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STRUCTURAL DETERMINATION OF THE CAPSULAR POLYSACCHARIDE OF *Streptococcus pneumoniae* TYPE* 19A (57)**

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ABSTRACT

The structure of the *Pneumococcus* type 19A (57) capsular polysaccharide has been reinvestigated by using methylation analysis and n.m.r. spectroscopy. It is composed of residues of 2-acetamido-2-deoxy-D-mannose, D-glucose, L-rhamnose, and phosphate in the molar ratios of 1:1:1:1. The polysaccharide is linear, and is composed of these components in a repeating unit of the following structure.

$$\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{NAc-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\text{-}PO_4^-)\rightarrow$$

The type 19A polysaccharide (Na^+ salt) was depolymerized by heating it in water at 100°, conditions that also hydrolyzed the newly formed phosphoric monoesters.

INTRODUCTION

The pneumococcal polysaccharides have recently assumed importance due to their use in a multivalent vaccine against pneumococcal infections[1,2]. Because of the diversity of pneumococcal types, eighty-three serologically defined, capsular polysaccharide types having been described to date, the choice of capsular polysaccharide used in the vaccine was based on serological surveys of the most prevalent types among pneumococcal isolates[2,3]. The vaccine was further simplified by using only one type to represent a number of cross-reacting, pneumococcal strains[3]; thus, type 9N (9) was used to represent types 9A (33), 9L (49), and 9V (68); type 6A (6) to represent type 6B (26); and type 19F (19) to represent type 19A (57). Recent human studies[4] have, however, shown that the type 9N polysaccharide is an immunologically inadequate representative of the group 9 polysaccharides. Therefore

*The Danish system of nomenclature, followed by the U.S. system in parentheses, is used throughout.
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a better understanding of the structure of the pneumococcal polysaccharides could assist in the formulation of more effective and stable human vaccines.

In recent studies, a complex structure was proposed for the type 19A polysaccharide in which it had the same trisaccharide phosphate repeating unit as the type 19F polysaccharide, but with additional, extensive branches involving structurally unrelated moieties[5]. In such a highly branched structure, the common backbone would probably be too relatively inaccessible to account for the strong cross-reactivity exhibited by the types 19A and 19F polysaccharides[5]. Therefore, we reinvestigated the structure of the type 19A polysaccharide and found[5–7] that it is structurally identical to that of type 19F, except that its α-L-rhamnopyranosyl residues are linked at O-3, instead of O-2.

RESULTS AND DISCUSSION

Analyses of the type 19A polysaccharide demonstrated that it contains 2-acetamido-2-deoxy-D-mannose, D-glucose, L-rhamnose, and phosphate in the molar ratios of 1:1:1:1, indicating that it is composed of a trisaccharide phosphate repeating unit similar to that of the type 19F polysaccharide[5–7]. A more complex structure, containing galactose and fucose in addition to the aforementioned sugar components, had previously been proposed for the type 19A polysaccharide[5]; however, we could find no evidence for the presence of these additional sugars in the type 19A polysaccharide.

Hydrolysis of the type 19A polysaccharide, performed with deamination, yielded D-glucose, L-rhamnose, and phosphate in the molar ratios of 2.0:1.3:1.1, respectively, the additional D-glucose originating from the deamination of the 2-amino-2-deoxy-D-mannose residue[6]. All hydrolyses using mildly acidic conditions gave lower glucose contents, due to the acid-stability of the glycosidic linkage of the amino sugar, indicating that the 2-acetamido-2-deoxy-D-mannose was linked glycosidically to the D-glucose residue.

