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STRUCTURAL STUDIES OF THE CAPSULAR POLYSACCHARIDE FROM *Streptococcus pneumoniae* TYPE 17A

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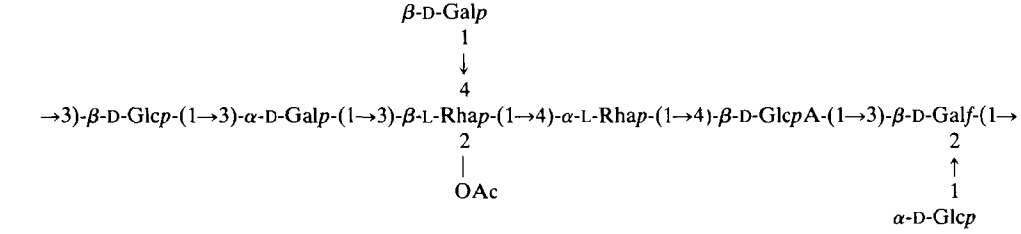
ABSTRACT

The structure of the capsular polysaccharide from *Streptococcus pneumoniae* type 17A has been investigated. Using n.m.r. spectroscopy, methylation analysis, and specific degradations as the principal methods of structural investigation, it is concluded that the polysaccharide is composed of octasaccharide repeating-units having the following structure.



INTRODUCTION

There are 82 types of *Streptococcus pneumoniae,* each elaborating its own, type-specific, capsular polysaccharide[1]. Some of the types have been combined into groups because of their antigenic relationship. Thus, group 17 consists of two types: 17F and 17A according to the agreed[2] Danish nomenclature, or 17 and 78 according to the American nomenclature. The antigenic formulas for 17F and 17A are 17a,17b and 17a,17c, respectively, and the capsular polysaccharides should contain common structural features. The structure of the 17F polysaccharide (S17F) has been determined by Perry *et al.*[3] and is of the teichoic acid type. We now report structural studies of the 17A polysaccharide (S17A).

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RESULTS AND DISCUSSION

Crude S17A was purified by chromatography on DEAE-Sepharose, giving the pure polysaccharide, $[\alpha]_{578}^{22}$ +17°. On hydrolysis with acid, S17A yielded L-rhamnose, D-galactose, and D-glucose in the relative proportions 35:34:31. The absolute configurations of the sugars were determined by the method of Leontein *et al.*[4]. Qualitative analysis also revealed that the polysaccharide contained D-glucuronic acid, the absolute configuration of which was determined by the method of Gerwig *et al.*[5].

Methylation analysis[6] of S17A gave the seven sugars listed in Table I, column A. Methylation analysis of the carboxyl-reduced[7] polysaccharide (obtained by using sodium borodeuteride) gave the same sugars and 2,3,6-tri-*O*-methyl-D-glucose, dideuterated at C-6 (Table I, column B). These results indicated that S17A is composed of octasaccharide repeating-units containing the following sugar residues: D-Glc*p*-(1→, D-Gal*p*-(1→, →3)-D-Glc*p*-(1→, →3)-D-Gal*p*-(1→, →2)-D-Gal*f*-(1→, →4)-L-Rha*p*-(1→, →3)-L-Rha*p*-(1→, →4)-D-Glc*p*A-(1→.

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3                                   4
↑                                   ↑
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It is not clear from the methylation analysis that the L-rhamnosyl residues and the D-glucosyluronic acid residue are pyranoid, but this was established from n.m.r. and other evidence discussed below.

The ^{13}C-n.m.r. spectrum of S17A (Table II) contained signals for eight

TABLE I

METHYLATION ANALYSES OF NATIVE AND MODIFIED S17A

Sugar[a]	T_1[b]	T_2[b]	*Mole %*				
			A[c]	B	C	D	E
1,2,5-Rha	0.34	0.39				19[d]	
2,3-Rha	0.92	0.93	18	15			23
2,3,4,6-Glc	1.0	1.0	19	14	16	16	18
2,3,4,6-Gal	1.19	1.14	13	11	18	22	14
2-Rha	1.37	1.42	15	16	19		
2,4,6-Glc	1.82	1.72	14	15	19	14	20
3,5,6-Gal	1.94	1.83			13[e]	13[e]	
2,4,6-Gal	2.03	1.94	14	15	15	16	21
2,3,6-Glc	2.22	1.94		8[d]			
5,6-Gal	2.50	2.48	7	6			4

[a]1,2,5-Rha = 1,2,5-tri-*O*-methyl-L-rhamnose, *etc.* [b]Retention time of the derived alditol acetate on an OV-225 column at 170° (T_1) and on an SP-1000 column at 220° (T_2), respectively, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [c]Key: *A*, Native material. *B*, Carboxyl-reduced. *C*, Uronic acid degradation 1. *D*, Uronic acid degradation 2. *E*, Location of *O*-acetyl groups, methylation performed with methyl triflate. Comparable amounts of non-methylated sugars were obtained. [d]Dideuterated at C-6. [e]Trideuteriomethylated at O-3. [f]Monodeuterated at C-1, trideuteriomethylated at O-1 and O-5.

