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THE CONFORMATION OF *Salmonella* O-ANTIGENIC OLIGOSACCHARIDES OF SEROGROUPS A, B, AND D_1 INFERRED FROM ^1H- AND ^{13}C-NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY*

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ABSTRACT

The conformational model derived by the HSEA calculation method was used to interpret the n.m.r. data for solutions of oligosaccharides corresponding to the *Salmonella* serogroups A, B, and D_1 antigenic determinants. The favored conformer, derived by calculation, accounted for the observed, chemical-shift changes and accurately predicted the existence and magnitude of inter-ring proton n.O.e.'s. Extensive proton-density and compression of proton, Van der Waals radii were correlated with deshielding of specific proton-resonances. The model of lipopolysaccharide conformation accounts for the known antigenic properties of *Salmonella* O-antigens.

INTRODUCTION

Molecular modeling of *Salmonella* lipopolysaccharides (LPS), based on HSEA calculations, was presented in the preceding paper[1]. Since n.m.r. spectroscopy is currently the only experimental technique capable of assessing the validity of these calculations and also the actual preferred conformation of oligosaccharides in aqueous solution, we describe herein the experimental evidence for the proposed conformation. These inferences are based upon analyses of the n.m.r. parameters of a large number of synthetic glycosides varying in size from mono- up

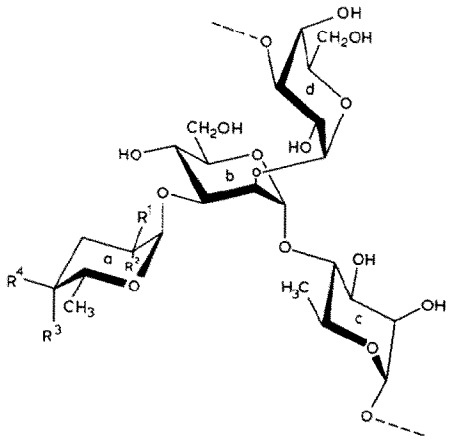
*Dedicated to Professor Raymond U. Lemieux.

to tetra-saccharides. In addition, larger oligosaccharides, from single repeating-units up to icosasaccharides, containing five repeating units were studied. These were obtained by phage-mediated hydrolysis of the Serogroup B lipopolysaccharide[2].

Oligosaccharides and polysaccharides are not rigid bodies and n.m.r. measurements provide data that may only be interpreted in terms of a time-averaged conformation, a hypothetical entity, which is the composite of all those conformational states that are appreciably populated. Since hexopyranose sugars exist in stable chair-conformations, which are adequately represented as a rigid entity, the description of oligosaccharide conformation may be reduced to a definition of permissible ϕ and ψ values, *i.e.*, the amplitude of the glycosidic torsional-angles. These angles were defined by semiempirical calculations in the preceding paper (HSEA calculations)[1], and it is possible to generate sets of interatomic distances from the model repeating-unit, which corresponds to the preferred conformation. The interatomic distances may be compared with nuclear Overhauser enhancements (n.O.e.'s) and specific deshielding-effects in ^{1}H-n.m.r. spectra, and, together with ^3J heteronuclear coupling-constants, which provide an estimate of the ψ torsional angle, these n.m.r. data define the "average" conformation of the polysaccharide repeating unit in aqueous solution. To date, agreement between ^{1}H- and ^{13}C-n.m.r. data and HSEA-derived conformations for oligosaccharides corresponding to the human, blood-group determinants has been remarkably good[3–5]. LPS O-antigens of *Shigella flexneri*[6] and glycan chains of glycoproteins have also been studied[7,8]. As the HSEA approach may now be considered a predictive tool, we have chosen to apply this approach to a set of structurally related and immunologically important molecules. These are the O-antigens of *Salmonella* Serogroups A, B, and D_1 for which a considerable body of serological data exists[9,10]. A large portion of these data may be rationalized on the basis of the primary structure[10–12], and, it is to be hoped, the conformation. Reliable experimental support for the empirically derived conformations of these structures is therefore most desirable, and this evidence is provided by the following n.m.r. measurements.

RESULTS AND DISCUSSION

The preferred conformations of oligosaccharides in solution may be reliably inferred from n.m.r. data only after rigorous assignment of all ^{1}H and ^{13}C resonances. This is most easily achieved for complex oligosaccharides by examination of model compounds which constitute essential elements of the parent structure. When this process is completed, measurement of n.O.e.'s for specific proton-resonances provides information simultaneously on intra- and inter-residue, proton–proton distances. These data are compared with interatomic distances derived from the coordinates of the preferred conformation, calculated by the HSEA method, and further extrapolations are possible by correlation of short oxygen–proton distances with specific deshielding-effects. It is also possible to obtain an estimate of



Scheme 1. The chemical repeating-unit →3)-α-D-Gal*p*-(1→2)-[3,6-dideoxy-α-D-hexopyranosyl-(1→3)]-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→ [d→(a→)b→c] of *Salmonella* A, B, and D_1 *O*-antigens. The source and identity of the model compounds **1–31** with respect to their saccharide and aglycon components are given below.

Compound	*Saccharide sequence*	*Aglycon*	*3,6-Dideoxyhexose*				*Name*	*Ref.*
			R^1	R^2	R^3	R^4		
1	a→b→c→d	*p*-$NO_2C_6H_4$	OH	H	H	OH	Par	14
2	a→b→c→d	*p*-$NO_2C_6H_4$	OH	H	OH	H	Abe	14
3	a→b→c→d	*p*-$NO_2C_6H_4$	H	OH	H	OH	Tyv	14
4	d→(a→)b→c	$(CH_2)_8CO_2Me$	OH	H	H	OH	Par	15
5	d→(a→)b→c	$(CH_2)_8CO_2Me$	OH	H	OH	H	Abe	16
6	d→(a→)b→c	H	OH	H	OH	H	Abe	2
7	[d→(a→)b→c]$_2$	H	OH	H	OH	H	Abe	2
8	d→(a→)b→c	$(CH_2)_8CO_2Me$	H	OH	H	OH	Tyv	15
9	d→(a→)b→c	$(CH_2)_8CO_2Me$	H	OH	H	OH	Asc	15
10	d→(a→)b	$(CH_2)_8CO_2Me$	OH	H	H	OH	Par	17
11	d→(a→)b	$(CH_2)_8CO_2Me$	OH	H	OH	H	Abe	18
12	d→(a→)b	$(CH_2)_8CO_2Me$	H	OH	H	OH	Tyv	17
13	d→b→c	$(CH_2)_8CO_2Me$						16
14	b→c→d	*p*-$NO_2C_6H_4$						14
15	a→b	*p*-$NO_2C_6H_4$	OH	H	H	OH	Par	19
16	a→b	*p*-$NO_2C_6H_4$	OH	H	OH	H	Abe	20,22
17	a→b	$(CH_2)_8CO_2Me$	OH	H	OH	H	Abe	21,23
18	a→b	*p*-$NO_2C_6H_4$	H	OH	H	OH	Tyv	24
19	b→c	*p*-$NO_2C_6H_4$						14
20	b→c	$(CH_2)_8CO_2Me$						16
21	c→d	*p*-$NO_2C_6H_4$						14
22	d→b	$(CH_2)_8CO_2Me$						17
23	a	Me	OH	H	H	OH	Par	
24	a	Me	OH	H	OH	H	Abe	
25	a	Me	H	OH	H	OH	Tyv	
26	d	*p*-$NO_2C_6H_4$						
27	d	$(CH_2)_8CO_2Me$						
28	b	*p*-$NO_2C_6H_4$						
29	b	$(CH_2)_8CO_2Me$						
30	c	*p*-$NO_2C_6H_4$						
31	c	$(CH_2)_8CO_2Me$						