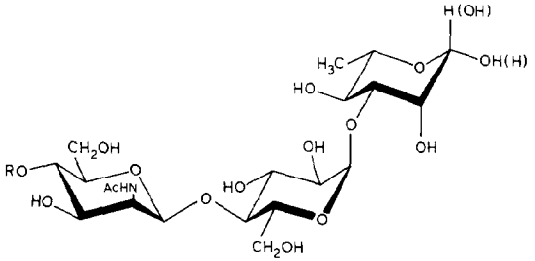
The ^{1}H-n.m.r. spectrum of the type 19A polysaccharide was consistent with the foregoing, analytical results. The spectrum exhibited three anomeric signals in the intensity ratios of 0.9:1.0:1.1, a doublet of doublets at δ 5.49 ($J_{1,2}$ 1.2, $^3J_{H1,P}$ 6.8, and $^2J_{C1,H1}$ 174 Hz), and doublets at δ 5.13 ($J_{1,2}$ 3.7, $^2J_{C1,H1}$ 170 Hz) and δ 4.98 ($J_{1,2}$ 1.2, $^2J_{C1,H1}$ 164 Hz) which were respectively assigned, by comparison with previous assignments made on the structurally similar, type 19F polysaccharide[6], to the L-rhamnose, D-glucose, and 2-acetamido-2-deoxy-D-mannose residues. A methyl (acetamido) singlet at δ 2.12 and a methyl (rhamnose) doublet at δ 1.39 ($J_{5,6}$ 8.3 Hz), in the intensity ratio of 1.0:1.0, were also detected. The intensity ratio of the former anomeric signals to that of the methyl signals was 1:2, which is consistent with the type 19A polysaccharide's being composed of a trisaccharide phosphate repeating-unit.

Treatment of the type 19A polysaccharide with 48% HF yielded oligosaccharide **1**, which contained no phosphate, and which, on hydrolysis, yielded sugar

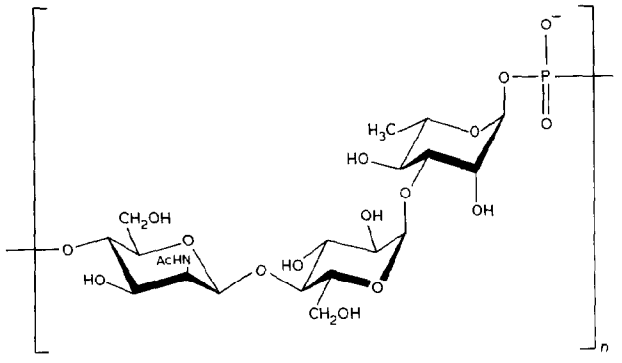
components identical to those contained in the original polysaccharide. In the methylation analysis of **1**, the individual, methylated sugars detected were 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,4-di-*O*-methyl-L-rhamnose. This result, together with the fact that no rhamnose could be detected colorimetrically following reduction of **1**, indicated that **1** had the structure depicted.

The anomeric configurations of the component sugars in **1** were assigned on the basis of ^{1}H-n.m.r. data obtained for the type 19A polysaccharide. The coupling constants of the respective, anomeric doublets of the glucopyranosyl residue at δ 5.13 ($J_{1,2}$ 3.7, $^2J_{C1,H1}$ 170 Hz), and the 2-acetamido-2-deoxymannopyranosyl group at δ 4.98 ($J_{1,2}$ 1.2, $^2J_{C1,H1}$ 164 Hz), are consistent only with their presence in the α and β configuration, respectively.

In the type 19A polysaccharide, **1**, is linked through a phosphoric diester bond between the rhamnosyl and 2-acetamido-2-deoxymannosyl residues. The position of linkage of the bond to the 2-acetamido-2-deoxy-mannosyl residues was determined by methylation of the phosphorylated oligosaccharide **2** according to the method of Fiege and Radziejeweska-Lebrecht[8]. Oligosaccharide **2** was ob-