TABLE II

N M R DATA FOR S17A: ALL SPECTRA WERE RECORDED AT 85°

	^1H				^{13}C		
	δ (p.p.m.)	$J_{1,2}$ (Hz)	Integral	Assignment	δ (p.p.m.)	$^1J_{C,H}$ (Hz)	Assignment
S17A native	5.67	*[a]	1 H	H-2 of →3)-β-Rha-(4↑	174.7		C=O of Uronic acid and of acetate
	5.46	*	1 H	→3)-β-Gal*f*-(2↑	108.4	175	→3)-β-Gal*f*-(2↑
	5.12	4	2 H	α-Glc-, →3)-α-Gal-	105.5	161	→3)-β-Glc-[c]
	4.90	*	1 H	→4)-α-Rha-	104.5	164	β-Gal-[c]
	4.78	*	1 H	→3)-β-Rha-(4↑	103.7	161	→4)-β-GlcA-[c]
	4.65	8	1 H	→3)-β-Glc-[b]	101.9	171	→4)-α-Rha-
	4.63	8	1 H	β-Gal-[b]	100.6	160	→3)-β-Rha-(4↑
	4.55	8	1 H	→4)-β-GlcA-[b]	99.9	171	α-Glc-
	2.13	s	3 H	CH_3 of OAc	94.8	171	→4)-α-Gal-
	1.37	6	3 H	CH_3 of Rha	64.9		C-6 of β-Gal*f*
	1.19	6	3 H	CH_3 of Rha	22.0		CH_3 of OAc
					19.0		C-6 of Rha
	Minor peaks						
	5.27				18.2		C-6 of Rha
	4.80						
	4.71						

[a] *Not resolved. [b,c]These assignments may be reversed.

anomeric carbons, two methyl groups of L-rhamnosyl residues, one methyl group of an acetic acid residue, and carboxyl groups. The $^1J_{C,H}$ coupling constants for the eight anomeric carbons were determined from the 100-MHz spectrum. From the coupling constants[8] and chemical shifts, it was concluded that the polysaccharide is composed of octasaccharide repeating-units containing one β-D-galactofuranosyl residue (δ 108.4), four β-pyranosyl residues ($^1J_{C,H}$ ~160 Hz), and three α-pyranosyl residues ($^1J_{C,H}$ ~170 Hz). On deacetylation, one of the high-field signals (δ 99.9 or δ 100.6) shifted to δ 102.0 (Tables II and III). This was by far the largest shift observed, and may indicate that the *O*-acetyl group is located on C-2 of a β-L-rhamnopyranosyl residue, or one of the α-hexopyranosyl residues.

One of the signals for anomeric carbons appears at an unusually high field (δ 94.8). Similar high fields have been observed for the C-1 signal of an α-D-galactopyranosyl residue linked to O-3 of a D-galactopyranosyl residue, as in methyl 3-*O*-α-D-galactopyranosyl-β-D-galactoside[9], or to O-3 of an L-rhamnopyranosyl residue, as in the *Klebsiella* K55 capsular polysaccharide[10]. The steric situation at the

TABLE III

N.M.R. DATA[a] FOR *O*-DEACETYLATED S17A

	^1H				^{13}C	
	δ (p.p.m.)	$J_{1,2}$ (Hz)	Integral	Assignment	δ (p.p.m.)	Assignment
S17A de-*O*-Ac	5.47	+[b]	1 H	→3)-β-Gal*f* 2 ↑	108.5	→3)-β-Gal*f* 2 ↑
	5.28	4	1 H	→3)-β-Gal-	105.6	→3)-β-Glc-[d]
	5.14	4	1 H	α-Glc-	104.8	β-Gal-[d]
	4.81	*	1 H	→4)-α-Rha-	104.0	→4)-β-GlcA-[d]
	4.72	*	1 H	→3)-β-Rha- 4 ↑	102.0 (2 C)	→3)-β-Rha-, →4)-α-Rha- 4 ↑
	4.66	8	2 H	→3)-β-Glc-[c], β-Gal-[c]	100.2	α-Glc-
	4.56	8	1 H	→4)-β-GlcA-[c]	95.0	→3)-α-Gal-
	1.35	6	3 H	CH_3 of Rha	65.1	C-6 of β-Gal*f*-
	1.23	6	3 H	CH_3 of Rha	19.2	C-6 of Rha
					18.5	C-6 of Rha

[a]All spectra were recorded at 85°. [b,c]Not resolved. [d]These assignments may be reversed.

glycosylated carbon and its neighbours on both sides is the same in these examples. It is possible that a similar situation prevails in S17A.

The ^{1}H-n.m.r. spectrum of deacetylated S17A at 500 MHz (Table III and Fig. 1) showed, *inter alia*, eight signals for anomeric protons and two signals for methyl groups of L-rhamnosyl residues. Three of the signals for anomeric protons showed coupling constants of 8 Hz, indicating that they derived from pyranosides having the *β-gluco* or *β-galacto* configuration. Thus, one of the four β-glycopyranosyl residues demonstrated by ^{13}C-n.m.r. spectroscopy is a β-L-rhamnopyranosyl residue, and the other L-rhamnopyranosyl residue is consequently α-linked. The H-1 signal for the α-linked rhamnose appears at an unusually high field (δ 4.81). Normally, this signal for α-rhamnosides appears at $\delta \geq 5.0$, but exceptions have been observed.

