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A 2D-^{1}H-N.M.R. STUDY OF SOME *Shigella flexneri* O-POLYSACCHARIDES

PER-ERIK JANSSON, LENNART KENNE, AND THOMAS WEHLER

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

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ABSTRACT

A ^{1}H-n.m.r. study of the O-polysaccharides from different types of *Shigella flexneri* has been performed. With the aid of 2D-n.m.r. techniques, namely, *J*-resolved, spin–spin correlation, and NOESY experiments, most of the structural features of these polysaccharides could be deduced. Sequences could generally be obtained from the NOESY experiments. When using a prolonged mixing time in these experiments, cross-peaks due to spin diffusion from one anomeric proton to the anomeric proton of an adjacent residue could be obtained, thereby giving complementary sequence information.

INTRODUCTION

Some years ago, we determined the structures of the O-polysaccharides from different types of *Shigella flexneri*[1]. The simplest, from type Y, is composed of tetrasaccharide repeating-units (**1**). In the repeating unit of the other 11 types, α-D-glucopyranosyl and/or *O*-acetyl groups are added to different positions of **1**. Various specific degradations were used in order to locate these substituents.

→2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→

1

It seemed possible that new serotypes with other combinations of the same substituents may be found. We have therefore investigated if some of these structures could be defined from ^{1}H-n.m.r. spectroscopy in combination with sugar and methylation analysis.

RESULTS AND DISCUSSION

The structures of the *Sh. flexneri* O-polysaccharides investigated are given in Fig. 1.

The ^{1}H-n.m.r. chemical shift data for the polysaccharides are given in Table I. Signals could be assigned directly from COSY- or relayed COSY-spectra. Good agreement with the values given by Bock *et al.*[2] for type Y were obtained. The

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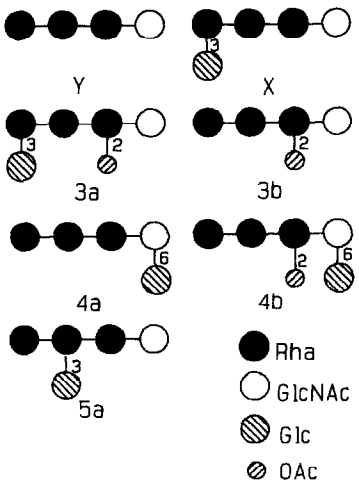

Fig. 1. Structures of the *Sh. flexneri* serotypes investigated.

small differences for some signals probably arise from different recording conditions. In the following, the sugar residues will be designated RhaI, RhaII, RhaIII, GlcNAc, and Glc in the Tables and in the text.

The glycosylation-induced chemical shift differences are given in Table II. They were obtained by subtraction of the chemical shifts for the monomeric reducing sugar residues from the chemical shifts for the corresponding residues in the polymer. For most sugar residues in the polysaccharides, differences >0.1 p.p.m. are observed for the proton on the linkage carbon and/or for the adjacent protons. For the 3-substituted RhaIII in type Y, however, large chemical shift differences are observed only for H-4 and H-5. The chemical shift differences for the anomeric protons can be either positive or negative. These effects may be attributed to different inter-residue atom–atom interactions.

Introduction of a Glc group in the 3-position of RhaI, as in type X, caused a major downfield shift for H-2 in RhaI. For the neighbouring GlcNAc, the signal for H-3 is shifted upfield. If a Glc group substitutes the 3-position of RhaII instead of RhaI, as in type 5a, major downfield shifts are observed for H-2 and H-4 in RhaII only. In types 4a and 4b, a Glc group substitutes the 6-position of GlcNAc which causes downfield shifts for H-4 and H-5 in that residue. The signal for the anomeric proton of Glc is shifted ~0.25 p.p.m. upfield when this group is linked to the 6-position in GlcNAc.

In studies of mono-*O*-acetylated methyl D-gluco- and D-galacto-pyranosides, it has been observed[3] that the signals of the proton on the acetoxylated carbon and the neighbouring protons, the α- and the β-protons, undergo downfield shifts of 1.10–1.50 p.p.m. and ~0.20 p.p.m., respectively. These chemical shift differences may differ if conformational changes of the *O*-acetyl group are induced by neighbouring glycosyl residues. In type 3b, the tetrasaccharide repeating-unit of

TABLE I

^{1}H-N.M.R. CHEMICAL SHIFTS[a] (δ) FOR *Shigella flexneri* TYPE Y, X, 3a, 3b, 4a, 4b, AND 5a O-POLYSACCHARIDES