1 R = H
2 R = PO_3^{2-}



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tained by the controlled, acid hydrolysis of the type 19A polysaccharide[6]. Following the methylation of **2**, the phosphate groups were removed with hydrogen fluoride, to generate a partially methylated oligosaccharide, which was methylated with trideuteriomethyl iodide in order to label the hydroxyl group originally involved in the phosphate linkage. In g.l.c.–m.s. studies on the hydrolyzed, methylated oligosaccharide obtained from **2**, 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-mannose labelled with deuterium (CD_3) at O-4 was identified, together with 2,3,6-tri-*O*-methyl-D-glucose and 2,4-di-*O*-methyl-L-rhamnose. Thus, O-4 of the 2-acetamido-2-deoxy-D-mannopyranosyl residue corresponds to the original linkage position of the phosphoric ester, and the structure of **2** was obtained by ^{13}C-n.m.r. spectroscopy.

The chemical shifts of some pertinent signals in the ^{13}C-n.m.r. spectra of **1**, reduced **1**, and **2**, and their assignments are listed in Table I. The anomeric signal at 100.6 p.p.m. was assigned to C-1 of the 2-acetamido-2-deoxy-D-mannopyranosyl group, because it was the only anomeric signal unaffected by structural variations in the reducing-end rhamnose residues. The anomeric signals at 94.6 and 94.8 p.p.m., in the intensity ratio of 2:3, were respectively assigned to the β-L and α-L forms of C-1″ of the reducing-end rhamnose residues[9] in **1** and **2**. These signals were replaced by a hydroxymethyl signal at 60.8 p.p.m. in the spectrum of reduced **1**.

Interestingly, the anomeric carbon atoms (C-1′) of the internal α-D-glucopyranosyl residues of **1** and **2** were also sensitive to change in the anomeric configuration of the L-rhamnopyranose residues, despite the fact that this long-range effect takes place over four bonds. This could be inferred by the fact that, in the ^{13}C-n.m.r. spectra of **1** and **2**, C-1′ gave two signals, at 96.4 and 96.7 p.p.m., having the same intensities as those of the two C-1″ signals, which were replaced by one signal, at 99.2 p.p.m., on reduction of **1**. Thus, when the orientation of the OH group on C-1″changes from axial to equatorial, it induces a 0.3-p.p.m. change in chemical shift in the signal of C-1′. This conformational effect is probably also dependent on the α-D configuration of the D-glucopyranosyl residue, because no such effect was observed in the ^{13}C-n.m.r. spectrum of the related disaccharide 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose[9].

The linkage of the phosphoric diester bond to the 2-acetamido-2-deoxy-D-mannopyranosyl residue in the type 19A polysaccharide could also be confirmed from ^{13}C-n.m.r. data. The signal at 67.9 p.p.m. in the spectrum of **1** and reduced **1** was[10] assigned to C-4. This signal did not appear in the ^{13}C-n.m.r. spectra of **2** and the type 19A polysaccharide. Although it was impossible to locate the exact position of the C-4 signal in the latter spectra, owing to signal complexity, it could be deduced, by comparison of the spectra of the dephosphorylated **1** and reduced **1** with those of **2** (phosphorylated **1**) and the type 19A polysaccharide, that it had been displaced downfield by at least +3.4 p.p.m. for the phosphorylated compounds. This evidence is consistent with the presence of a substituent at C-4 of **2** and the type 19A polysaccharide[11,12].