In the ^{1}H-n.m.r. spectrum of native S17A at 500 MHz (Table II and Fig. 2), nine signals in the region for anomeric protons were observed, one of which should be given by the methine hydrogen on the acetoxylated carbon-atom. This signal appeared at δ 5.67 and the coupling constants were low, demonstrating that the proton is equatorial and the *O*-acetyl group consequently axial. This, in conjunction with results discussed above, indicates that the acetyl group is linked to O-2 of the β-L-rhamnopyranosyl residue. In the spectrum of methyl 2-*O*-acetyl-β-D-mannopyranoside[11], the signal for H-2 appears at δ 5.41 ($J_{1,2}$ 1.5, $J_{2,3}$ 3 Hz). In addition to the major signals, minor signals coinciding with those in the ^{1}H-n.m.r. spectrum of the deacetylated polysaccharide were observed, indicating that some of the repeating units in S17A do not contain *O*-acetyl groups. The signals for three of the anomeric protons shifted significantly upon acetylation, which may indicate that

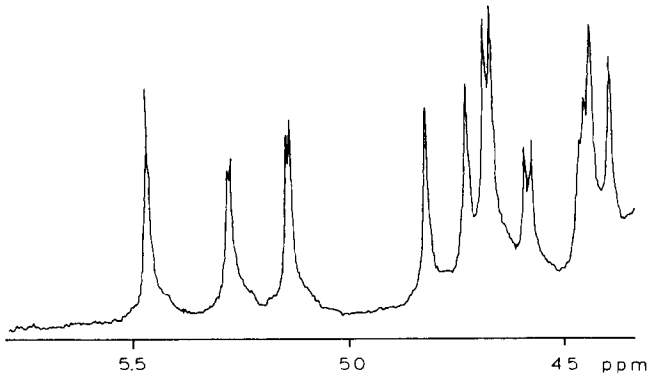


Fig. 1. ^{1}H-N.m.r. spectrum of *O*-deacetylated S17A (anomeric region).

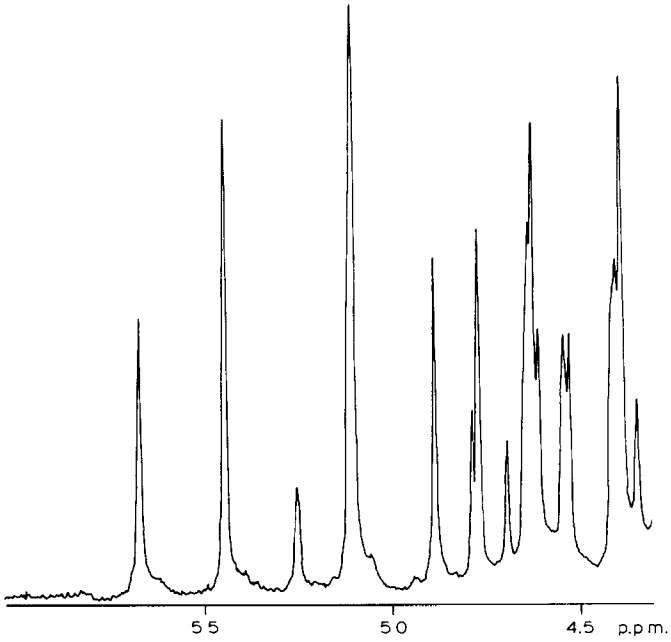


Fig. 2. ^{1}H-N.m.r. spectrum of native S17A (anomeric region).

the *O*-acetyl group is located on the branching residue and that conformational changes occur on introduction of this group.

In order to determine the sequence and the anomeric configurations of the sugar residues in S17A, it was subjected to different specific-degradations. Partial hydrolysis of deacetylated S17A with acid yielded an aldobiouronic acid, composed of D-glucuronic acid and D-galactose. The derived alditol had $[\alpha]^{22}_{578}$ +7°. Methylation, carboxyl-reduction (lithium borohydride), and hydrolysis yielded a mixture of 2,3,4-tri-*O*-methyl-D-glucose and 1,2,4,5,6-penta-*O*-methyl-D-galactitol (Table IV, column B). In the n.m.r. spectra of the alditol (Table V), the signals for the

TABLE IV

METHYLATION ANALYSES OF OLIGOSACCHARIDES OBTAINED ON DEGRADATION OF S17A

Sugar[a]	T_1[b]	T_2[b]	*Mole %*[c]				
			A[c]	B	C	D	E
1,2,5-Rha	0.34	0.39	22				
1,2,4,5,6-Gal	0.43	0.47		39			
1,4,5,6-Gal	0.82	0.91		4	23		
2,5-Ara	0.84	0.91				18	
2,3,4,6-Glc	1.00	1.00	25	9	45	5	42
2,4-Rha	1.10	1.00				27	28
2,3,4,6-Gal	1.19	1.14	28				
2,4,6-Glc	1.82	1.72				24	
2,4,6-Gal	2.03	1.94	25			26	30
2,3,4-Glc	2.22	2.00		48	32		

[a] 1,2,5-Rha = 1,2,5-tri-*O*-methyl-L-rhamnose, *etc.* [b] Retention time of the derived alditol acetate on an OV-225 column at 170° (T_1) and on an SP-1000 column at 220° (T_2), respectively, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [c] Key: Reducing oligosaccharides were transformed into alditols, and when relevant, carboxyl reduced after methylation. A, Neutral tetrasaccharide (**4**) from partial, acid hydrolysis. B, Acidic disaccharide (**1**) from partial, acid hydrolysis. C, Acidic trisaccharide (**2**) from partial, acid hydrolysis. D and E, Oligosaccharides obtained on Smith degradation (see text).