Type	*(1→2)-α-L-Rhap-(1→2) (RhaI)*						*(1→2)-α-L-Rhap-(1→3) (RhaII)*						*(1→3)-α-L-Rhap-(1→3) (RhaIII)*					
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
Y	5.16	4.14	3.87	3.34	3.69	1.26	5.15	4.06	3.93	3.49	3.76	1.31	4.88	3.87	3.79	3.55	4.01	1.26
X	5.10	4.39	3.93	3.36	3.72	1.25	5.16	4.05	3.91	3.46	3.74	1.30	4.86	3.84	3.77	3.53	4.00	1.23
3a	5.10	4.39	3.94	3.38	3.73	1.27	5.13	4.03	3.76	3.45	3.58	1.29	4.90	5.01	3.95	3.59	4.08	1.27
3b	5.15	4.13	3.86	3.34	3.69	1.26	5.10	4.02	3.76	3.45	3.58	1.28	4.91	5.02	3.96	3.59	4.07	1.27
4a	5.11	4.14	3.87	3.32	3.68	1.24	5.14	4.05	3.92	3.47	3.75	1.31	4.89	3.87	3.79	3.55	4.00	1.25
4b	5.10	4.14	3.87	3.33	3.68	1.26	5.10	4.02	3.76	3.45	3.58	1.28	4.93	5.03	3.95	3.60	4.08	1.28
5a	5.14	4.14	3.89	3.34	3.70	1.25	5.22	4.25	4.01	3.61	3.79	1.34	4.88	3.87	3.80	3.56	4.01	1.27

	(1→3)-β-D-GlcNAcp-(1→2) (GlcNAc)								*α-D-Glcp-(1→X) (Glc)*							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*NAc*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*OAc*
Y	4.75	3.84	3.66	3.55	3.46	3.91	3.76	2.06								
X	4.81	3.85	3.50	3.51	3.43	3.86	3.75	2.09	5.16	3.71	3.82	3.48	4.02	3.82	3.75	
3a	4.81	3.87	3.48	3.52	3.43	3.87	3.75	2.11	5.16	3.71	3.83	3.49	4.03	3.83	3.76	2.18
3b	4.73	3.84	3.64	3.54	3.44	3.90	3.75	2.06								2.18
4a	4.77	3.85	3.66	3.67	3.63	3.98	3.81	2.05	4.97	3.56	3.75	3.42	3.71	3.87	3.75	
4b	4.76	3.86	3.66	3.67	3.62	3.97	3.83	2.06	4.98	3.57	3.76	3.43	3.72	3.88	3.75	2.18
5a	4.78	3.83	3.66	3.49	3.48	3.94	3.76	2.07	5.09	3.60	3.80	3.46	3.92	3.86	3.78	

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*
α-D-Glc*p*	5.22	3.54	3.74	3.41	3.82	3.84	3.75
α-L-Rha*p*	5.12	3.92	3.81	3.45	3.86	1.28	
β-D-GlcNAc*p*	4.70	3.66	3.52	3.44	3.45	3.90	3.74

[a]Recorded at 70°, using TSP = 0.00 as internal reference.

TABLE II

GLYCOSYLATION-INDUCED ^{1}H-N.M.R. CHEMICAL SHIFT DIFFERENCES[a] FOR *Sh. flexneri* O-POLYSACCHARIDES

Type	*(1→2)-α-L-Rhap-(1→2) (RhaI)*						*(1→2)-α-L-Rhap-(1→3) (RhaII)*						*(1→3)-α-L-Rhap-(1→3) (RhaIII)*					
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
Y	0.04	0.22	0.06	−0.11	−0.17	−0.02	0.03	0.14	0.12	0.04	−0.10	0.03	−0.24	−0.05	−0.02	0.10	0.15	−0.02
X	−0.02	0.47	0.12	−0.09	−0.14	−0.03	0.04	0.13	0.10	0.01	−0.12	0.02	−0.26	−0.08	−0.04	0.08	0.14	−0.05
3a	−0.02	0.47	0.13	−0.07	−0.13	−0.01	0.01	0.11	−0.05	0.0	−0.28	0.01	−0.22	1.09	0.14	0.14	0.22	−0.01
3b	0.03	0.21	0.05	−0.11	−0.17	−0.02	−0.02	0.10	−0.05	0.0	−0.28	0.0	−0.21	1.10	0.15	0.14	0.21	−0.01
4a	−0.01	0.22	0.06	−0.13	−0.18	−0.04	0.02	0.13	0.11	0.02	−0.11	0.03	−0.23	−0.05	−0.02	0.10	0.14	0.03
4b	−0.02	0.22	0.06	−0.12	−0.18	−0.02	−0.02	0.10	−0.05	0.0	−0.28	0.0	−0.19	1.11	0.14	0.15	0.22	0.0
5a	0.02	0.22	0.08	−0.11	−0.16	−0.03	0.10	0.33	0.20	0.16	−0.07	0.06	−0.24	−0.05	−0.01	0.11	0.15	−0.01