TABLE I

CARBON-13 CHEMICAL-SHIFTS[a] OF THE TYPE 19A PNEUMOCOCCAL POLYSACCHARIDE AND ITS CONSTITUENT OLIGOSACCHARIDES

	C-1	*C-1′*	*C-1″*	*C-4′*	*C-5*	*C-3″*	*C-3*	*C-4*	*C-2″*	*C-6*	*C-6′*	*C-2*	CH_3	CH_3 $NHCOCH_3$	$C{=}O$ $NHCOCH_3$
Type 19A polysaccharide (**3**)	100.5	96.8	97.2 (4.9)[b]	79.7	76.8 (4.9)[c]	76.6	73.4 (7.3)[c]	NA	68.4 (9.8)[c]	61.6[d]	60.9[d]	54.3	18.0	23.2	176.6
Phosphorylated trisaccharide (**2**)	100.6	96.7 96.4	94.8α 94.6β	79.8	76.9 (−)[c,e]	76.9α 78.9β	72.7 (−)[c,e]	NA	NA	61.6[d]	60.9[d]	54.2	18.1	23.2	176.4
Dephosphorylated trisaccharide (**1**)	100.5	96.7 96.4	94.8α 94.6β	79.7	77.7	76.9α 78.9β	73.2	67.9	NA	61.6[d]	60.9[d]	54.5	18.1	23.2	176.4
Reduced **1**	100.5	99.2	60.8	79.8	77.8	NA	NA	67.9	NA	61.6[d]	61.1[d]	54.5	20.2	23.2	176.7

[a]In p.p.m. from external tetramethylsilane. [b]$^2J_{^{13}C,^{31}P}$. [c]$^3J_{^{13}C,^{31}P}$. [d]Tentative assignments. [e]Not resolved.

All of this evidence is consistent with the concept that the type 19A polysaccharide is composed of repeating units of **2**, and the remaining question, as to the mode of linkage of the phosphoric diester bonds to the α-L-rhamnopyranosyl residues, was resolved from the ^{13}C- and ^{1}H-n.m.r. spectra of the type 19A polysaccharide. That the phosphoric diester bond was linked anomerically to C-1″ was deduced from the ^{13}C–^{31}P couplings[11,12] observed in the ^{13}C-n.m.r. spectrum of the type 19A polysaccharide, in which the doublets at 97.2 p.p.m. ($^{2}J_{C\text{-}1'',P}$ 4.9 Hz) and 68.4 p.p.m. ($^{2}J_{C\text{-}2'',P}$ 9.8 Hz) could be assigned to C-1″ and C-2″, respectively. This was also confirmed for the ^{1}H-n.m.r. spectrum of the type 19A polysaccharide, in which the anomeric H-1″ signal of the rhamnopyranosyl residues was a doublet of doublets due to the presence of 3-bond, ^{1}H–^{31}P coupling ($^{2}J_{H\text{-}1'',P}$ 6.7 Hz). Although $^{3}J_{H\text{-}1,H\text{-}2}$ values are relatively insensitive to the anomeric configuration of sugars having the D-*manno* configuration, the quartet at δ 5.49 ($^{3}J_{H\text{-}1'',H\text{-}2''}$ 1.2 Hz) could be assigned to an α-L-rhamnopyranosyl residue because of its low-field position in the spectrum[6,13], and this assignment was confirmed by the characteristic $J_{C\text{-}1'',H\text{-}1''}$ coupling constant (174 Hz) in the proton-coupled ^{13}C-n.m.r. spectrum of the type 19A polysaccharide. Thus, the type 19A polysaccharide has structure **3**, and differs from the type 19F polysaccharide[6] only in being linked at O-3, instead of O-2, to the α-L-rhamnopyranosyl residue.

Depolymerization of the type 19A polysaccharide (Na^+ salt) was achieved in aqueous solution at 100°, conditions previously used to depolymerize sialic acid homopolymers[14]. However, on prolonged heating, instead of the minimum unit expected, namely, the phosphorylated trisaccharide (**2**), dephosphorylated trisaccharide **1** was obtained as the major product, indicating concomitant hydrolysis of the phosphoric monoester. A rate study (see Table II) indicated that depolymerization occurred fairly rapidly at 100°, the glycosylic phosphoric diester bonds being hydrolyzed faster than the newly formed phosphoric monoesters. Depolymerization and dephosphorylation were virtually complete after 16 h, at which time the solution had pH 5.2. Except for a slight increase in rate, a comparable result was also obtained when the depolymerization was conducted in buffer at pH 7.0. The dephosphorylation results are consistent with previous studies on the hydrolysis of

TABLE II

RATE OF DEPHOSPHORYLATION AND DEPOLYMERIZATION OF THE TYPE 19A POLYSACCHARIDE IN WATER[a] AT 100°

Time (h)	*Dephosphorylation (%)*	*Depolymerization (%)*
2	7 (15)[b]	52
4	18 (38)[b]	58
8	52 (68)[b]	79
16	85 (91)[b]	87

[a]Solution had pH 5.2 after 16 h. [b]In 0.1M Tris · HCl at pH 7.0.