TABLE V

NMR DATA FOR OLIGOSACCHARIDES OBTAINED AFTER PARTIAL, ACID HYDROLYSIS OF S17A

	^1H[a] δ (*p.p.m.*)	$J_{1,2}$ (*Hz*)	*Integral*	*Assignment*	^{13}C[a] δ (*p.p.m.*)	Assignment
1	4.55	8	1 H	β-GlcA-	103.7	β-GlcA-
2	5.19	4	1 H	α-Glc-	103.6	β-GlcA-
	4.58	8	1 H	β-GlcA-	99.6	α-Glc-
4	5.25	3	1 H	→3)-α-Gal-	105.3	β-Glc-[b]
	4.68	8	1 H	β-Glc-[b]	104.9	β-Gal-[b]
	4.58	8	1 H	β-Gal-[b]	100.7	→3)-α-Gal-
	1.36	6	3 H	CH_3 of Rha	20.3	C-6 of Rha

[a] ^{13}C spectra were recorded at ambient temperature, ^1H spectra were recorded at 70°. [b] These assignments may be reversed.

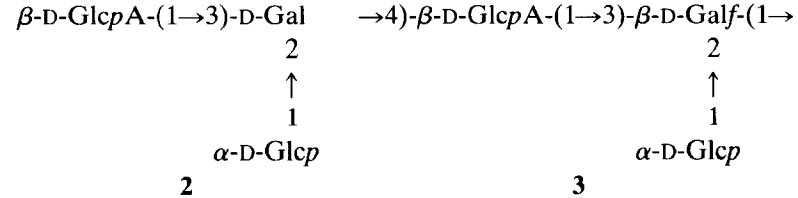
anomeric proton and carbon appeared at δ 4.55 ($J_{1,2}$ 8 Hz) and 103.7, respectively, indicating a β-pyranosidic linkage. The aldobiouronic acid consequently has structure **1**.

β-D-Glc*p*A-(1→3)-D-Gal

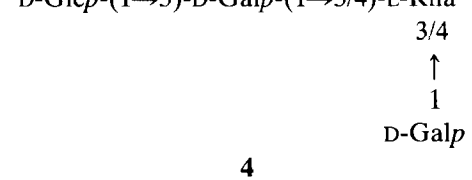
1

Acid hydrolysis under milder conditions yielded a mixture of oligosaccharides which, after reduction with sodium borohydride, was fractionated on

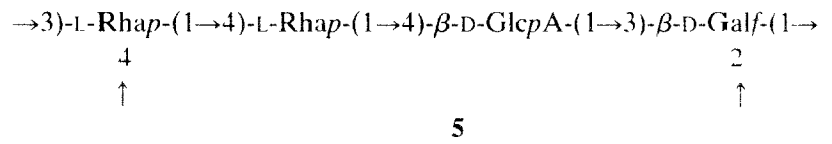
Sephadex G-15. Two major compounds, a neutral and an acidic oligosaccharide, were obtained. The latter, on methylation analysis with carboxyl-reduction of the methylated product, gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose, and 1,4,5,6-tetra-*O*-methyl-D-galactitol (Table IV, column C). In the ^{1}H-n.m.r. spectrum of the acidic alditol (Table V), signals for two anomeric protons appeared at δ 5.19 ($J_{1,2}$ 4 Hz) and 4.58 ($J_{1,2}$ 8 Hz), demonstrating the presence of an α- and a β-D-glycopyranosyl residue. In agreement with this finding, signals at δ 99.6 and 103.6 were observed in the ^{13}C-n.m.r. spectrum. As the aldobiouronic acid **1** is an integral part of the aldotriouronic acid, the latter consequently has structure **2**. This result, in conjunction with the n.m.r. evidence and the methylation analysis of S17A, demonstrates the presence of the structural element **3**. In principle, the D-glucopyranosyl group in **3** could also have been derived from the 3-linked D-glucopyranosyl residue which, however, is accounted for in the neutral oligosaccharide.



On methylation analysis, the neutral oligosaccharide-alditol, $[\alpha]_{578}^{22}$ +13°, yielded comparable amounts of 1,2,5-tri-*O*-methyl-L-rhamnitol, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,4,6-tri-*O*-methyl-D-galactose (Table IV, column A). N.m.r. evidence (Table V) indicates that the tetrasaccharide-alditol contains one α- and two β-glycopyranosyl residues. It is not possible from these results to assign an unequivocal structure to this oligosaccharide. However, it is clear that it contains the branching L-rhamnopyranosyl residue, the 3-linked D-glucopyranosyl and D-galactopyranosyl residues, and the terminal D-galactopyranosyl group of S17A; **4** is one possible structure, and another involves insertion of the 3-linked D-galactopyranosyl residue between the L-rhamnosyl residue and the D-galactopyranosyl group. The only residue in the octasaccharide repeating-unit that is not accounted for in oligosaccharides **2** and **4** is the 4-linked L-rhamnopyranosyl residue, which therefore should be interposed between these residues.