TABLE I

^{1}H-NMR CHEMICAL SHIFTS (δ)[a] AND COUPLING CONSTANTS (Hz)[b] FOR COMPOUNDS 1–31

Comp.	Structure	3,6-Didehex	Unit																							
			a							b							c						d					
			H-1	H-2	H-3	H-3'	H-4	H-5	H-6	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-1	H-2	H-3	H-4	H-5	H-6	H-6'
1	a→b→c→d	Par	5.05	3 83	1 80	2 16	3 40	3 78	1 23	5 00	4 13	3.94	3 91	4 03	3 88	3.81	5 12	4 13	4 00	3.60	3 98	1 34	5.70	4 15↔4.11		4 10	3 73	3.69
			3 8	4 5	11 0	4 5	9 5	6 3		1 0					2 0	5 0, 12 0	1.1	3.5	9 5	9 5	6 3		3.0				7.2	5 0, 12 0
				11 0	11.0																							
2	a→b→c→d	Abe	5 09	4.04	2 07	1 98	3 91	4 13	1.16	5 00	4 12	3 92	3 90	4.02	3.88	3 81	5 11	4.12	4 00	3 60	3 98	1 35	5 71	4.15↔4 11		4 09	3 73	3.70
			3 6	12 0	12 0	5 5	0 5	6 3		1 8					2 5	5.0, 12 5	1.8	3.5	9 5	9.3	6 3						7.5	5 0, 12.0
				3 0	2 5																							
3	a→b→c→d	Tyv	4 88	4 08	1 90	2 06	3 64	3 81	1 26	4 99	4 11	3 92	3.83	4 03	3 88	3 81	5 11	4.14	4 00	3 60	3 98	1 36	5 70	4 15↔4 11		4 08	3.73	3.69
			1 5	3 0	12 5	4.5	9 5	6 3		1 5	3 0	9 5	9.5		2 0	5 0, 12 0	1.5	3 2	9.5	9.5	6 3		3 0				7 5	5 0, 12 0
				3 0	11 5																							
4	a→b→c→d	Par	5 05	3.81	1 73	2 13	3 36	3 69	1 23	5 25	3 99	4 00	3 79	3 92	3 85	3.79	4 77	3 91	3 81	3 51	3 76	1.32	5 22	3.78	3 90	4 00	4.03	3 74	3 68
			3 8	12.0	11 8	4 3	9 5	6.4		1 6	2 9	9 4			2.8	4 5, 12 0	1 9	3 4	9 4	9 4	6 5		3 8	10 2	3 3			5 0	6 5, 11.3
				5 5	11 5																							
5	d→(a→)b→c	Abe	5 08	4 02	1 98	1 98	3 87	4 07	1 18	5 27	3 97	4 01	3 90	3 97	3 84	3.79	4.77	3 91	3 83	3 51	3 78	1 32	5.14	3 77	3.89	3 98	4 02	3 73	3.68
			3 5	2 5				6 6		2 0	2 5	9 8	9 6		2 2	4 5, 12.1	1.9	3 2	9.4	9.4	6 5		3 7	10 2	3 1			6 0	6 0, 12 0
				8 8																								
6	d→(a→)b→c	Abe	5 11	4 07	2 00↔1.96		3 90	4 14	1 18	5 31	4.03	3 92		4 07	3 88	3 83	5 12	3 94	3 92	3 54	3 99	1 33	5 17	3 80	3 94	4 01	4 09	3 76	3.72
								6 4		1 2	3 2	9.5			2 4	5 0, 12 0	1 6	3 2	9 5	9 2	6 2		4 0	10 0	3 2			4 4	8 0, 12 6
7	d→(a→)b→c	Abe	5 09	4.02	1 97	1 97	3 86	4 10	1 16	5 29	3 99	4 04	3.91	3 96	3 86	3 80	5 04	4 06	3 97	3.55	3 91	1 32	5 15	3 77	3.90	3.99	4 05	3 72	3 69
			3 8				1 7	6.3		1 9	3 4				2 6	4 7, 12 5	1 9	3 4	9 4	9 4	6 5		4 0	10 9	3 4	1 0		6 3	7 7, 12 3
7	d→(a→)b→c	Abe	5 09	4 02	1 97	1 97	3 86	4 10	1 16	5 30	3 99	4 04	3 91	3 96	3 86	3 80	5 09	3 92	3.92	3.51	3 96	1 30	5 16	3 90	3 94	4 06	4 02	3 72	3 69
			3 8			1 7	6 3			1 9	3 4				2 6	4 7, 12 5	1 9		9 4	9.4	6 5		4 0	10 9	3 4			6 3	7.7, 12 3
8	d→(a→)b→c	Tyv	4 88	4 05	1 79	2 04	3 60	3 75	1.25	5 26	3 98	4 03	3.94	3 93	3 84	3.78	4 77	3 91	3 81	3.51	3 78	1 31	5 19	3 79	3 89	3 98	4 03	3 74	3 68
			1.3	3 1	11 3	4 2	9 7	6 3		1 8	3 0	9 3			1 4	3.8, 11 7	1 9	3 6	9 4	9.4	6.5		3 9	10 4	3 2	0 8		4 1	6 3, 11 0
				3 2	13 7																							
9	d→(a→)b→c	Asc	4 77	3.95	1 86	2 04	3 61	3 89	1 23	5 27	4 15	3 96	3.90	3 96	3 85	3.80	4 77	3 91	3.84	3.50	3.76	1 31	5 10	3 76	3.88	3.94	4 02	3 73	3 68
			1 9	5 8	11 5	3 7	9.2	6 3		1 8	2 9	9 4	9 4		1 8	6 0, 11 7	2 0	3 8	9.6	9.6	6.4		3 9	10 2	3 3			5 1	6 5, 12 5
				3 1	13 4																							
10	d→(a→)b	Par	5 06	3 81	1 71	2 12	3.35	3 75	1 23	5 11	3 95	3 97	3 90	3 65	3 85	3.76							5 18	3 78	3 89	3 96	4 03	3 74↔3 70	
			3 8	11.4	11 4	4 5	9 3	6 3		0 5	2 4	9 5	9 5		2.1	5 7, 12 4							4 0	10.4	3 6	0 5	6 0, 6 0		
				4 5	9 3																							
11	d→(a→)b	Abe	5 09	4 01	1 97	1 97	3.85	4 09	1 17	5 14	3.94	3 99	3.94	3 72	3 87	3 77							5 12	3 77	3.89	3 98	4 05	3 73	3 70
			3 8	6 4			1.3	6 7		1 9	3.0	9.6	9 6		2.3	5 9, 12 4							4 0	10 4	3.5	1 2		4 7	7 3, 12 0
				7 4																								
12	d→(a→)b	Tyv	4 89	4 04	1 81	1 99	3.61	3 73	1 29	5 12	3.93	4 01	3 90	3 63	3.84	3 79							5 18	3 79	3 89	4 00	4 03	3 75	3 68
				3 0	14 1	4 6	9 5	6 2		1.9	3 5	10 0	10 0		2 2	5 5, 12 0							3 8	10 4	3.6	1 0		6.5	7 5, 11 7
				3 9	11 2																							