D-glucopyranose 6-phosphate[15,16], in which it was shown that inorganic phosphate was slowly released under similar conditions. It was proposed that the reactive species in the hydrolysis in this pH range is the monoanion, and that the hydrolysis occurs with cleavage of the O–P bond. The results, shown in Table III, of heating other phosphorylated polysaccharides under similar conditions indicated that all those containing anomerically linked phosphoric diester bonds [*S. pneumonia* type 19 F (refs. 5–7) and *N. meningitidis* groups A (ref. 17) and X (ref. 17)] were depolymerized, whereas the one containing nonglucosylically linked phosphoric diesters [*N. meningitidis* group Z (ref. 18)] remained stable. These depolymerizations proceeded at different rates, and, in fact, as had previously been observed[19], the type 19F polysaccharide was depolymerized more slowly than that of the type 19A, which could have some significance as regards the relative stabilities of these two polysaccharides when considered as candidates for human vaccines[5,19].

In addition to being a controlled method of depolymerization of these acid-labile, phosphorylated polysaccharides, this procedure can also be used to remove phosphoric monoesters under extremely mild and reagent-free conditions. Also, the method might find utility in the preferential hydrolysis of glycosylically linked phosphoric diesters in the presence of other types of phosphoric diesters.

EXPERIMENTAL

Materials. — Pneumococcal capsular polysaccharides type 19A (57) and 19F (19) were the generous gift of Dr. J. Carlo of Merck Sharp and Dohme, Rahway, N.J. The polysaccharide was purified on an ion-exchange column (25 × 2 cm) of DEAE-Sepharose 6B, using a sodium chloride gradient (0 → 0.6M) in 0.01M sodium phosphate buffer at pH 7.0. The eluate was monitored for hexose[20] and phosphate[21], and the pure, type 19A polysaccharide was eluted at a gradient concentration of 0.3M. The polysaccharides of *N. meningitidis* groups A, X, and Z were obtained as previously described[22].

Instrumental methods. — Gas–liquid chromatography (g.l.c.) was performed with a Hewlett–Packard 5830A instrument equipped with a flame-ionization detector and a model 18850A electronic integrator. The glass columns used contained

TABLE III

DEPOLYMERIZATION OF PHOSPHORYLATED POLYSACCHARIDES FROM *N. meningitidis* AND *S. pneumoniae*[a]

Polysaccharide	*Degree of depolymerization inorganic phosphate, %)*
S. pneumoniae type 19 A	90
S. pneumoniae type 19 F	46
N. meningitidis group A	92
N. meningitidis group X	35
N. meningitidis group Z	0

[a]Depolymerizations were conducted in 0.1M Tris · HCl buffer at pH 7.0 for 16 h at 100°.

the following liquid phases on Chromosorb Q (100–200 mesh): (*i*) 3% of SP2340 (120 × 0.15 cm) at 180→240° (4°/min) for alditol acetates, and at 160→240° (4°/min) for partially methylated alditol acetates, and (*ii*) 3% of OV-17 (180 × 0.15 cm) at 190° for partially methylated alditol acetates. Combined g.l.c.–mass spectrometry (g.l.c.–m.s.) was conducted in a Hewlett–Packard 5985 system, using the same columns.