	(1→3)-β-D-GlcNAcp-(1→2) (GlcNAc)							*α-D-Glcp-(1→X) (Glc)*						
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*
Y	0.05	0.18	0.14	0.11	0.01	0.01	0.02							
X	0.11	0.19	−0.02	0.07	−0.02	−0.04	0.01	0.06	0.17	0.08	0.07	0.20	0.02	0.0
3a	0.11	0.21	−0.04	0.08	−0.02	0.03	0.01	−0.06	0.17	0.09	0.08	0.21	−0.01	0.01
3b	0.03	0.18	0.12	0.10	−0.01	0.0	0.01							
4a	0.07	0.19	0.14	0.23	0.18	0.08	0.07	−0.25	0.02	0.01	0.01	0.11	0.03	0.0
4b	0.06	0.20	0.14	0.23	0.17	0.07	0.09	0.24	0.03	0.02	0.02	0.10	0.04	0.0
5a	0.08	0.17	0.14	0.05	0.03	0.04	0.02	−0.13	0.06	0.06	0.05	0.10	0.02	0.03

[a]Relative to the respective monomeric sugars.

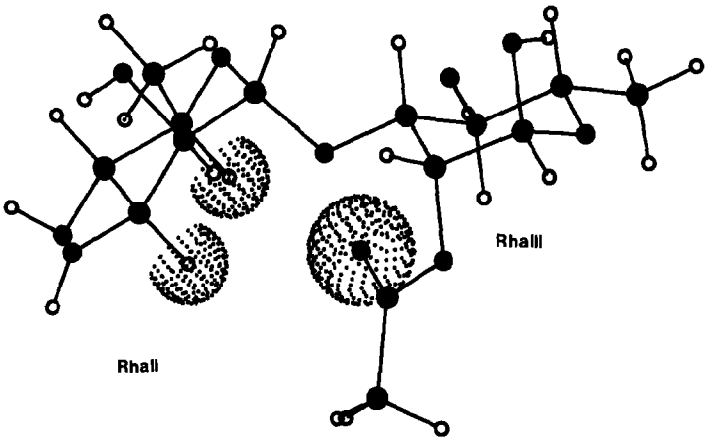

Fig. 2. Rhamnopyranosyl residues II and III in *Sh. flexneri* type 3b. The dot-surfaced atoms (70% of the van der Waals radii) indicate the proximity of the carbonyl oxygen and H-3 and H-5 in the neighbouring residue.

type Y is substituted with an *O*-acetyl group at the 2-position of RhaIII, which causes a downfield shift of 1.14 p.p.m. for H-2 in this residue. Only one of the two β-protons, H-3, shows a significant downfield shift, 0.16 p.p.m. In addition, upfield shifts of the signals for H-3 and H-5 in RhaII are observed. Assuming the φ- and ψ-angles to be 50° and 15°, respectively, the disaccharide element containing RhaII, RhaIII, and the *O*-acetyl group was constructed (Fig. 2). The *O*-acetyl group has been added in such a way that the α-proton and the carbonyl group eclipse each other, approximately as found in crystal structures[4]. In this conformation, H-3 and H-5 of RhaII will be adjacent to the carbonyl group. For types 3a and 4b, in which also a Glc group is linked to RhaI or GlcNAc, respectively, the changes in chemical shifts caused by the *O*-acetyl group are almost the same as those observed for type 3b. Thus, the conformation of the disaccharide element in question should not be significantly changed on introduction of a Glc group in either of the positions indicated.

TABLE III

RELAXATION TIMES[a] (T_1) FOR THE ANOMERIC PROTONS OF THE *Shigella flexneri* TYPE X, Y, 3a, 3b, 4a, 4b, AND 5a O-POLYSACCHARIDES

Residue	*Y*	*X*	*3a*	*3b*	*4a*	*4b*	*5a*
(1→2)-α-L-Rha*p*-(1→2)	1.0	1.2	1.2	1.1	1.0	1.0	1.0
(1→2)-α-L-Rha*p*-(1→3)	1.0	1.0	1.0	0.9	1.0	1.0	1.0
(1→3)-α-L-Rha*p*-(1→3)	1.2	1.4	1.4	1.2	1.2	1.4	1.4
(1→3)-β-D-GlcNAc*p*-(1→2)	0.9	1.0	0.9	0.9	0.8	0.8	1.0
α-D-Glc*p*-(1→X)		1.0	1.0		1.1	1.2	1.0

[a]Standard deviations did not exceed ±0.07 s.

