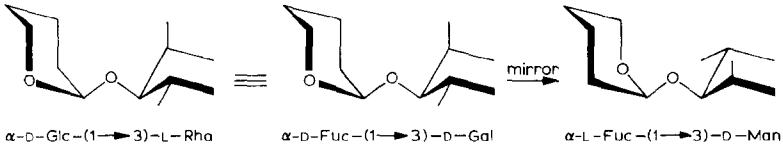
TABLE IV

CHEMICAL SHIFT DIFFERENCES FOR DISACCHARIDES DISCUSSED IN THE TEXT[a]

Type	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-L-Rha*p*-(1→2)-α-L-Rha*p*-OMe	7.9	−0.8	−0.2	−0.4	0.3	−0.2	−1.4	8.0	−0.4		−0.2	
α-L-Rha*p*-(1→3)-α-L-Rha*p*-OMe	7.9	−0.9		−0.3	0.2	−0.2	−0.3	−0.2	7.5	−0.9		0.1
α-L-Rha*p*-(1→3)-β-D-GlcNAcOR[b]	7.2	−1.0		−0.5	0.1	−0.6	[c]	0.1	7.8	−1.2	0.5	0.3
β-D-GlcNAc-(1→2)-α-L-Rha*p*-OR	7.3	−1.3	−0.7	−0.6	−0.6	−0.5	[c]					
β-D-GlcNAc-(1→2)-α-L-Rha*p*[d]							−1.2	8.5	−0.4	0.3	0.1	−0.1
α-L-Fuc*p*-(1→3)-β-D-Glc*p*	7.2	0.2	0.3		0.7	−0.2	−0.2	0.3	7.2	−1.4		
α-D-Fuc*p*-(1→3)-α-D-Man*p*	8.5	0.4	0.2	−0.1	0.7	−0.2	−0.2		8.0	−0.8	0.1	
α-L-Fuc*p*-(1→3)-α-D-Man*p*	3.8	−0.2	0.2		0.6	−0.3	−0.3	−3.2	5.7	−1.7	0.1	
α-D-Fuc*p*-(1→3)-α-D-Gal*p*	3.2	−0.2	0.2		0.6	−0.2		−1.6	5.2	−3.5	−0.2	
α-D-Glc*p*-(1→6)-β-D-Glc*p*	5.8	−0.1	0.3	−0.1	0.4	−0.2	0.1		0.2	−0.2	−1.6	5.0

[a]The substituent shifts were obtained by comparison of the chemical shifts for the disaccharide with those of the relevant monosaccharide which were obtained from ref. 14. [b]R = $(CH_2)_8CO_2Me$. [c]Not applicable due to lack of relevant monomer. [d]Obtained by comparison between β-D-GlcNAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p* and α-L-Rha*p*-(1→2)-α-L-Rha*p*.

Comparison of the spectra from type X and type Y shows that resonances for C-2 to C-4 in RhaI and for C-1 in the GlcNAc residue have significant shifts (Table III). In order to obtain a correct comparison of substituent shifts in the side-chain group, the disaccharide α-D-Glc*p*-(1→3)-α-L-Rha*p*, or a similar disaccharide, should be used. As this was not available, suitable disaccharides are α-L-Fuc*p*-(1→3)-α-D-Man*p*[4] and α-D-Fuc*p*-(1→3)-α-D-Gal*p*[4] (Table IV), which have similar stereochemistry around the glycosidic linkage.



The substituent shifts are of the same order of magnitude but still differ significantly. Thus, in type X, the induced shifts are −4.5, 4.1, and −1.5 p.p.m. for C-2 to C-4 in the RhaI residue, compared to −3.5, 5.2, and −1.6 p.p.m. and −3.2, 5.7, and −1.7 p.p.m., respectively, for the corresponding signals in the disaccharides. For the C-1 resonance in the Glc group, the shift is 2.6 p.p.m. for the polysaccharide and 3.8 p.p.m. and 3.2 p.p.m., respectively, for the disaccharides. The deviations for resonances from C-2 and C-3 in RhaI and the linkage carbons C-1 in GlcNAc and Glc and C-2 in RhaII are derived from additional interactions between the Glc, RhaI, RhaII, and GlcNAc residues due to the vicinal disubstitution.