13	d→b→c									5 28	3 99	3 90	3 79	3 92	3 84	3 78		4 76	3 91	3.79	3 50	3.77	1 33	5.12	3.77	3 90	3.97	4 08		3.73	3 68
										1 6	3 1	9 8	9 8		2 3	4 7, 12 6		2.0	3 8	9 3	9 3	6 0		3 8		3 6				5.0	7.7, 12 0
14	b→c→d									4 99	4.00	3 84	3 72↔3 87			3 78		5 10	4 11	3.97	3 59	3 95	1 33	5.69		4 15↔4 11		4 07		3.74↔3.71	
										1 8	3 2	9 4	9 4	2 2		5 3, 11 6		2 0	3 3	9 4	9 4	6 2						6.0, 6 0			
15	a→b	Par	5 12	3 84	1 83	2 18	3 40	3.79	1 25	5.61	4.28	4.12	3 96		3.82↔3 73																
			3 9	12 1	12 0	4.5	9.5	6 6		1 8	3.3	9 6	9.6																		
16	a→b	Abe	5 17	4.05	2 09	2 01	3 90	4.16	1 18	5.61	4.27	4 12	3 95		3.83↔3 73																
			3 8	11 7	12 6	3 5	1 3	6.6		1 9	3 2	9 6	9 6																		
				5 4	2 8																										
17	a→b	Abe	5 09	3 99	2 05	1 95	3 86	4.10	1 16	4 74	4 05	3.83	3 82	3.65	3 89	3 69															
			3.9	12 5	14 1	3 0	1 4	6.4		1 8	2 8	9 3	9 4		2 3	5 6, 11 9															
				5.6	3 4																										
18	a→b	Tyv	4 96	4 09	1 91	2 07	3 64	3 85	1 27	5 61	4 26	4 12	3 87		3.81↔3 73																
			1 0	3 9	13 0	3.9	9 0	6 3		2 0	3 2	9.5	9 5																		
				3 4	10 6																										
19	b→c									4 99	3 98	3.83	3 73	3 96	3 87	3 80		5.69	4 18	4.08	3 62	3 77	1 25								
										1 8	3 3	9 5	9 5		2.8	5 5, 12 0		2 1	3.6	9.5	9 5	6 0									
20	b→c									4 97	3 97	3 76	3 72	3.92	3 85	3 78		4 76	3.91	3 76	3 53	3.70	1 30								
										1 5	2.8	9 4	9 4		2 6	5 2, 12 2		1 1	2.8	9.4	9 4	6 0									
21	c→d																	5.11	4 12	3 89	3.50	3 85	1 31	5 71	4 13↔4.11			4 09		3 78↔3.71	
																		1.8	3 3	9 6	9 6	6.3									
22	c→d									5 16	3 92	3 90	3 73	3 60	3 87	3.76								5.12	3 81	3.89	3 98	4 07		3 74↔3.71	
										1 8	3.8	9.7	9 7		2.4	5 5, 12 2								3 6	10.4	3.6	1 0	6.3, 6 3			
23	a	Par	4 65	3 82	1 67	2 12	3 36	3 58	1 24																						
			3 6	12 0	12 0	11 4	9 6	6 3																							
				5.0	4 5																										
24	a	Abe	4 69	4 01	1 92	1 92	3 84	3 94	1 17																						
			3 9	11 1	4 0	4 0	1 2	6 3																							
				6.4																											
25	a	Tyv	4 50	3 92	1 77	2 04	3 59	3 67	1.27																						
			1.6	4.0	13 6	10.3	10 0	6 4																							
				3.5	3 6																										
26																								5.67	4 01	4 08	4 06	4.04		3 74↔3.70	
																								3 6	9 6	2.6		6.0, 6.0			
27																								4 92	3 83	3.80	3 96	3 92		3 73	3 70
																								3 1	10.2	3.3	1 0			6 2	6 2, 12.4
28										5 62	4 17	4 04			3 86↔3 69																
										1 8	3 2	9.0																				
29										4 84	3 92	3.77	3.66	3.63	3 86	3 75															
										1 6	3 2	9.3	9 0		1.8	5 1, 12 0															
30																		5.71	4 19	4 02	3 53	3.73	1.23								
																		1 9	3 4	9.4	9 4	6.3									
31																		4.78	3 92	3 72	3.44	3.66	1.27								
																		1 6	3 5	9.5	9 5	6.2									

[a]Upper line. [b]Lower line(s).

TABLE II

^{13}C-N M R CHEMICAL SHIFTS (δ) FOR COMPOUNDS **1–5** AND **8–31**

Comp.	*Structure*	*3,6-Dudches*	*Unit*																					
			a						*b*						*c*						*d*			
			C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1	a→b→c→d	Par	100.8	68 3	35.7	70.9	70 3	17.6	102.5	71.6	79.6	67.0	74 5	61.6	103 3	71.6	70 3	82.8	69 3	18 2	98.7	68.6	78.5	70.3	73 0	62.1
2	a→b→c→d	Abe	101.6	64.8	34.3	69.6	68 1	16.8	102.6	71.7	79.6	67.0	74 5	62.0	103.3	71.7	70 3	82.9	69.3	18.3	98 8	68.6	78.5	70.3	73 0	62.1
3	a→b→c→d	Tyv	102.5	68.7	34.7	68.2	71 6	18.1	102.5	71 5	79 3	67.1	74.6	61 9	103 3	71.5	70.4	82.9	69.3	18.3	98.7	68.7	78.5	70 3	73.0	62.1
4	d→(a→)b→c	Par	100.7	68.2	35.9	70 2	71 0	17.8	101 4	79.7	78 3	67.5	74 7	61.7	100.8	71.7	70.5	83.2	68.5	18 2	102.0	69.8	70.8	70.4	72.4	62.1
5	d→(a→)b→c	Abe	101.4	64 6	34.1	69.4	67 8	16 7	101 1	80.1	78 4	67.5	74.6	61 7	100.7	71 6	70.5	82.9	68 5	18.1	102.2	69.8	70 6	70.3	72.4	62 1
8	d→(a→)b→c	Tyv	102 1	70 4	34.6	68.0	71.5	17.9	100.9	79 7	78.0	67.4	74.6	61.6	100.6	71 4	70.4	82 8	68.4	18 1	102.2	69.8	70.6	70.3	72.4	62.1
9	d→(a→)b→c	Asc	96.6	68.7	34.6	71 0	66.1	17.9	101 0	76.1	75.7	68.0	74.2	61.7	100.7	71 6	70.4	82 7	68.5	18.1	102 1	69.8	70.5	70.3	72.5	62.1
10	d→(a→)b	Par	100.2	67.9	35.4	70.7	70.0	17 3	99 0	79.4	78.2	67.6	73.8	61 6							101 7	69 5	70 2	70.2	72.2	62 1
11	d→(a→)b	Abe	101.5	64.5	34.0	69.4	67.9	16.5	99 3	80 2	78.9	67.7	74.0	61.9							102.3	69.8	70.5	70.4	72.4	62.4
12	d→b	Tyv	101.9	68 2	34.2	67 8	71 1	17 6	99 0	79.5	78.2	67.4	73.8	61.6							101.9	69.6	70.1	70.2	72.2	62 1
13	d→b→c								100.8	80 7	71 5	68.0	74 1	61.9	100.5	71 5	70.4	82 5	68.5	18.0	102 3	69.8	70 4	70.4	72.3	62.1
14	b→c→d								102 5	71.6	71 6	67.7	74.2	61.9	103.1	71 6	70.1	82 5	69.3	18.1	98.7	70.1	78.5	68.5	72 8	61.9
15	a→b	Par	100 8	68 4	35.8	71 0	70.4	17 6	99.5	71.1	79.5	67.0	74 9	61.8												
16	a→b	Abe	101 7	64 8	34.3	69 6	68.1	16.6	99.5	71.2	79.5	67.0	74.9	61.9												
17	a→b	Abe	101 5	64.7	34 1	69 6	68.0	16.5	102.0	71.2	79.6	67 2	73.9	62.1												
18	a→b	Tyv	102 4	68 6	34.6	68.1	71.4	17.9	99.4	70 9	79.1	67.0	74.9	61.8												
19	b→c								102.5	71.6	71.4	67.7	74.3	61.9	98.6	71.1	70.0	82.2	69 8	18.0						
20	b→c								102.4	71.3	71.5	67.5	74.0	61 7	100.5	71.5	70.3	82 4	68.4	17.8						
21	c→d														103 7	71.5	71.5	73 4	70.6	18.0	98.8	68 7	78.6	70.1	73.2	62 2
22	d→b								99 1	80.5	71 6	68.0	73 2	61.7							102 1	69.7	70 2	70 2	72.2	62 1
23		Par	99.1	67.4	35 4	70 6	69.2	17.3																		
24		Abe	99 9	63 9	33 9	69.2	67 0	16.2																		
25		Tyv	100 8	68.4	34 6	68 0	70.7	17 9																		
26																					98.6	69.2	70.6	70.3	72 9	62.2
27																					99 2	69.4	70.6	70 3	71.9	62.2
28									99 2	70.7	71 2	67.4	74.3	61.5												
29									100.5	71.0	71 5	67.6	73.6	61.7												
30															98.6	70.6	70.9	72.8	70.6	17.5						
31															100.6	71 1	71.3	72.9	69.4	17 5						