^{13}C-N.m.r. spectra were recorded in 10-mm tubes at 37° with a Varian CFT20 and a Bruker CXP300 instrument operated at 20 MHz and 75.47 MHz, respectively, in the Fourier-transform mode with complete proton-decoupling. Chemical shifts are reported in parts per million (p.p.m.) downfield from external tetramethylsilane, and the ^{2}H resonance of deuterium oxide was used as the field-frequency lock-signal. Polysaccharides and oligosaccharides were examined as solutions in deuterium oxide (25–50 mg/mL). ^{1}H-N.m.r. spectra were recorded at 80° with a Varian 400-MHz spectrometer (Regional High Resolution N.m.r. Laboratory, University of Montreal, Quebec) in the pulsed, Fourier-transform mode. The polysaccharide (3 mg) was twice lyophilized from 99.7% D_2O, and examined in the same solvent. The apparent, first-order coupling-constants (Hz) were measured directly, and the chemical shifts (δ) are expressed relative to external sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate.

Analytical methods. — Rhamnose was determined by the method of Gibbons[23], and phosphorus by the method of Chen[21]. 2-Amino-2-deoxymannose was assayed by the method of Ludowieg and Benmamen[24] as modified by Romanowska and Reinhold[25]. The polysaccharide was hydrolyzed with 4M HCl at 100° at intervals between 4 and 24 h, and the total content of 2-amino-2-deoxymannose was determined by extrapolation to zero time. D-Glucose was estimated by using D-glucose oxidase and peroxidase[26] following hydrolysis of the polysaccharide with 0.5M HCl for 16 h at 100°.

Thin-layer chromatography was conducted on plates precoated with silica gel (60F-254) with 60:45:4:30 (v/v/v/v) 1-butanol–pyridine–acetic acid–water as the solvent. Sugars were detected by using an ammonium molybdate spray.

Sugar analysis. — The polysaccharide was hydrolyzed with 0.5M HCl in a sealed ampoule for 16 h at 100°. The HCl was removed by codistillation with water, and the residue was divided into two parts, one of which was deaminated[27] with a mixture of water (0.4 mL), 33% acetic acid (0.24 mL), and 5% $NaNO_2$ (0.24 mL) for 1 h at room temperature. Under these conditions, 2-amino-2-deoxy-D-mannose is converted into D-glucose. The solution was passed through a column of Dowex 50 (H^+) ion-exchange resin, and lyophilized. The aldoses were then converted into alditol acetates[28], and these were analyzed by g.l.c.–m.s. in column (*i*). The rest of the residue was subjected to the same procedure, but omitting the deamination step. The D configuration was assigned to glucose and the L configuration to rhamnose by g.l.c. analysis of their (+)-2-octyl glycosides as described by Leontein *et al.*[29]. The D configuration was assigned to the 2-acetamido-2-deoxyglucose residue by g.l.c. analysis of its (−)-2-butanol glycosides as described by Gerwig *et al.*[30].

Methylation analysis. — The type 19A polysaccharide and associated oligosaccharides were methylated with methyl iodide in the presence of methylsulfinyl anion according to the method of Hakomori[31]. The methylated products were recovered by partitioning the reaction mixture between water and chloroform, and the chloroform phase was evaporated to dryness. The location of the phosphate groups in the type 19A polysaccharide was achieved by a double-methylation technique, essentially as described by Feige and Radziejeweska-Lebrecht[8]. The methylated, reduced, phosphorylated oligosaccharide repeating-unit of the type 19A polysaccharide was treated for 72 h with 48% hydrogen fluoride, in order to remove[32] the phosphate groups, and the hydrogen fluoride was removed by evaporation in a desiccator over NaOH pellets. The dephosphorylated, methylated oligosaccharide was then remethylated as already described, but using trideuteriomethyl iodide.