In type 5a, the RhaII residue in the basic structure is substituted in the 3-position by a Glc group (Fig. 1). Again, steric crowding is expected since RhaII is also substituted in the 2-position. The natures of the substituting sugars differ from those in corresponding positions in type X, however. Thus, substituents in the 3- and the 2-position are an α-D and an α-L sugar in type 5a, but an α-D and a β-D sugar residue in type X. This difference is not as large as it may seem, however, because, in the preferred conformation, the ring oxygen is located on the same side in the α-L- and the β-D-glycosyl residue. Similar substituent shifts were also observed for disaccharide glycosides with α-D and β-L, as well as for β-D and α-L, stereochemistry of the glycosidic linkage[3,5].

Some of the accessible space for the β-D-glycosyl residue is not available for the α-L-glycosyl residue, however, because H-5 in the latter interacts with RhaII, resulting in restricted mobility. By comparison of spectra of the 5a and Y polysaccharides, substituent shifts for the resonances of C-2 to C-4 in the RhaII residue are obtained. Substituent shifts for the Glc group are compared with those for the Fuc group in α-L-Fuc*p*-(1→3)-α-D-Man*p*[4] for which a different shift of the C-1 resonance is observed. The shift for the C-1 resonance in the RhaI residue, which corresponds to the GlcNAc residue in type X, is small. The shifts altogether are similar to those in type X, thus the indicated similarity between α-L and β-D- sugars resulted in similar substituent shifts in the "branched" trisaccharides.

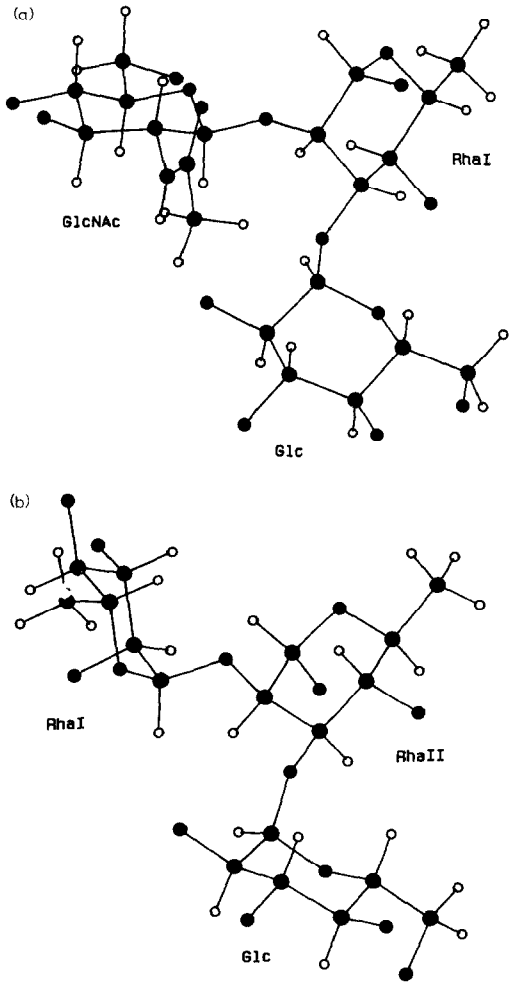


Fig. 2. Minimum energy conformations of trisaccharides representing the branching region in *Sh. flexneri* types X (a) and 5a (b).