TABLE IV

OBSERVED n.O.e.-CONTACTS OF THE ANOMERIC PROTONS OF THE *Sh. flexneri* TYPE Y, X, 3a, 3b, 4a, 4b, AND 5a O-POLYSACCHARIDES

Anomeric proton	*N.O.e. contact*
Type Y	
5.16 (RhaI)[a]	4.14 (RhaI, H-2), 4.06 (RhaII, H-2)
5.15 (RhaII)	4.06 (RhaII, H-2), 3.79 (RhaIII, H-3)
4.88 (RhaIII)	3.87 (RhaIII, H-2), 3.66 (GlcNAc, H-3), 3.55 (RhaIII, H-4)
4.75 (GlcNAc)	4.14 (RhaI, H-2), 3.66 (GlcNAc, H-3)
Type X	
5.16 (RhaII)[b]	5.10 (RhaI, H-1), 4.86 (RhaIII, H-1), 4.05 (RhaII, H-2), 3.84 (RhaIII, H-2), 3.77 ((RhaIII, H-3)
5.16 (Glc)[b]	4.39 (RhaI, H-2), 3.93 (RhaI, H-3), 3.71 (Glc, H-2)
5.10 (RhaI)	4.39 (RhaI, H-2), 4.05 (RhaII, H-2)
4.86 (RhaIII)	3.84 (RhaIII, H-2), 3.50 (GlcNAc, H-3)
4.81 (GlcNAc)	4.39 (RhaI, H-2), 3.50 (GlcNAc, H-3)
Type 3a	
5.16 (Glc)	4.81 (GlcNAc, H-1), 4.39 ((RhaI, H-2), 3.95 (RhaI, H-3), 3.71 (Glc, H-2)
5.13 (RhaII)	4.03 (RhaII, H-2), 3.95 (RhaIII, H-3)
5.10 (RhaI)	4.39 (RhaI, H-2), 4.03 (RhaII, H-2)
4.90 (RhaIII)	5.01 (RhaIII, H-2), 3.59 (RhaIII, H-4), 3.48 (GlcNAc, H-3)
4.81 (GlcNAc)	4.39 (RhaI, H-2)
Type 3b	
5.15 (RhaI)	5.10 (RhaII, H-1), 4.73 (GlcNAc, H-1), 4.13 (RhaI, H-2), 4.02 (RhaII, H-2)
5.10 (RhaII)	4.02 (RhaII, H-2), 3.96 (RhaIII, H-3)
4.91 (RhaIII)	5.02 (RhaIII, H-2), 4.73 (GlcNAc, H-1), 3.64 (GlcNAc, H-3), 3.59 (RhaIII, H-4)
4.73 (GlcNAc)	5.15 (RhaI, H-1), 4.91 (RhaIII, H-1), 4.13 (RhaI, H-2), 3.86 (RhaI, H-3), 3.64 (GlcNAc, H-3)
Type 4a	
5.14 (RhaII)	4.05 (RhaII, H-2), 3.87 (RhaIII, H-2), 3.79 (RhaIII, H-3)
5.11 (RhaI)	4.14 (RhaI, H-2), 4.05 (RhaII, H-2)
4.97 (Glc)	3.56 (Glc, H-2)
4.89 (RhaIII)	3.87 (RhaIII, H-2), 3.66 (GlcNAc, H-3), 3.55 (RhaIII, H-4)
4.77 (GlcNAc)	4.14 (RhaI, H-2), 3.66 (GlcNAc, H-3)
Type 4b	
5.10 (RhaI)[b]	4.14 (RhaI, H-2), 4.02 (RhaII, H-2)
5.10 (RhaII)[b]	4.02 (RhaII, H-2), 3.95 (RhaIII, H-3)
4.97 (Glc)	3.57 (Glc, H-2)
4.93 (RhaIII)	5.03 (RhaIII, H-2), 3.66 (GlcNAc, H-3), 3.60 (RhaIII, H-4)
4.76 (GlcNAc)	4.14 (RhaI, H-2), 3.66 (GlcNAc, H-3)
Type 5a	
5.22 (RhaII)	5.14 (RhaI, H-1), 4.88 (RhaIII, H-1), 4.25 (RhaII, H-2), 3.87 (RhaIII, H-2), 3.80 (RhaIII, H-3)
5.14 (RhaI)	5.22 (RhaII, H-1), 5.09 (Glc, H-1), 4.78 (GlcNAc, H-1), 4.25 (RhaII, H-2), 4.14 (RhaI, H-2)
5.09 (Glc)	5.14 (RhaI, H-1), 4.25 (RhaII, H-2), 4.01 (RhaII, H-3), 3.60 (Glc, H-2)
4.88 (RhaIII)	5.22 (RhaII, H-1), 4.78 (GlcNAc, H-1), 3.87 (RhaIII, H-2), 3.66 (GlcNAc, H-3), 3.56 (RhaIII, H-4)
4.78 (GlcNAc)	5.14 (RhaI, H-1), 4.88 (RhaIII, H-1), 4.14 (RhaI, H-2)