TABLE III

^{13}C-N.M.R. CHEMICAL SHIFTS (δ) FOR PHAGE-DERIVED OLIGOSACCHARIDES (a = Abe)

Compound (unit)	*Atom*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Tetrasaccharide						
d	102.3	69.9	70.5	70.6	72.6	62.3
b	101.6	80.3	78.6	67.6	74.7	61.9
c; α	94.9	72.3	70.2	83.0	68.4	18.5
c; β	94.7	72.9	72.8	82.6	72.2	18.5
a	101.0	64.7	34.2	69.5	68.1	16.7
Octasaccharide						
d[a]	102.3	69.8	70.5	70.5	72.5	62.2
d	102.3	69.1	78.2	70.2	72.5	62.1
b (2)	101.6	80.4	78.6	67.5	74.6	61.8
c	102.9	71.5	70.2	82.7	69.1	18.2
c; α	94.8	72.2	70.2	82.9	68.4	18.3
c; β	94.6	72.8	72.7	82.5	72.2	18.3
a (2)	100.8	64.5	34.1	69.4	68.0	16.6
Dodecasaccharide						
d[a]	102.3	69.8	70.4	70.4	72.5	62.2
d (2)	102.3	69.1	78.2	70.2	72.5	62.1
b (3)	101.6	80.5	78.5	67.5	74.6	61.7
c (2)	102.9	71.5	70.2	82.7	69.1	18.2
c; α	94.8	72.2	70.2	82.9	68.4	18.2
c; β	94.6	72.8	72.7	82.5	72.2	18.2
a (3)	100.8	64.5	34.1	69.4	68.0	16.6
Hexadecasaccharide						
d[a]	102.4	69.9	70.5	70.6	72.6	62.2
d (3)	102.4	69.2	78.3	70.3	72.6	62.1
b (4)	101.7	80.5	78.7	67.6	74.7	61.9
c (3)	103.0	71.6	70.3	82.8	69.1	18.3
c; α	94.8	72.3	70.3	82.9	68.5	18.4
c; β	94.6	73.0	72.9	82.7	72.9	18.2
a (4)	100.9	64.6	34.2	69.5	68.1	16.7
Icosasaccharide						
d[a]	102.4	69.9	70.5	70.6	72.5	62.3
d (4)	102.3	69.2	78.3	70.3	72.6	62.2
b (5)	101.7	80.4	78.7	67.6	74.7	61.9
c (4)	103.0	71.6	70.3	82.8	69.1	18.3
c; α	94.9	72.3	70.3	83.0		18.3
c; β	94.7	72.7		82.6	72.3	18.3
a (5)	100.9	64.6	34.2	69.5	68.1	16.7

[a]Terminal nonreducing d unit.

the ψ torsional angle by measuring the heteronuclear ^3J coupling across the glycosidic linkage. In this way, a self-consistent conformational model is built up that agrees with HSEA calculations, and more importantly, accounts reliably and consistently for all features of the n.m.r. data[13].

The n.m.r. measurements reported herein were performed first on synthetic saccharides[14–23], and then on oligosaccharides[2] derived from the *Salmonella* serogroup B polysaccharide. The oligosaccharides **1–3** (ref. 14) and **4–8** (refs. 2, 15, 16) correspond to the biological and chemical repeating-units of *Salmonella* Serogroups A, B, and D_1, and in conjunction with the component tri- and di-saccharides were the subject of the initial n.m.r. studies. Although a related repeating-unit bearing an α-D-glucopyranosyl side-group was modeled in the preceding paper[1], no related experimental data are presented herein. Unambigous assignment of all ^1H and ^{13}C resonances for the repeating units **1–9** was made possible through the wide range of component saccharides[17–24] **10–31** (Tables I and II). The second set of oligosaccharides studied were those[2] derived by phage-mediated hydrolysis of the intact O-chain of serogroup B LPS. These compounds possess the branched, chemical, repeating-unit sequence d→(a→)b→c, rather than the biological repeat unit a→b→c→d which terminates the O-chain. These compounds ranged in size from a single-tetrasaccharide repeating-unit up to an icosasaccharide composed of five repeating units. The ^1H-n.m.r. data for two of these compounds, tetrasaccharide **6** and octasaccharide **7**, are recorded in Table I. The n.m.r. data accumulated for the synthetic compounds **1–31** (Tables I and II) permitted assignment of all aspects of the ^1H-n.m.r. data for **6** and **7**, and ^{13}C-n.m.r. data (Table III) for the tetra- to icosa-saccharides derived by phage-mediated hydrolysis.

The assignment of proton chemical-shifts for oligosaccharides is a routine procedure, and the methods employed have been adequately described in recent work[6] and a detailed review article[25]. In general, the approach has been to use double-resonance techniques and partly relaxed spectra, occasionally in conjunction with simultaneous double-resonance, and finally 2-D-*J*-resolved and 2-D-scalar-coupled experiments to construct a general framework of assignments. This served as the basis for complete assignments. In this regard, it may be noted that the deoxy groups of the 3,6-dideoxyhexose and L-rhamnose units permit particularly convenient connections to be established with the adjacent ring protons. The importance of this should not be overlooked since, even at the high magnetic-field-strengths employed, the concentration of 15–20 multiplets within the range δ 3.2–4.1 complicates the assignment task. Following ^{13}C chemical-shift assignment, heteronuclear ^{13}C–{^1H} decoupling confirmed existing assignments and established the identity of the remaining unassigned signals.