In order to appreciate similarities and differences between the trisaccharides representative for the branching sugar residue and its two glycosyl substituents in type X and 5a, energy minimisation of the glycosidic bonds using the GESA program[10] was performed. The resulting conformations are shown in Fig. 2. The φ/ψ-values obtained for the disaccharide elements α-L-Rha*p*-(1→2)-α-L-Rha*p*, β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*, and α-D-Glc*p*-(1→3)-α-L-Rha*p*, 47°/15°, 53°/8°, and −48°/−27°, respectively, are essentially maintained in the polysaccharide. Thus, for the type X polysaccharide, the values are 47°/6° and −51°/−29° for the 2- and the 3-linkage, respectively. The corresponding values for the 5a polysaccharide are 44°/9° and −54°/−32°. Thus, the observed changes in chemical shift differences are

not due to large changes in conformation. Restricted rotation around the glycosidic bonds will, however, occur due to interactions between the two vicinally substituting residues.

Trisaccharides, representative for the branching sugar residue and its two glycosyl substituents in type X and 5a, have been synthesised[11]. On comparison of the chemical shifts of the resonances from linkage carbons C-1′ (Rha or GlcNAc), C-1″ (Glc), and C-2 and C-3 (Rha), in the polysaccharides and in the trisaccharides, deviations of less than 0.5 p.p.m. are observed for type X. For type 5a, however, deviations up to 1.5 p.p.m. are observed, and it thus seems possible that interactions additional to those observed in the trisaccharide are present in the 5a polysaccharide.

The substituent shift for "branched" trisaccharides with long-range interactions cannot be predicted at present, but studies of a number of such trisaccharides are now in progress in the authors' laboratory.

The influence of the O*-acetyl group.* — The only difference between the polysaccharide pairs Y-3b and 4a-4b, respectively, is the presence of an *O*-acetyl group in 3b and 4b (Fig. 1). This *O*-acetyl group is located in the 2-position of RhaIII. The substituent shifts caused by the *O*-acetyl group are given in Table III. The induced shifts for both polysaccharides are similar, indicating that the Glc group in the 6-position of the GlcNAc residue does not significantly interact with the *O*-acetyl group.

The influence on chemical shifts of an *O*-acetyl group in methyl gluco- and galacto-pyranosides has been studied[12]. It was concluded that, *inter alia,* the position of the *O*-acetyl group and the presence of eventual axial substituents were essential for the shifts of resonances from the substituted α-carbon and the neighbouring β-carbons. No mannopyranosides were investigated, but suggested approximation rules give an indication of the magnitude of the shifts on α- and β-carbon resonances in the 3b and 4b polysaccharides. Substituent shifts for the C-1–C-3 resonances are calculated[12] to be −2.4, 2.7, and −1.4 p.p.m., respectively. The fit for the signals given by the β-carbons (C-1 and C-3) is good, but the deviation for the C-2 resonance is ~1 p.p.m. This may be due to the fact that the *O*-acetyl group is axial and near the anomeric center, or to the influence of the glycosyl residue in the 3-position. Small shifts for all resonances of atoms in the RhaII residue support the latter explanation. Some other signals also shift slightly, but the only pronounced value is observed for the C-3 resonance in the GlcNAc residue, namely, 0.6 p.p.m.

EXPERIMENTAL

N.m.r. spectra were recorded on a JEOL GX-400 instrument at 70°. The O-polysaccharides were dissolved in D_2O (40–80 mg/mL), using 1,4-dioxane (δ 67.40) as internal reference. Chemical shifts were taken from the 1D-n.m.r. spectra with a digital resolution of 0.6 Hz. For complete assignment of all ^{13}C resonances,

2D C–H shift-correlation spectroscopy was performed. For the assignment of signals from anomeric and ring carbons, 128 spectra were accumulated, each consisting of 1024 data points, and zero-filled into a 1024 × 256 data matrix with a frequency range of 5000 Hz and 800 Hz for the f_2 and the f_1 dimension, respectively. For assignment of the 6-deoxy carbons, 64 spectra, each consisting of 1024 data points, were accumulated and zero-filled into a 1024 × 128 data matrix with a frequency range of 700 Hz and 300 Hz for the f_2 and the f_1 dimension, respectively.

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