[a]For explanation of residue coding, see Table I. [b]Contacts assigned by the cross-peak pattern.

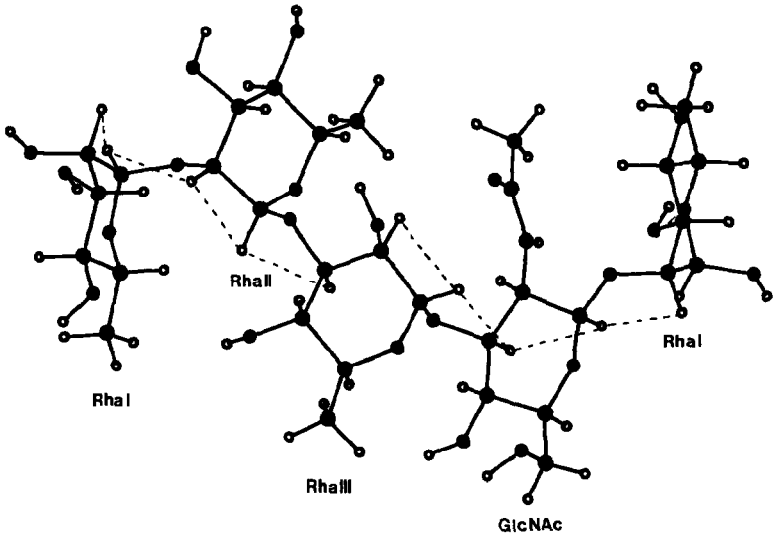

Fig. 3. The n.O.e. contacts (----) observed for *Sh. flexneri* type Y.

As all of the coupling constants, which were obtained by resolution-enhanced ^{1}H-n.m.r. spectra and 2D-JRES experiments, are of the expected order of magnitude, the pyranosidic rings seem to have maintained their normal chair conformations. Spin–lattice relaxation times for all anomeric protons are given in Table III. Knowledge of these is needed for the 2D-n.O.e. experiment.

In the n.O.e. spectra, all expected intra-residual n.O.e. contacts for the anomeric protons can be observed (Table IV) except that between H-1 and H-5 in the GlcNAc residue. This may be due to the multiplicity of the signal or to loss of intensity due to spin diffusion from H-5. The presence of cross-peaks due to n.O.e. contacts between H-1 and H-3 in the GlcNAc residue, but to H-2 in the Glc group and in the Rha*p* residues, further corroborates the β and α configurations assigned to these residues. In addition to the intra-residue contacts, most of the expected inter-residue contacts are also observed. For the basic tetrasaccharide structure **1**, the complete sequence is evident from the cross-peaks due to n.O.e. contacts between the anomeric protons and the protons on the linkage carbons (Fig. 3).

Somewhat more complicated spectra are observed for the polysaccharides having an additional Glc group. When this group is attached to the 3-position in RhaI, as in type X, or to the same position in RhaII, as in type 5a, n.O.e. contacts between H-1 of the Glc*p* group and both H-2 and H-3 in the Rha*p* residue are observed. An energy-minimised molecular model of the Glc-(1→3)-Rha disaccharide fragment also indicates the proximity of these protons. As the dynamics of the molecule may cause more distant protons to approach each other for shorter periods of time, more n.O.e.'s than anticipated from the energy-minimised molecu-