All of the permethylated products were treated with 90% formic acid for 2 h at 100°, and, after evaporation to dryness, were hydrolyzed with 0.25M H_2SO_4 for 16 h at 100°. Following neutralization of the acid with $BaCO_3$, and reduction of the sugars with $NaBH_4$, the resulting methylated alditols were acetylated with 1:1 (v/v) pyridine–acetic anhydride for 1 h at 100°, and the acetates analyzed by g.l.c.–m.s.[33] using columns (*i*) and (*ii*).

Treatment of the type 19A polysaccharide with hydrogen fluoride. — The phosphorylated polysaccharide (50 mg) was treated at 4° with 48% hydrogen fluoride (1 mL), a reagent that has been shown to promote the facile cleavage of phosphoric esters[32], and the release of free phosphate was monitored[21] at time intervals. Complete dephosphorylation had occurred after 24 h, and the hydrogen fluoride was removed, under diminished pressure, over sodium hydroxide in a desiccator. The residue was dissolved in water, and fractionated on a column (2.6 × 90 cm) of Sephadex G25, using water as the eluant and a Water Associated differential refractometer (model R403) to monitor the eluate. One major oligosaccharide fraction was identified which, on lyophilization, yielded **1** (25 mg). Oligosaccharide **1** was reduced with sodium borohydride for 6 h at room temperature, and the base was removed with Dowex-50 X-8 (H^+) ion-exchange resin, the solution evaporated to dryness, and the residue codistilled with methanol.

Partial hydrolysis of the type 19A polysaccharide. — The phosphorylated polysaccharide (40 mg) was hydrolyzed with 0.1M hydrochloric acid (2 mL) for 20 min at 100°, conditions that had yielded the phosphorylated oligosaccharide repeating-unit of the pneumococcal type 19F polysaccharide[6]. After neutralization of the acid with M sodium hydroxide, the solution was applied to a column (2.6 × 90 cm) of Sephadex G-25, and the oligosaccharide-containing fraction of the eluate was obtained as described[6] for the hydrogen fluoride-treated, type 19F polysaccharide. The fraction yielded **2**, which contained 5.2% of P and had a rhamnose:phosphate molar ratio of 1:1. Oligosaccharide **2** was also reduced with sodium borohydride as described for **1**.

Depolymerization of the type 19A polysaccharide. — A solution of the type

19A polysaccharide (Na^+ salt) (25 mg) in water (4 mL) was heated for 16 h in a sealed tube at 100°, cooled, and lyophilized; following dissolution of the residue in water (1 mL), the products of the depolymerization were fractionated on a Sephadex G-25 column (2.6 × 90 cm) as already described. The major product was an oligosaccharide, containing no phosphate, which was shown by analysis and ^{13}C-n.m.r. spectroscopy to be **1**.

A number of similar, smaller-scale depolymerizations were conducted on the type 19A and other phosphorylated polysaccharides at concentrations of 1 mg/mL in water and 0.1M Tris · HCl buffer at pH 7.0. The degree of dephosphorylation was determined by measuring the free phosphate. The degree of depolymerization of the type 19A polysaccharide as a function of time was determined by colorimetric assay of the total rhamnose before and after reduction of the depolymerized products with sodium borohydride.