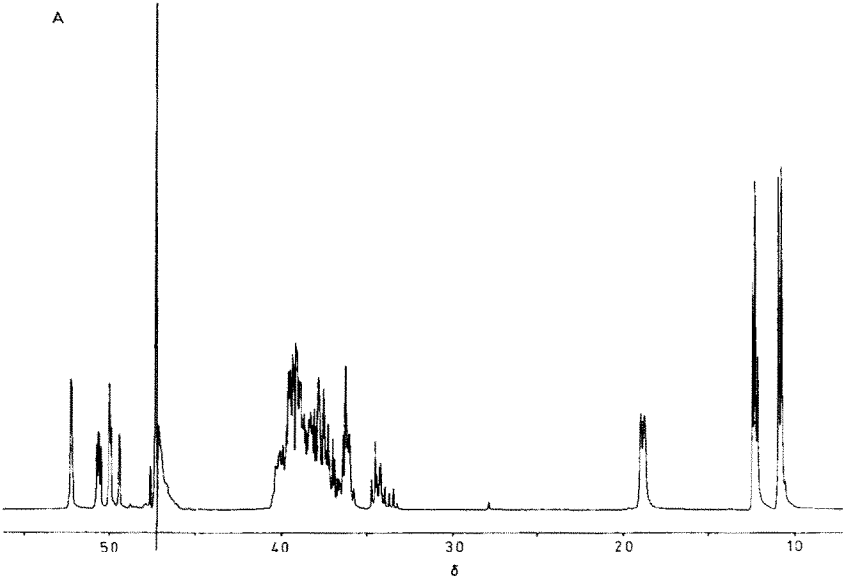
A basic assumption in the HSEA calculations of preferred oligosaccharide conformations is that the individual pyranose sugars adopt fairly rigid chain-conformations, which do not deviate appreciably from the orientations defined by the published coordinates of crystal structures. Inspection of proton ^3J values for each of the component saccharides of structures **1–31** (Table I) support this assumption.

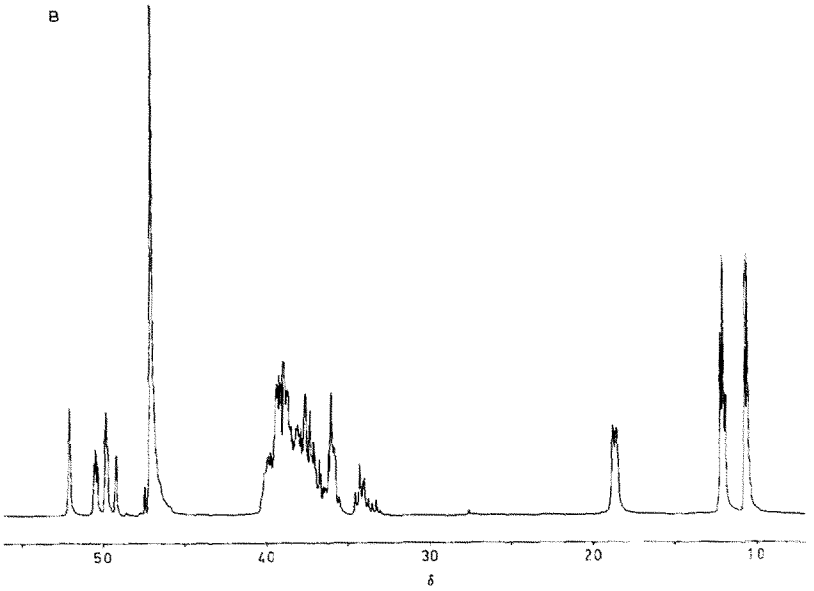
Examination of the proton chemical-shift data (Table I) revealed several examples of specific shielding of certain proton signals, and these may be correlated with short (<2.7 Å) proton–oxygen internuclear distances, which would exist in the preferred conformations calculated by the HSEA method[3,4]. In all compounds containing the disaccharide sequence Abe→Man (a→b) (**2**, **5–7**, **11**, **16**, and **17**), the H-1(a) signal showed a downfield shift of 0.4 p.p.m. when compared with that of the methyl glycoside **24**. Similar results were observed for the signals of H-1(a) of the paratose compounds **1**, **4**, **10**, and **15**, and also the tyvelose compounds **3**, **8**, **12**, and **18**, but not for the ascarylose isomer **9**, if these compounds are compared with their respective methyl glycosides **23** and **25**. In all the former sets of isomers, with the exception of the ascarylose-containing tetrasaccharide **9**, the results are consistent with the distance of 2.6 Å between O-4(b) and H-1(a). In comparison, no short oxygen-proton distances could be identified that would explain the respective 0.13- and 0.32-p.p.m. downfield shifts of the H-1(b) resonance in disaccharides b→c (**19**, **20**) or d→b (**22**) relative to the shift for the monosaccharide glycoside **29**. Both effects are compounded in trisaccharide d→b→c (**13**), where the signal of H-1(b) is deshielded by 0.45 p.p.m. relative to that of the same compound **29**. Again no short proton–oxygen distances were predicted by the calculations, but it is notable that several short proton–proton distances were identified. Thus, H-1(b) is situated at distances of 2.3, 2.5, and 2.6 Å from H-4(c), -6(c), and -5(d), respectively. The deshielding of H-1(b) was attributed to steric compression resulting from the concentration of protons in a compact, local area of the oligosaccharide surface. Support for this interpretation was found in the work of Winstein *et al.*[26], who showed that compression of proton, Van der Waals radii at the bridge head of fused-ring systems causes strong proton deshielding. Deshielding of similar origin was observed in the case of **9** where the signal of H-2(b) is shifted by 0.2 p.p.m. relative to the same resonance in **8**. Substitution of the D sugar (tyvelose) of **8** by the L enantiomer (ascarylose) (**9**) effectively interchanges the atoms of the 3,6 dideoxyhexose unit through a mirror plane defined by the atoms O-3(b), C-3(b), and H-3(b). Thus, H-1(a) and -2(a) are close to H-2(b), the signal of which was shifted downfield by 0.17 p.p.m. relative to the signal of H-2(b) in **8**, owing to the densely packed cluster of hydrogen atoms, H-1(a), -2(a), -6(c), and -1(d) which occur in its vicinity. Other downfield shifts associated with the proximity of oxygen atoms and protons included those due to H-5(b) and O-3(c) (2.4 Å) which occurred for all compounds containing the disaccharide sequence b→c, *i.e.*, **1–9**, **13**, **14**, **19**, and **20**. Their ^{1}H-n.m.r. spectra exhibited a 0.3–0.4-p.p.m. deshielding of the H-5(b) resonance relative to the corresponding chemical shift in the appropriate glycosides **28** or **29**. It is necessary to compare the shifts for *p*-nitrophenyl glycosides with those of other *p*-nitrophenyl glycosides because this aglycon has a significant influence both upon the shift values of the sugar ring atoms to which it is glycosidically linked and also upon some resonances of the neighboring ring atoms. ^{1}H-N.m.r. spectra of **1–8**, **14**, and **21**, which contain the disaccharide sequence c→d, exhibited a 0.3-p.p.m. deshielding of the H-1(c) resonance