Methylated S17A was subjected to a uronic acid degradation[12] by treatment with sodium methylsulfinylmethanide in dimethyl sulfoxide and the product was trideuteriomethylated. Hydrolysis yielded a mixture of methylated sugars (Table I, column C). The 2,3-di-*O*-methyl-L-rhamnose obtained in the methylation analysis of native S17A (Table I, column A) had disappeared and the 5,6-di-*O*-methyl-D-galactose was replaced by 5,6-di-*O*-methyl-3-*O*-trideuteriomethyl-D-galactose. When hydrolysis with acid under mild conditions and reduction with sodium borodeuteride preceded the trideuteriomethylation (Table I, column D), the 2-*O*-methyl-L-rhamnose in the former analysis was replaced by 2-*O*-methyl-1,5-di-*O*-trideuteriomethyl-L-rhamnitol-*1-d*. The inference of these results (Scheme 1) is that the 3-position of the β-D-galactofuranosyl residue and the 1-position of the 4-linked L-rhamnopyranosyl residue were released on treatment of methylated S17A with base. The reducing L-rhamnopyranose residue formed was further degraded by β-elimination, but was still linked to the adjacent L-rhamnopyranosyl residue. On treatment with acid, however, this modified sugar residue was split off and the branching L-rhamnopyranosyl residue became a reducing L-rhamnopyranose residue. The results therefore demonstrate the presence of the structural element **5** in S17A.



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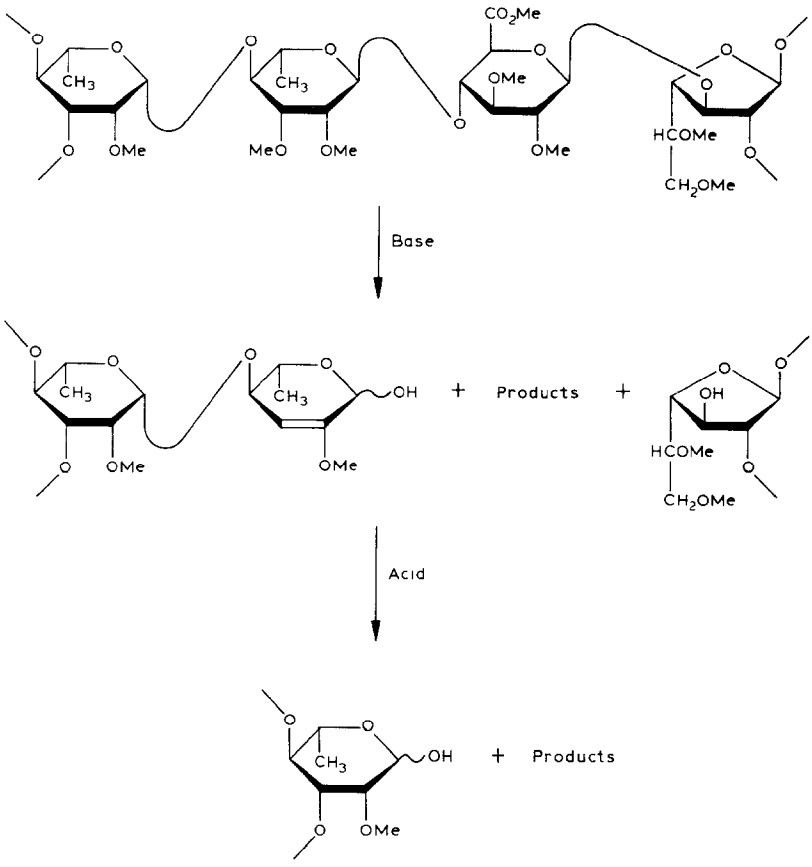
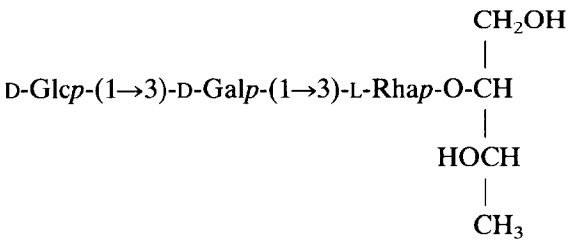
The structural information gained so far could not be combined to give a unique structure, and S17A was therefore subjected to a Smith degradation[13] (periodate oxidation, reduction with sodium borohydride, carboxyl-reduction of the "polyalcohol", and treatment with acid under mild conditions[14], during which the acetalic linkages of the modified residues but as few as possible of the glycosidic linkages, including the furanosidic linkages, should be cleaved). Two glycosides were isolated after chromatography on Sephadex G-15. The smaller of these, from the results of the methylation analysis (Table IV, column E), contained a D-glucopyranosyl group and one residue each of L-rhamnopyranose and D-galactopyranose, both linked through O-3. The ^{1}H-n.m.r. spectrum (Table VI) showed the presence of one α-linked and one β-linked hexopyranosyl residue and, presumably, one β-linked L-rhamnopyranosyl residue. Signals for two methyl groups were observed, one belonging to the L-rhamnopyranosyl residue and the other, consequently, to the "aglycon". The latter should be 4-deoxy-L-erythritol, derived from the 4-substituted L-rhamnopyranosyl residue. According to the results of the uronic acid degradation, the rhamnosyl residues are adjacent and therefore the glycoside must have structure **6**, indicating the presence of structure element **7** in S17A. In this structure, the terminal D-galactopyranosyl group must be linked directly to the L-rhamnopyranosyl residue, as all the seven other sugar residues are accounted for in **2** and **6**.



Scheme 1. Uronic acid degradation of S17A.