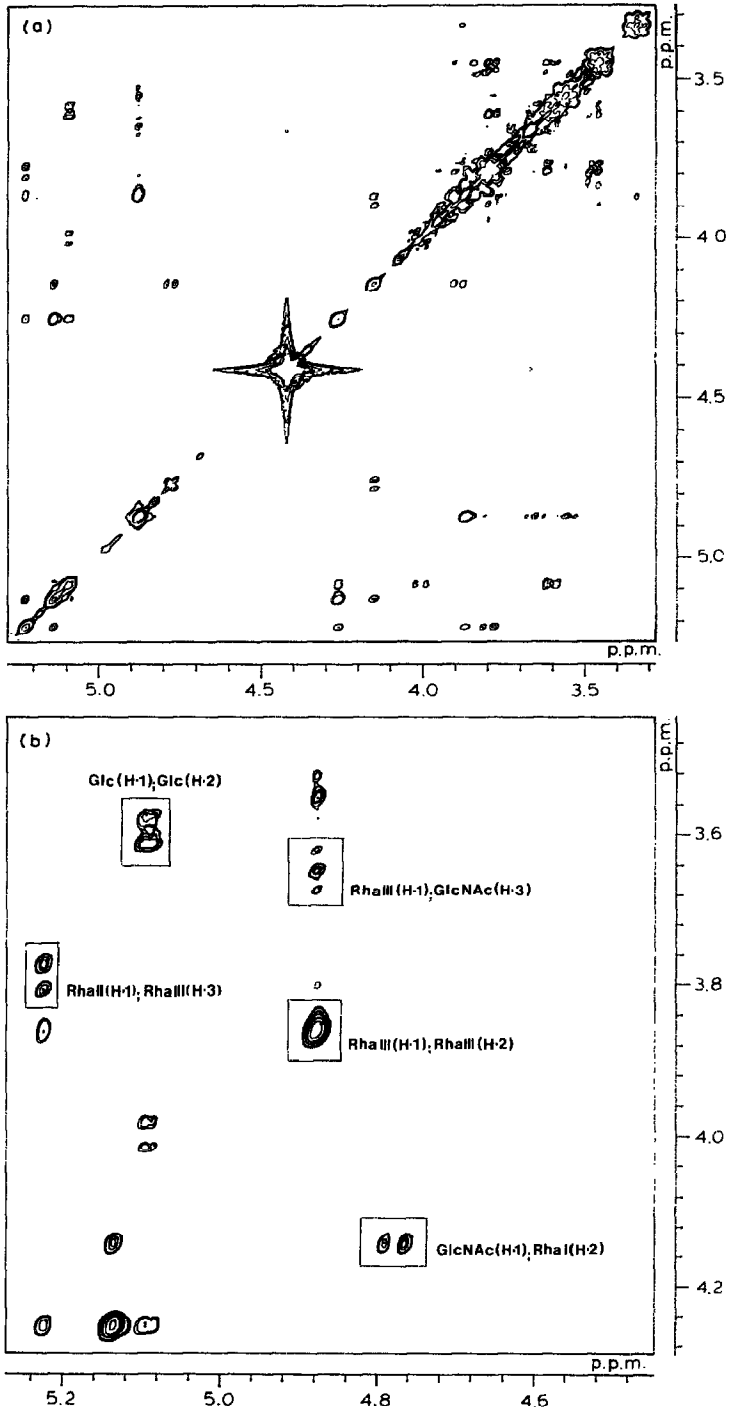

Fig. 4. NOESY-spectrum of *Sh. flexneri* type 5a and enlargement of the cross-peak region showing the different coupling patterns.

lar model may appear and complicate the assignment of sequences.

In the repeating units of types 4a and 4b, Glc is linked to the 6-position of GlcNAc, but no cross-peaks between H-1 and H-6 or H-6′ in GlcNAc are observed. This situation may depend upon the conformation or upon spin diffusion as discussed above. The n.O.e. experiment therefore gives only limited information on sequence for these types.

A complication in the analysis of the NOESY spectra occurs when the signals for the anomeric protons overlap. The protons involved in the n.O.e. contacts can, however, be derived by the pattern of the cross-peaks. The distance between the individual peaks in a cross-peak, derived from an anomeric proton, reflects the magnitude of $J_{1,2}$. Thus, different types of cross-peaks were derived from α-Rha, α-Glc, and β-GlcNAc (see Fig. 4). Consequently, analysis of the patterns of the cross-peaks helps the assignments when several signals have similar chemical shifts. When, due to low spectral quality, no apparent difference in the pattern of the cross-peaks is observed, alternative interpretations are possible.