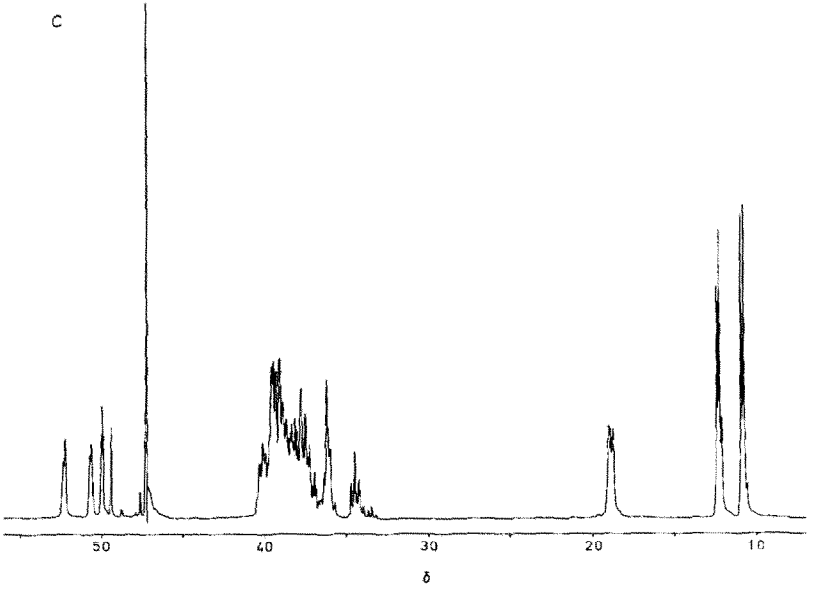
relative to the signal of the appropriate glycosides **30** or **31**, and this observation is consistent with the distance of H-1(c) to O-2(d) predicted by calculations. Finally, the signal of H-1(d), which is positioned at 2.5 Å from O-3(b) in **4–13** and **22**, showed a 0.2-p.p.m. deshielding relative to that of H-1(d) in **26** or **27**. Short distances between ring oxygen atoms and protons consistently failed to provide significant, specific deshielding of the corresponding proton resonances. Thus, despite such distances as H-2(b) to O-5(a) (2.6 Å), H-3(b) to O-5(a) (2.5 Å), or H-4(c) to O-5(b) (2.89 Å), the deshielding observed fell in the range of 0.1–0.15 p.p.m., a value that is considered to be below the resolving power of the HSEA and n.m.r. techniques to be adequately and consistently interpret[27]. A possible explanation is the reduced electronegativity of an acetal oxygen atom when compared to the oxygen atom of hydroxyl groups[28].

Examination of the proton spectra obtained for the mono- to penta-saccharides resulting from phage-mediated hydrolysis of the bacterial polysaccharide showed that, when consideration was made for the presence of a reducing residue in **6** and **7**, the chemical-shift values are in excellent agreement with those of the model compounds **2, 6,** and **11**. The proton shifts for the tetra- and octa-saccharides are recorded in Table I, and the proton spectra of **6** and **7** may be visually compared with those of the dodeca-, hexadeca-, and icosa-saccharides in Fig. 1.

Assignment of ^{13}C chemical shifts was achieved by comparison of model compounds **1–31**, as described in related work[6] and a review article[25]. Proton-coupled, ^{13}C spectra and selective proton-irradiation were the principal methods for confirming the assignments (Table II). Heteronuclear-shift-correlated 2D spectra, in a few instances, confirmed the validity and self-consistency of assignments




B
50
40
30
20
10
δ


C
50
40
30
20
10
δ

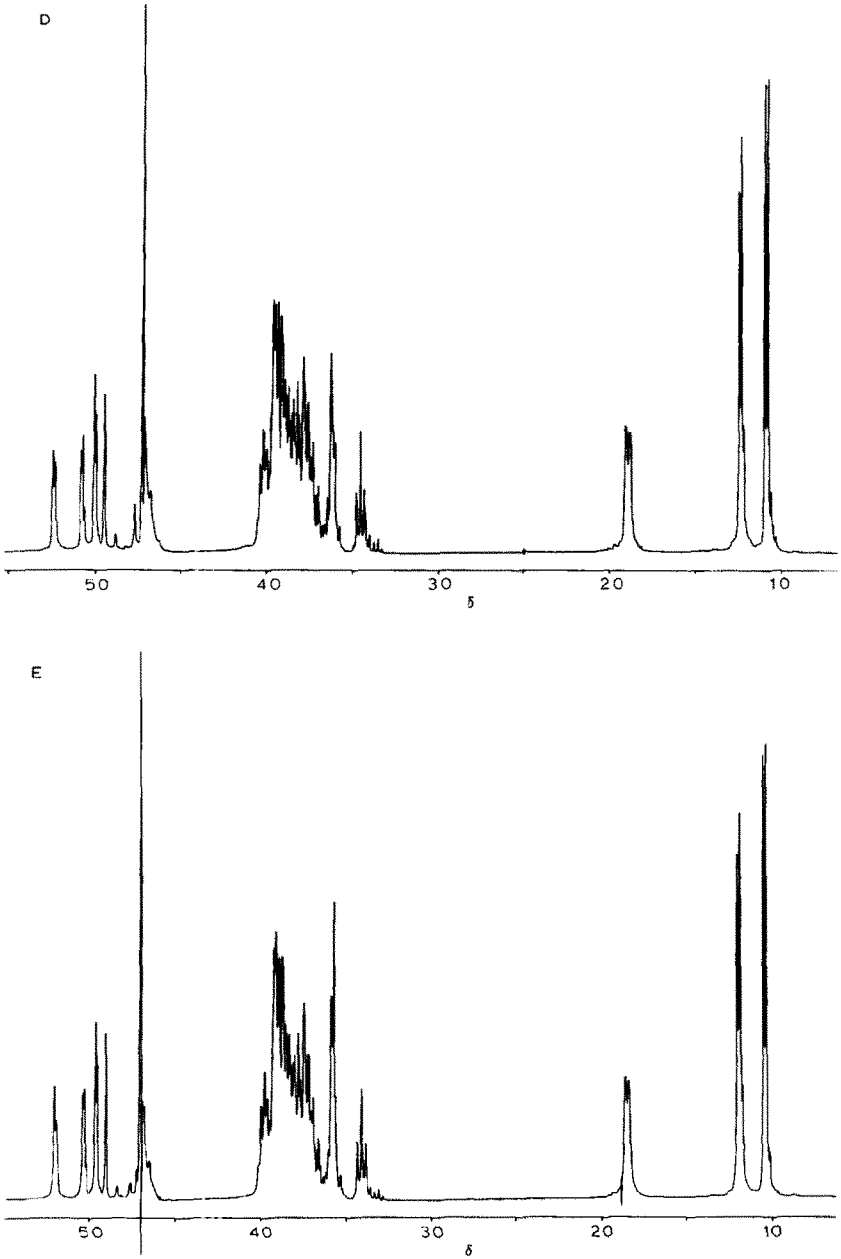


Fig. 1. (A) The 400-MHz ^{1}H-n.m.r. spectrum of the reducing tetrasaccharide d→(a→)b→c obtained by phage-associated enzyme hydrolysis of the serogroup B O-antigen. (B) The 400-MHz ^{1}H-n.m.r. spectrum of the octasaccharide, [d→(a→)b→c]$_2$, obtained in similar fashion to the tetrasaccharide. (C) ^{1}H-N.m.r. spectrum of the dodecasaccharide [d→(a→)b→c]$_3$. (D) ^{1}H-N.m.r. spectrum of the hexadecasaccharide. (E) ^{1}H-N.m.r. spectrum of the icosasaccharide showing the identical spectral features found for one to five repeating units.

for certain key structures. These assignments were readily extrapolated to provide complete identification of the phage-derived oligosaccharides (Table III). Relative-intensity differences allowed easy identification of the signals due to the terminal, reducing residues. The ^{13}C-chemical shifts displayed no unusual values with the exception of those of compound **9**, the ascarylose-containing tetrasaccharide d→(a→)b→c. Although HSEA calculations indicated that the a→b linkage would possess ϕ,ψ torsional angles within the normal range for compound **9** (50°, 35°), ^{13}C chemical shifts for C-1(a), -2(a), -4(a), and -5(a) of the ascarylose residue and also for C-2(b) of the D-mannose residue exhibited shift variations of 1.7–5.5 p.p.m. When compared to proton data, ^{13}C-chemical shifts are relatively insensitive to conformational changes, and the observed shieldings for compound **9** reflects the crowding about the anomeric center of the ascarylose residue and H-2(b) of the D-mannose residue, which was also demonstrated by the n.O.e. data. The very large shielding of C-1(a) (~5.5 p.p.m.) most likely finds its origin in small valence or bond-angle deformations which result from the proton crowding previously discussed. Such changes, which are accompanied by different hybridization of the ^{13}C-nucleus, are known to have a pronounced effect on ^{13}C-chemical shifts[29]. A similar change in ^{13}C-chemical shift has been observed when methyl α-D-mannopyranoside was glycosylated at O-3 with abequose (the D enantiomer) and colitose (the L enantiomer), respectively[23]. It is notable that no significant changes of ^{13}C-chemical shift were observed for either the L-rhamnose or the D-galactose residues of **9** relative to those of **8**.