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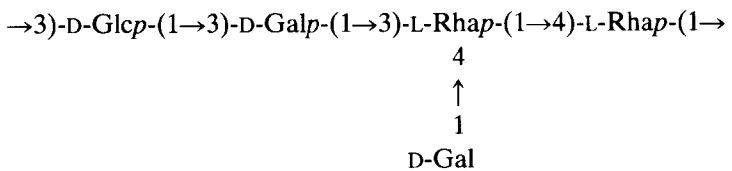

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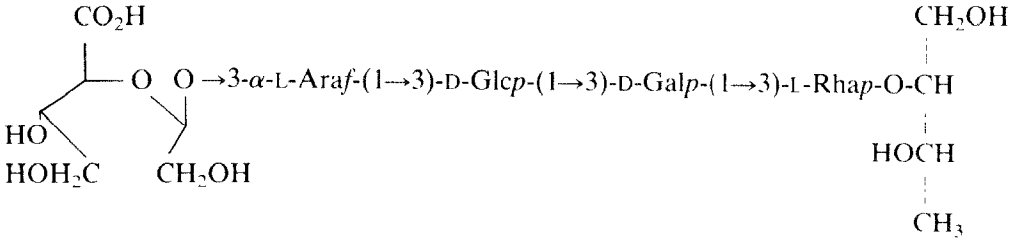
TABLE VI

N.M.R. DATA FOR OLIGOSACCHARIDES ISOLATED ON SMITH DEGRADATION OF S17A

	^1H[a]				^{13}C[a]	
	δ (p.p.m.)	$J_{1,2}$ (Hz)	Integral	Assignment	δ (p.p.m.)	Assignment
8	5.37	*[b]	1 H	→3)-α-Ara-	109.4	→3)-α-Ara-
	5.18	3	1 H	→3)-α-Gal-	104.8	→3)-β-Glc-
	4.76	*	1 H	→3)-β-Rha-	103.3	β-GlcA-
	4.70	**	1 H	β-GlcA-	100.9	→3)-β-Rha-
	4.61	8	1 H	→3)-β-Glc-	96.3	→3)-α-Gal-
	1.34	6	3 H	CH_3 of 4-deoxy-L-erythritol[d]	18.4	C-4 of 4-deoxy-L-erythritol[c]
					18.0	C-6 of Rha[c]
	1.26	6	3 H	CH_3 of Rha[d]		
6	5.18	3	1 H	→3)-α-Gal-		
	4.77	*	1 H	→3)-β-Rha-		
	4.72	8	1 H	β-Glc-		Not determined
	1.34	6	3 H	CH_3 of 4-deoxy-L-erythritol[f]		
	1.27	6	3 H	CH_3 of Rha[f]		

[a] ^{13}C spectra were recorded at ambient temperature; ^1H spectra were recorded at 70°. [b] *Not resolved **, Triplet, couplings obscured. [c,d,e,f] These assignments may be reversed.

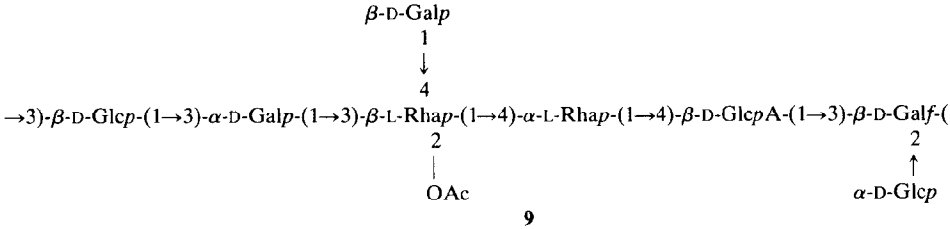
Methylation analysis of the larger glycoside showed that the 2,3,4,6-tetra-*O*-methyl-D-glucose in the corresponding analysis of **6** was replaced by 2,4,6-tri-*O*-methyl-D-glucose. 2,5-Di-*O*-methyl-L-arabinose (Table IV, column D), from the D-galactofuranosyl residue, was also found instead of the expected 2,3,5-tri-*O*-methyl-L-arabinose. The most reasonable explanation for this is that the carboxyl-reduction was incomplete and that the oligosaccharide contained the modified D-glucosyluronic group linked to O-3 of L-arabinose. The resistance of such residues to Smith hydrolysis has been observed[15]. In agreement with this assumption, five signals were observed in the region for anomeric protons and anomeric carbons, respectively (Table VI). The signal at δ 4.70 could be assigned to the modified D-glucosyluronic group. The oligosaccharide glycoside consequently has structure **8**.



8

The oligosaccharide glycoside **8** was acetylated, and treated with chromium trioxide in acetic acid. During this treatment, β-glycopyranosides of the component sugars should be oxidised but α-glycopyranosides should be resistant[16]. Sugar analysis of the product showed that 89% of the D-galactopyranosyl groups, but only 17 and 9% of the D-glucopyranosyl and L-rhamnopyranosyl residues, respectively, had survived. The results therefore suggest that the former residue is α-linked and the two latter β-linked. Of the L-arabinofuranosyl residues, ~70% also survived, which may be due to the preferred pseudo-axial orientation of the "aglycon"[17].

From the combined evidence, structure **9** is proposed for the octasaccharide repeating-units of S17A. The assignments of anomeric configurations to the different sugar residues and the location of the *O*-acetyl group will be commented on below.