Different mixing times (0.05–2.0 s) in 7 different experiments were used in the NOESY experiment with type 4a. At times shorter than 100 ms, no inter-glycosidic n.O.e.'s were observed. For short times (0.1–0.3 s), mainly cross-peaks derived from direct n.O.e.-contacts were observed, whereas, for longer times (0.6–1.0 s), cross-peaks caused by spin diffusion were also observed. Less informative spectra were obtained for mixing times >1.5 s, due to extensive spin-diffusion which gave cross-peaks of low intensity. Because sequential information was of primary interest in this study, a mixing time of 0.6 s was used, which also generated some useful spin-diffusion. Cross-peaks derived from spin diffusion between the

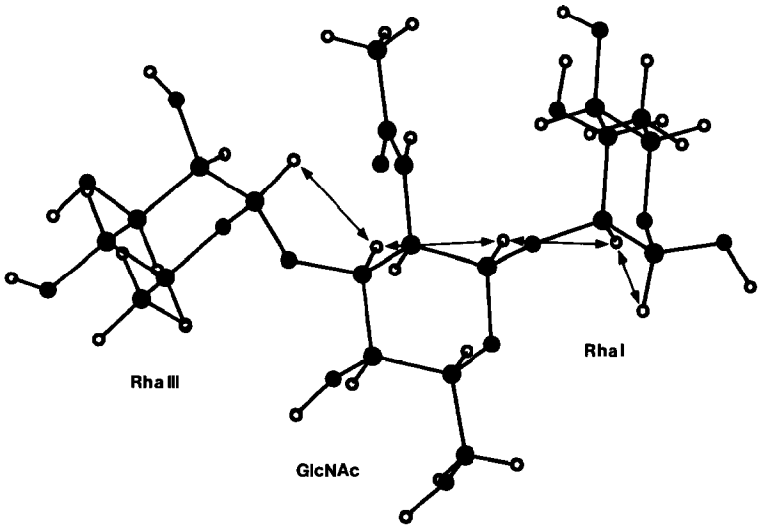

Fig. 5. The routes for spin diffusion in the →3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→2)-α-L-Rha-(1→ element.