The correlation of chemical-shift data with internuclear distances supports the conformation predicted by HSEA calculations; however, n.O.e. measurements provide the strongest evidence in support of such preferred conformers. The treatment of n.O.e. data in terms of the minimum-energy conformation has been described previously[3,5]. In brief, the magnitude of the n.O.e. depends upon the inverse sixth power of interproton distances, and these distances are, of course, available from the HSEA calculations. Summing the effects of these various H–H contributions, the anticipated relative n.O.e. values were calculated for the minimum-energy conformer by use of Equation *1*,

$$\text{n.O.e.}_d(s) = r_{ds}^{-6}/(2 \sum_{j \neq d} r_{dj}^{-6}) \qquad (1)$$

where the n.O.e. value refers to that observed on proton d when proton s is saturated. r_{ds} is the distance between protons d and s, and r_{dj} is the distance between proton d and other protons in the molecule.

These calculated values were compared (Table IV) with the observed n.O.e. values for model compounds **1–31** and the oligomeric structures. Experimentally, the n.O.e. values were determined in the difference mode, and it may be seen that the observed and calculated relative n.O.e. values are in good agreement (Table IV).

It is expected that, for glycosidic bonds whose conformation is dominated by

TABLE IV

N O E DATA FOR O-SPECIFIC ANTIGENS OF *Salmonella* SEROGROUPS A, B, AND D_1[a]

Comp	Proton saturated	1(a)			1(b)			2(b)			1(c)		6(c)[b]							1(d)				
	Proton observed	2(a)	3(b)	2(b)	2(b)	4(c)	5(d)	1(a)	1(b)	1(d)	2(c)	3(d)	1(b)	2(b)[c]	4(c)	5(c)	3(d)	4(d)	5(d)	1(a)	2(a)	5(a)	2(b)	2(d)
1		53(14)	47(13)		28(6)	72(14)					50(9)	50(9)												
		50	50	0	24	76	0				54	46												
2		55(20)	45(16)		37(8)	63(14)					50(12)	50(12)												
		59	41	0	23	77	0				54	46												
3		39(5)	61(9)		29(4)	71(9)					50(10)	50(10)												
		48	52	0	23	77	0				54	46												
4[d]		45(3)	55(4)		24(2)	54(4)	21(1)						9	6	23	59			4			26(3)	27(3)	46(5)
		50	50	0	15	62	33													0	0	21	19	60
5		55(13)	45(11)		18(9)	54(26)	28(14)						13	6	25	41	5		11			19(6)	27(9)	54(18)
		59	41	0	15	62	23													0	0	20	18	62
7[d]			100(37)		22(9)	48(20)	30(12)				53(20)	47(18)	15	14	17	31			7			9(6)	34(23)	57(38)
			100	0	15	62	23				54	46								0	0	20	18	62
8[d]		48(2)	52(2)		12(1)	56(3)	31(2)						12	6	28	46			7			13(1)	30(2)	57(4)
		48	52	0	15	62	23													0	0	20	18	62
9		33(2)	41(2)	26(1)	17(6)	48(16)	35(12)	40(10)	22(7)	38(9)			10	8	20	35	6	16	4	16(5)	16(5)		27(7)	41(11)
		31	29	38	15	62	23	47	18	35										14	19	0	17	50
10		49(16)	51(17)		41(5)		59(7)															20(5)	34(9)	46(12)
		50	50	0	43		57													0	0	21	19	60
11[d,e]		64(6)	36(4)		40(2)		60(3)															24(2)	34(2)	38(3)
		59	41	0	43		57													0	0	20	18	62
12		43(8)	57(10)		48(8)		52(8)															29(10)	34(12)	37(13)
		48	52	0	37		63													0	0	21	19	60
13					20(5)	60(16)	20(5)						12	8	18	43	19						53(14)	47(13)
					21	58	21																72	28
14					32(7)		68(15)					100(19)												
					32		68					100												
15		50(16)	50(16)																					
		49	51																					
16		57(15)	43(12)																					
		58	42																					
17		53(18)	47(16)																					
		58	42																					
18		39(10)	61(16)																					
		48	52																					
20					41(8)	59(12)																		
					38	62																		
21											50(13)	50(13)												
											53	47												
22																							56(10)	44(8)
																							59	41

[a]For each compound, relative observed n O e (%) for each saturation (upper line), experimental n.O.e. (%) (in parentheses, upper line), and relative calculated n O.e (%) by use of Equation *1* (lower line) [b]Calculation of relative n O e.'s was prohibited owing to rotation of the methyl group. [c]Overlap of signals prohibited distinction of contributions from H-2(b) and -4(d), except in the case of compound **9** where the contribution to H-4(d) amounted to 16% [d]Obtained at 400 MHz, all other values recorded at 270 MHz [e]Simultaneous irradiation of H-3*a*(a) and -3*e*(a) gave considerable enhancement of anomeric H-1(d) in addition to the enhancements within the a unit.

the *exo*-anomeric effect, the anomeric proton will be in close contact with the proton linked to the aglycon carbon atom[30]. Consequently, a strong n.O.e. would be anticipated between these two protons spanning the glycosidic bond. This has been demonstrated so often[3–8] that it is now regarded as common knowledge. Indeed, the effect is sufficiently general that it may be used to sequence oligosaccharides[31,32]. Thus, irradiation of each anomeric resonance H-1(a)–(d) resulted in significant n.O.e.'s for H-3(b), -4(c), -3(d), and -2(b) (Table IV, compounds **1–22**). Simultaneously, a n.O.e. was observed for H-2 of the ring whose anomeric proton was irradiated. This follows as this H-2 and the aglycon proton are at approximately equal distances from the anomeric hydrogen atom. The relative magnitudes of the n.O.e. are, however, not always equal (Table IV) because H-2 is enhanced in a manner dependent upon the orientation of neighboring protons (Equation *1*), and this differs between hexopyranosides. In addition to these expected n.O.e.'s, others were observed that served to unambiguously define the relative disposition of the sugar rings. Thus, irradiation of H-1(d) (of the D-galactose unit) established the proximity of H-1(d) to H-5(a) (2.25 Å), thereby unequivocally identifying the disposition of the 3,6-dideoxyhexose ring in conjunction with the H-1(a)–H-3(b) distance of 2.42 Å, which was verified by the just mentioned n.O.e. Dispositions of hydrogen atoms about the glycosidic linkage d→b were further assisted by the n.O.e.'s observed on saturation of the H-1(b) resonance. In this case, H-5(d) showed an n.O.e. consistent with its 2.36-Å spacing from H-1(b) (compound **5** or **6**). Irradiation of H-6(c) for the same compounds and also compound **13** showed that H-1(b), -2(b), -4(c), -5(c), and -5(d) receive comparable and significant n.O.e.'s. As previously stated in the section on ^{1}H-chemical-shift differences, H-1(b) is situated between 2.3–2.6 Å from each of the protons H-4(c) and -6(c). However, H-6(c) is also situated ~3.0 Å from H-5(d), but the latter proton is not the only one of the D-galactose (d)-unit to receive a n.O.e. when H-6(c) is saturated and the spectrum recorded at 270 MHz. Protons H-3(d) and -4(d), which are both positioned at least 5.5 Å from H-6(c), showed variable (depending upon the compound), but consistent n.O.e.'s. These may be attributed to the almost linear alignment of H-6(c), -5(d), and -3(d), or in some instances -4(d). This arrangement is known[33] to favor relayed n.O.e.'s. The spatial proximity of H-5(d), -4(d), and -3(d) and the potentially linear arrangement on the D-galactopyranoside ring of the pairs H-5(d)–H-4(d) and H-5(d)–H-3(d) require that these protons experience strong, mutual dipole–dipole relaxation. In addition a dipole–dipole relay may be established *via* the proximity of H-5(d) to H-6(c), when the H-6(c) resonance is saturated. In this manner, the n.O.e. is distributed to H-3(d) or -4(d), or both. This effect disappeared when the experiment was conducted at 400 MHz. Inspection of the stereo plots depicting the serogroup B repeating-unit in its preferred conformation (ref. 1, Fig. 6a) provided an appreciation of the arrangement of these atoms. With the exception of the L-rhamnose linkage c→d, two inter-ring n.O.e.'s were observed for each of the glycosidic bonds a→b, b→c, and d→b. Calculations predicted only one such n.O.e. for c→d, H-1(c) to H-3(d), and this was observed