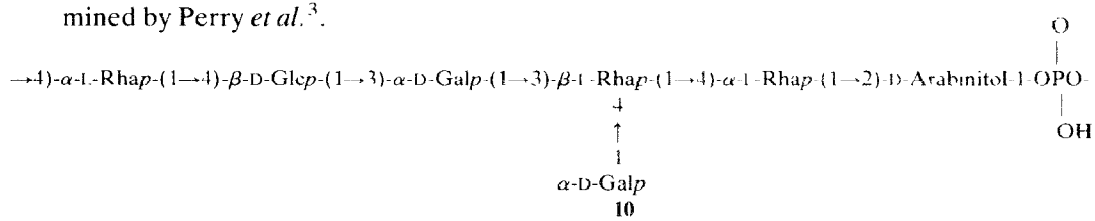


9

According to the n.m.r. evidence discussed above, S17A should contain one β-D-galactofuranosyl residue, one β-L-rhamnopyranosyl residue, and three β-pyranosyl residues having the *gluco* or *galacto* configuration, the three remaining residues being α-pyranosidic. As the terminal D-glucopyranosyl group and the 3-substituted D-galactopyranosyl residue have been shown to have the α configuration, the other pyranosyl residues having the *gluco* or *galacto* configuration are consequently β-linked. For similar reasons, the 4-substituted L-rhamnopyranosyl residue should be α-linked. This is consistent with the assignments (some of which are tentative only) of the signals in the ^{13}C- and ^{1}H-n.m.r. spectra of the degradation products (Tables V and VI).

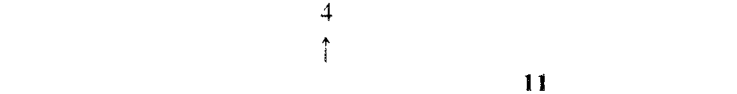
As discussed above, the *O*-acetyl group was assigned, from n.m.r. evidence, to O-2 of the β-rhamnopyranosyl residue. This was confirmed by methylation analysis of S17A, using methyl triflate and 2,6-di-*tert*-butyl-4-methylpyridine in trimethyl phosphate for the methylation[18]. *O*-Acetyl groups are stable under these conditions, but are hydrolysed during Hakomori methylation. As seen from Table I (column E), 2-*O*-methyl-L-rhamnose, present in the conventional analysis (Table I, column A), was absent, demonstrating that the *O*-acetyl group is linked to position 2. The presence of L-rhamnose in this analysis, as required from the fully substituted L-rhamnopyranosyl residue, was observed, but is less significant because part of the polysaccharide was not methylated, probably because it was not solubilised. However, no sugars resulting from a partially methylated polysaccharide were observed.

The structure of S17F, with the repeating unit **10**, has recently been determined by Perry *et al.*[3].



10

When structures of two capular antigens belonging to the same group have been determined, as for S6A and S6B[19], S9A[20] and S9V[21], and 12F[22] and 12A[23], the structures have proved to be so similar that one or two mutations may suffice for going from one of the structures to the other. The difference between S17F and S17A is far more profound, the former being of the teichoic acid type and the latter being a polysaccharide proper. The structural similarity, with element **11** common to the two antigens, may be quite accidental and may not necessarily indicate an intimate biological relationship between the two types.



11

EXPERIMENTAL

General. — Concentrations were performed under diminished pressure at bath temperatures not exceeding 40°. For g.l.c., Perkin–Elmer 990 and Hewlett-Packard 5830 A instruments, fitted with flame-ionisation detectors, were used. Separations of alditol acetates were performed on glass columns containing 3% of OV-225 on Gas Chrom Q at 190°, and of partially methylated alditol acetates on the same columns at 170° or on SP-1000 W.C.O.T. glass-capillary columns at 220°. The relative proportions of components were determined from peak areas without the use of response factors. G.l.c.–m.s. was performed on a Varian MAT 311 instrument, using the same two phases. All identifications of mass spectra were unambiguous and will not be discussed. Optical rotations were measured at 22° with a Perkin–Elmer 241 polarimeter. A differential refractometer was used for monitoring the Sephadex-column effluents. Methylations were performed according to Hakomori[6,24] with sodium methylsulfinylmethanide–methyl iodide in dimethyl sulfoxide. Methylated products were isolated by dialysis against water, followed by freeze-drying. For materials of low molecular weight, dimethyl sulfoxide was removed by freeze-drying, and the product was isolated by partition between chloroform and water. When necessary, it was further purified by chromatography on Sephadex LH-20. Absolute configurations of the neutral sugars and of the glucuronic acid were determined by the procedures of Leontein *et al.*[4] and Gerwig

et al.[5], respectively. Carboxyl-reductions were performed as described by Taylor *et al.*[7].

For n.m.r. spectroscopy, a JEOL FX-100 or a GX-400 spectrometer was used. Chemical shifts are reported in p.p.m. downfield from external tetramethylsilane (^{13}C) or internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^{1}H). Spectra were recorded at various temperatures; see Tables.

S. pneumoniae type 17A was grown and the capsular polysaccharide was isolated essentially as described earlier[25]. It was further purified by chromatography on a column (3 × 30 cm) of DEAE-Sepharose CL-6B, irrigated first with water and then with a linear gradient of aqueous sodium chloride (0 → M, 1 L). The fractions were monitored polarimetrically, and the polysaccharide was eluted at a salt concentration of 0.3–0.4M. The material was recovered by dialysis and freeze-drying. The yield of pure S17A, $[\alpha]_{578}^{22}$ +17° (*c* 1, water), was 80%.

Deacetylation and partial hydrolysis of S17A. — A solution of S17A (92 mg) in aqueous sodium hydroxide (0.1M, 25 mL) was kept at room temperature for 15 h and then neutralised with 0.1M hydrochloric acid, and the polysaccharide (85 mg), $[\alpha]_{578}^{22}$ +23° (*c* 1, water), was isolated by dialysis and freeze-drying.