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REFERENCES

1 P. SMIT, D. OBERHOLZER, S. HAYDEN-SMITH, H. J. KOORNHOF, AND M. R. HILLEMAN, *J. Am. Med. Assoc.*, 238 (1977) 2613–2616.
2 R. AUSTRIAN, *J. Infect. Dis.*, 136 (1977) S38–S42.
3 H. J. JENNINGS, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 155–206.
4 S. C. SZU, C.-J. LEE, J. C. PARKE, JR., G. SCHIFFMAN, J. HENRICHSEN, R. AUSTRIAN, S. C. RASTOGI, AND J. B. ROBBINS, *Infect. Immun.*, 35 (1982) 777–782.
5 C.-J. LEE AND B. A. FRASER, *J. Biol. Chem.*, 255 (1980) 6847–6853.
6 H. J. JENNINGS, K.-G. ROSELL, AND D. J. CARLO, *Can. J. Chem.*, 58 (1980) 1069–1074.
7 N. OHNO, T. YADOMAE, AND T. MIYAZAKI, *Carbohydr. Res.*, 80 (1980) 297–304.
8 V. FIEGE AND J. RADZIEJEWESKA-LEBRECHT, *Proc. Int. Symp. Glycoconjugates*, (1979) 12–13.
9 P. COLSON AND R. R. KING, *Carbohydr. Res.*, 47 (1976) 1–13.
10 D. R. BUNDLE, H. J. JENNINGS, AND I. C. P. SMITH, *Can. J. Chem.*, 51 (1973) 3812–3819.
11 H. J. JENNINGS AND I. C. P. SMITH, *Methods Enzymol.*, 50 (1978) 39–50.
12 H. J. JENNINGS AND I. C. P. SMITH, *Methods Carbohydr Chem.*, 8 (1980) 97–105.
13 J. V O'CONNOR, H A. NUNEZ, AND R. BARKER, *Biochemistry*, 18 (1979) 500–507.
14 H. J. JENNINGS AND C. LUGOWSKI, *J. Immunol.*, 127 (1981) 1011–1018.
15 C. DEGANI AND M. HALMANN, *J. Am. Chem. Soc.*, 88 (1966) 4075–4082.
16 C. A. BUNTON AND H. CHAIMOVICH, *J. Am. Chem. Soc.*, 88 (1966) 4082–4089.
17 H. J. JENNINGS, A. K. BHATTACHARJEE, D. R. BUNDLE, C. P. KENNY, A. MARTIN AND I. C. P. SMITH, *J. Infect. Dis.*, 136 (Suppl.) (1977) S78–S83.
18 H. J. JENNINGS, K.-G. ROSELL, AND C. P. KENNY, *Can. J. Chem.*, 57 (1979) 2902–2907.
19 G. ZON, S. C. SZU, W. EGAN, J. D. ROBBINS, AND J B. ROBBINS, *Infect. Immun* , 37 (1982) 89–103.
20 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
21 P. S. CHEN, J. Y. TORIBA AND H. WARNER, *Anal. Chem.*, 28 (1956) 1756–1758.
22 D. R. BUNDLE, H. J. JENNINGS AND C. P. KENNY, *J. Biol. Chem.*, 249 (1974) 4797–4801
23 Z. DISCHE, *Methods Carbohydr. Chem.*, 1 (1962) 501–503.
24 J. LUDOWIG AND J. D. BENMAMEN, *Anal. Biochem.*, 19 (1967) 80–88.
25 E. ROMANOWSKA AND V. REINHOLD, *Eur. J. Biochem.*, 36 (1973) 160–166

26 H. U. BERGMEYER AND E. BERNT, in H. U. BERGMEYER (Ed.), *Methoden der Enzymatischen Analyse*, Verlag Chemie, Weinheim, 1962, pp. 123–130.
27 L. V. DMITRIEV, V. L. BACKINOWSKI, V. L. LVOV, N. K. KOCHETKOV, AND I. L. HOFMAN, *Eur. J. Biochem.*, 50 (1975) 539–547.
28 J. S. SAWARDEKER, J. H. SLONEKER, AND A. R. JEANES, *Anal. Chem.*, 37 (1965) 1602–1604.
29 K. LEONTEIN, B. LINDBERG, AND J. LÖNNGREN, *Carbohydr. Res.*, 62 (1978) 359–362.
30 G. J. GERWIG, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *Carbohydr. Res.*, 77 (1979) 1–7.
31 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
32 D. LIPKIN, B. E. PHILLIPS, AND J. W. ABRELL, *J. Org. Chem.*, 34 (1969) 1539–1547.
33 B. LINDBERG, *Methods Enzymol.*, 28B (1972) 178–195.