(Table IV). Finally, the fraudulent structure **9**, in which ascarylose replaces tyvelose, exhibited a significant n.O.e. that differs from those observed for **8**. Irradiation of H-1(a) of **9** resulted in a significant n.O.e. for H-2(b), in addition to H-3(b), which is a consequence of the reversed configuration of the dideoxyhexose of **9** relative to **8**. Selective saturation of H-2(b), now made possible by deshielding due to high-proton density[26] as discussed earlier, gave, in addition to the predicted n.O.e.'s for H-1(b) and -1(d), a strong n.O.e. to H-1(a), thereby confirming the related arguments based on chemical-shift differences.

CONCLUSIONS

The ^{1}H- and ^{13}C-chemical-shift data discussed herein, in conjunction with a substantial body of proton n.O.e. data, provided convincing evidence in support of the preferred conformation developed by HSEA calculations. Indeed, the number of connections established by the n.O.e.'s and specific chemical-shift differences require a very rigid arrangement of pyranose rings and glycosidic bonds with the exception of the L-rhamnose-to-D-galactose bond, which is the most flexible linkage.

That the anticipated conformation or conformers close to it are the most predominant is well demonstrated by the markedly different n.O.e.'s observed for **9**. The substitution of an L- for a D-3,6-dideoxyhexose alters the conformation about a single linkage a→b, but also dramatically changes the pattern of observable n.O.e.'s. In connection with this, it should be noted that the possible conformation for the D-mannose-to-L-rhamnose (b→c) linkage discussed by Lipkind and Kochetkov[34] is specifically excluded as a significantly populated conformer on the basis of the n.O.e.'s established by irradiation of H-6(c). If the ϕ_{bc} −13°, ψ_{bc} −170° conformer proposed by these authors were appreciably present, H-6(c) would no longer be close to H-1(b), -2(b), and H-5(d) [*cf.* Figs. 3 (ref. 1) with Figs. 2 herein], and the observed n.O.e.'s would be different.

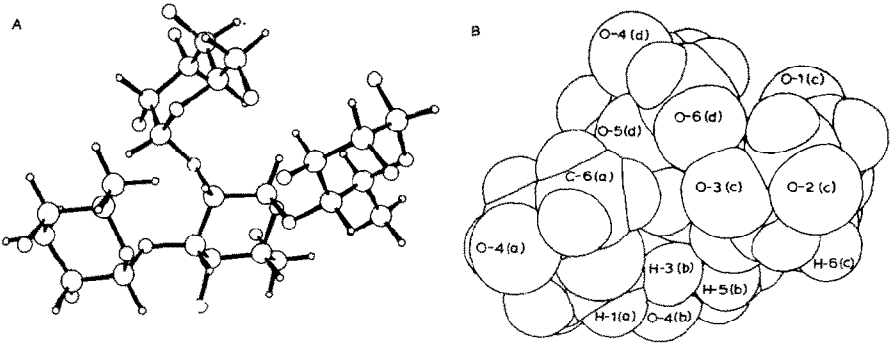


Fig. 2. The conformation of the *O*-specific tetrasaccharide d→(a→)b→c (a, Abe) of *Salmonella* serogroup B as determined by Lipkind and Kochetkov[34] with the following ϕ, ψ angles: $(\phi, \psi)_{ab}$ = −43.6, 6.0°; $(\phi, \psi)_{bc}$ = −12.8, −169.1°; and $(\phi, \psi)_{db}$ = −65.1, −14.5°. (A) Stick and ball model; (B) CPK model.

The conclusion reached for the monomeric repeating-unit also applies to the oligomeric structures, since all features of ^{1}H- and ^{13}C-chemical shifts of the monomers are duplicated in these structures as are the n.O.e.'s. Therefore the O-chain would, over a short range, closely correspond to the depicted model. As the chain extends beyond a relatively small number of repeating-units, the permissible amplitude for each glycosidic linkage and especially the c→d linkage would contribute to an overall flexibility consistent with the random-coil description of polysaccharide-solution characteristics[35].

This model adequately describes the immunodominance associated with 3,6-dideoxyhexose structure, and highlights potentially interesting features for further study. Thus the 6-deoxy group of the 3,6-dideoxyhexose residue is involved in an extensive hydrophobic surface that includes H-1 of the D-galactose residue, the α face of the mannose residue, and the 6-deoxy group of the L-rhamnose residue in combination with H-4 of this residue. At the same time, O-4 of abequose (Serogroup B) or O-2 of tyvelose (Serogroup D_1) are exposed on the edge of this surface. This would permit the known stereochemical dependence of serological specificity to be readily expressed.

EXPERIMENTAL

Compounds **1**–**31** and the phage-degraded oligosaccharides were available from previously reported work, as referenced in the legend to Scheme 1. ^{1}H-N.m.r. spectra were obtained at 310 K, for ~20mM solutions in D_2O samples, with a Bruker HX-270 instrument operating at 270 MHz. The use of a spectral width of 3 kHz with a data memory of 32 k gave a digital resolution of ±0.2 Hz. The pulse width used was 12 μs (90°). Acetone (0.5%) was used as internal reference (δ = 2.225). Assignments were made by use of methods previously published[25]. The nuclear-Overhauser-enhancement experiments were performed by the difference method[36] and are considered accurate to ±10%.

The assignments of compounds **1** to **12** were confirmed by spectra obtained at 400 MHz or 500 MHz with Bruker WM-400 and WM-500 instruments, respectively. The assignment of compound **7** was confirmed by a 2-D-scalar-coupled experiment performed at 500 MHz. ^{13}C-N.m.r. spectra were obtained at 310 K, for 50mM solutions in D_2O samples, with a Bruker HX-270 instrument operating at 67.89 MHz. The use of a spectral width of 10 kHz with a data memory of 32 k gave a digital resolution of ±0.6 Hz. The pulse width used was 12 μs (90°). 1,4-Dioxane (1%) was used as internal reference (δ 67.4). Assignments were made as previously published[25]. The ^{13}C-n.m.r. spectra of the phage-degraded oligosaccharides (5–10 mg) were obtained at 100 MHz with a Bruker WM-400 instrument. The assignment of compound **12** was confirmed by a 2-dimensional, proton–carbon correlated spectrum performed with a Bruker WM-500 n.m.r. instrument.

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