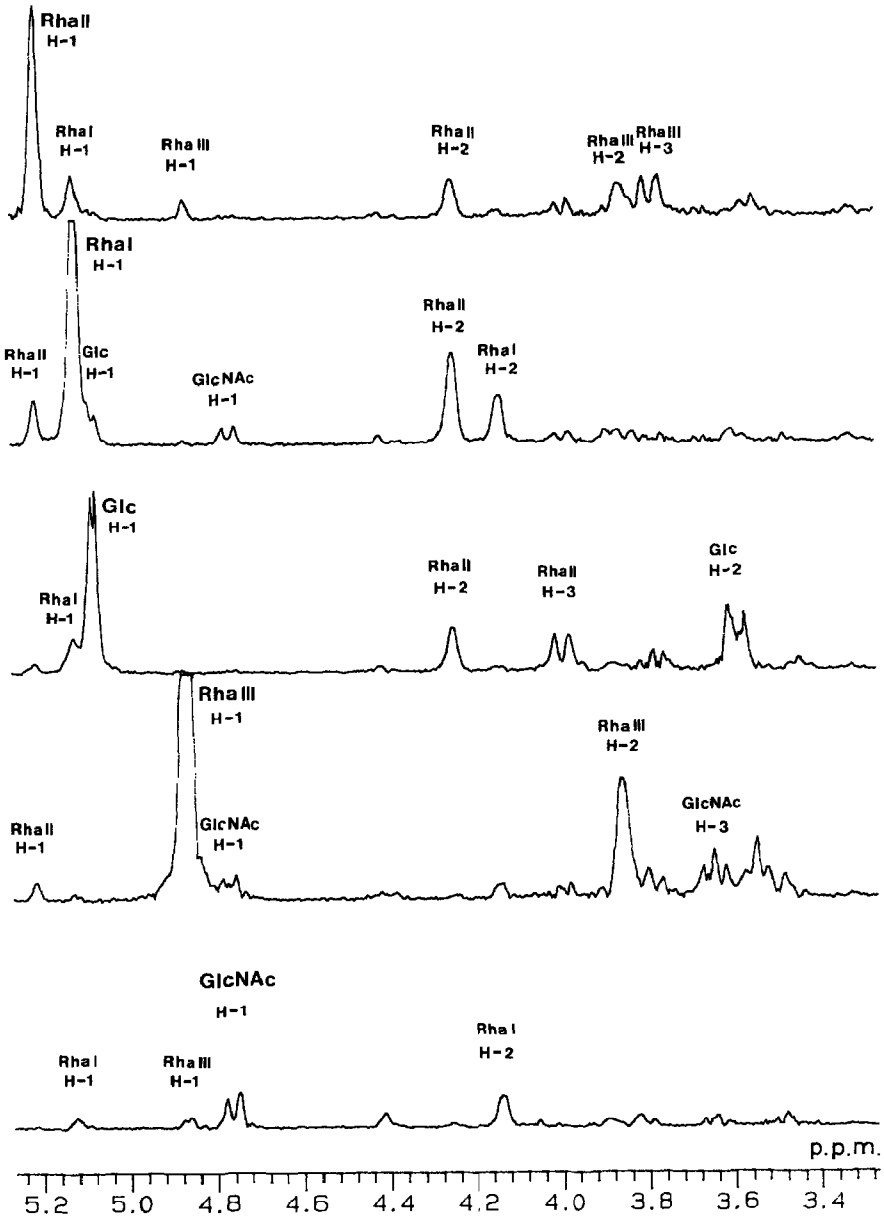

Fig. 6. Cross-sections of the NOESY-spectrum for *Sh. flexneri* type 5a.

anomeric protons of neighbouring residues are mainly observed when the proton on the glycosyloxylated carbon has a direct n.O.e.-contact with the anomeric proton of the neighbouring residue. Consequently, only minor cross-peaks were observed between the anomeric protons of RhaII and RhaIII where H-3 and H-1

of RhaIII have no direct contact. A sequence of residues in which this direct n.O.e.-contact occurs is given in Fig. 5. Cross-sections from the NOESY spectrum of *Sh. flexneri* type 5a showing signals deriving from both neighbouring residues are given in Fig. 6.

The substituent shifts for the α- and β-protons depend upon the stereochemistry around the glycosidic bond. Further n.O.e.-contacts may be observed for more than one inter-residue proton or may not be observed at all. For these reasons, it may be difficult to obtain complete information on components, linkages, and sequences from ^{1}H-n.m.r. spectra. If components and linkage positions are already determined by sugar and methylation analyses, the structural analysis from ^{1}H-n.m.r. spectra is facilitated.

CONCLUSION

Although ^{1}H-n.m.r. spectroscopy, using 2D-n.m.r. techniques, is a powerful tool in structural polysaccharide chemistry, there are obvious limitations. The identification of linkages by glycosidation shifts is not yet straightforward, but may be safer when more ^{1}H-n.m.r. data for oligosaccharides having different types of glycosidic linkages become available. Inter-residue n.O.e. contacts, when observed, will give sequences, and cross-peaks caused by spin diffusion may give further such information. The use of energy-minimised molecular models may facilitate the interpretation of the n.O.e. spectra.

It seems to be impractical today to solve complicated polysaccharide structures using ^{1}H-n.m.r. spectroscopy only. A combination with sugar and methylation analysis and ^{13}C-n.m.r. spectroscopy seems to be preferable.

EXPERIMENTAL

The polysaccharides were prepared from the lipopolysaccharides as described earlier[1].

N.m.r. spectroscopy was performed at 400 MHz with a JEOL GX-400 instrument for ~2% solutions in D_2O [internal sodium 3-(trimethylsilyl)propanoate-d_4 (TSP)]. All spectra were recorded at 70°. Relaxation times (T_1) were measured by the inversion recovery method and calculated with a three-parameter non-linear fit. Samples were not degassed. 2D-N.m.r. spectroscopy was performed with standard JRES, COSY, relayed COSY, and NOESY pulse sequences. For the correlation experiments, a 90° mixing pulse was used. In the NOESY experiments, a mixing time of 0.6 s was used, except for type 4a, for which seven spectra with mixing times varying from 0.05 to 2.0 s were obtained. The relaxation delay was 6 s. For COSY and NOESY experiments, a total of 128 spectra, each consisting of 512 data points, were accumulated and zero-filled into 256 data points with a frequency range of 800 Hz in each dimension. When the pattern of the cross-peaks was to be analysed, the size of the matrix was 1K × 256 zero-filled to 512 points.

Resolution enhancement in both dimensions was performed by a non-shifted sine-bell or a trapezoidal function, and the physical presentation of the spectra was given in the power display mode. The analysis of spectra was performed by a combination of 1D-n.m.r. spectra, COSY-spectra, NOESY-spectra, and NOESY-cross-sections.

The molecular model was constructed with the aid of CHEM-X (Chemical Design Oxford) and minimised with respect to non-bonded interactions.

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