A solution of deacetylated S17A (50 mg) in 0.1M trifluoroacetic acid (20 mL) was kept at 100° for 2 h and then freeze-dried. A solution of the product in water containing sodium borohydride (100 mg) was kept at room temperature overnight, excess of borohydride was decomposed by adding 50% aqueous acetic acid, the solution was concentrated, and boric acid was removed by codistillation with methanol (3 × 10 mL). The product was fractionated on a column (3 × 80 cm) of Sephadex G-15 by elution with water. Three main fractions were obtained, one of which (17 mg) was eluted in the void volume, and two in the oligosaccharide region. The latter were rechromatographed on the same column, to give the pure alditols of **4** (9 mg), $[\alpha]_{578}^{22}$ +13° (*c* 0.8, water), and **2** (4 mg).

In a separate experiment with a longer hydrolysis time (16 h), the alditol of the aldobiouronic acid **1** (7 mg), $[\alpha]_{578}^{22}$ +7° (*c* 0.7, water), was the main oligosaccharide formed. The oligosaccharide-alditols were methylated, those containing uronic acid residues were reduced with lithium borohydride in tetrahydrofuran, the products were hydrolysed, and the resulting methylated sugars analysed (Table IV, columns A, B, and C; and Table V).

Uronic acid degradation of S17A[12]. — (*a*) Methylated S17A (5 mg) was dissolved in dimethyl sulfoxide (2 mL) and, in order to remove any water present, 2,2-dimethoxypropane (0.1 mL) and a trace of *p*-toluenesulfonic acid were added. Sodium methylsulfinylmethanide (2M) in dimethyl sulfoxide (1 mL) was then added, and the mixture was agitated in an ultrasonic bath for 30 min and then kept at room temperature for 15 h. Trideuteriomethyl iodide (1 mL) was added with external cooling and the mixture was agitated in an ultrasonic bath for 30 min. Excess of trideuteriomethyl iodide was distilled off and the mixture was freeze-dried. The product was purified on a column (1.5 × 10 cm) of Sephadex LH-20 by elution with

chloroform–acetone (1:1), and hydrolysed, and the mixture of methylated sugars was analysed (Table I, column *C*).

(*b*) Methylated S17A was treated with sodium methylsulfinylmethanide as in (*a*). Aqueous acetic acid (50%, 2 mL) was added to the solution, and the product was recovered by partition between chloroform and water. The material obtained on concentration of the chloroform phase was treated with 10% aqueous acetic acid (5 mL) at 100° for 1 h. The product was recovered by freeze-drying, dissolved in 50% aqueous ethanol (5 mL), reduced with sodium borodeuteride, and methylated with trideuteriomethyl iodide essentially as described above. The analysis of the methylated sugars obtained on hydrolysis of the degraded material is given in Table I (column D).

Smith degradation of S17A[13]. — A solution of deacetylated S17A (50 mg) in 27mM sodium metaperiodate in sodium acetate buffer (0.1M, 30 mL; pH 3.9) was kept in the dark at 4° for 95 h. Excess of periodate was reduced with ethylene glycol, and the product was isolated by chromatography on a column (3 × 80 cm) of Sephadex G-15. Sodium borohydride (100 mg) was added to the aqueous solution (25 mL) of the oxidised polysaccharide and, after 15 h, the "polyalcohol" was recovered by chromatography (as described above) and freeze-drying. A small sample of the "polyalcohol" was used for sugar analysis and the major part was carboxyl-reduced (two treatments). This material was then treated[14] with 0.2M trifluoroacetic acid at room temperature for 40 h. The product was isolated by freeze-drying and fractionated by gel chromatography (as described above). One fraction (5 mg), $[\alpha]_{578}^{22}$ +12° (*c* 0.5, water), was eluted in the void volume, and two fractions in the oligosaccharide region. One of these (11 mg), $[\alpha]_{578}^{22}$ +13° (*c* 0.7, water), consisted of **8**; the other (3 mg), $[\alpha]_{578}^{22}$ +34° (*c* 0.3, water), was **6** (Table IV, columns D and E; and Table VI).

Chromium trioxide oxidation[16]. — A solution of the oligosaccharide glycoside **8** (4 mg) in a mixture of pyridine and acetic anhydride (1:1, 2 mL) was kept at room temperature for 20 h and then concentrated. The acetylated material and *myo*-inositol hexa-acetate (1 mg) were dissolved in chloroform, and part of the solution was withdrawn for sugar analysis. The main part was concentrated, the residue was dissolved in anhydrous acetic acid (300 μL), and chromium trioxide (30 mg) was added. The mixture was agitated in an ultrasonic bath at 50° for 1 h. Water (2 mL) was added, the product was extracted with chloroform (5 × 1 mL), and the extract was subjected to sugar analysis. The percentages of L-arabinose, D-galactose, D-glucose, and L-rhamnose were reduced to 68, 89, 17, and 9%, respectively, of the values obtained for the reference sample.

Location of O*-acetyl groups*[18]. — S17A (5 mg) was suspended in trimethyl phosphate (1 mL) containing 2,6-di-*tert*-butyl-4-methylpyridine (175 mg). Methyl trifluoromethanesulfonate (100 μL) was added and the mixture agitated in an ultrasonic bath for 3 h at 50°. The solution was dialysed, first against acetone and then against water, and freeze-dried, and the product was analysed (Table I, column E).

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