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STRUCTURAL STUDIES ON A POLYSACCHARIDE FROM *Shigella dysenteriae* TYPE 7

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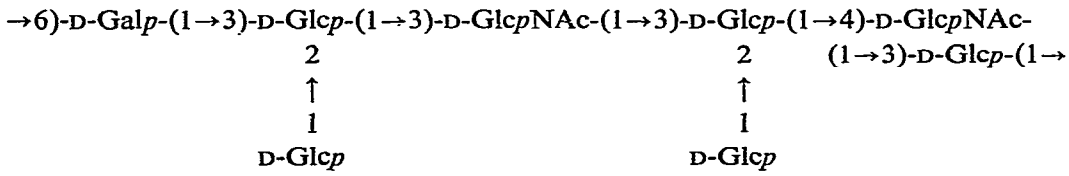
ABSTRACT

The polysaccharide obtained from the O-somatic antigen of *Shigella dysenteriae* type 7 (strain NCTC 519/66) contains D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose in the mole ratios of 2:1:1. From the results of methylation, periodate oxidation, graded hydrolysis, and deamination studies, the structure assigned to the repeating unit of the polysaccharide is as follows.



Oxidation studies with chromium trioxide revealed the nature of the anomeric linkages of some of the sugar residues in the polysaccharide.

INTRODUCTION

Shigella dysenteriae subgroup is known to contain ten serotypes of distinct, serological specificities[1]. The lipopolysaccharides from them differ in monosaccharide composition, and some contain unusual, acidic sugars[2–4]. Kochetkov *et al.*[2–13] established the structure of most of these O-somatic polysaccharides, and showed[14] that the polysaccharide of *Sh. dysenteriae* type 7 contains 2-acetamido-2-deoxy-D-glucuronic acid, 2-acetamido-2-deoxy-D-glucose, and glycine. We were interested in identifying the immunologically specific groupings in the polysaccharide molecule. The polysaccharide isolated from strain NCTC 519/66 of *Sh. dysenteriae* type 7 was found to contain a different sugar composition. Consequently, investigations have

been conducted to establish the structure of the repeating unit of this carbohydrate, and we now report the results.

EXPERIMENTAL

General. — Evaporations were conducted under diminished pressure in a rotary evaporator at 40° (bath temperature). Optical rotations were recorded with a Perkin–Elmer 141 polarimeter. Ultracentrifugations were performed in a Beckman Model L 5-65 ultracentrifuge. Descending paper chromatography was performed on Whatman No. 1 and 3 MM papers, using as solvent systems (v/v) (*A*) 9:2:2 ethyl acetate–acetic acid–water and (*B*) 1-butanol–acetic acid–water (4:1:5, upper phase). Chromatograms were developed with (*i*) alkaline silver nitrate[15], (*ii*) aniline oxalate[16], and (*iii*) ninhydrin reagent[17]. Elutions were monitored with a Water Associates Model R-403 differential refractometer, and polarimetrically and spectrophotometrically. I.r. spectra were recorded with a Beckman IR 20-A spectrophotometer. G.l.c. was performed with a Hewlett–Packard Model 5730 A gas chromatograph equipped with a f.i.d. detector. The columns (1.8 m × 6 mm) used were (*1*) 3% of ECNSS-M, (*2*) 3% of OV-225, and (*3*) Poly A, each on Gas Chrom Q (100–120 mesh); nitrogen was the carrier gas. N.m.r. spectra were recorded with a Varian-60 A instrument. Mass spectra were recorded with an AEIMS 3074 mass spectrometer. Paper electrophoresis was conducted in a Shandon high-voltage apparatus, Model L-24.

The micro-organism *Shigella dysenteriae* type 7, strain 519/66, obtained from NCTC, London, was grown on a large scale. Sterile media containing Difco bacto-agar impregnated with bacto brain-heart infusion (Difco) were used at pH 7.4 in Roux bottles. After 48 h of growth at 37°, the bacteria were collected by washing with saline. The cell suspension from 100 bottles was centrifuged at 19,000 r.p.m., and the sediment was washed successively with normal saline (thrice) and acetone (twice); the dry weight of the cells was 20 g.

Isolation of the lipopolysaccharide[18]. The dried cells (13 g) were swollen in water (150 mL) at 65–68°, and an equal volume of 90% phenol at 65–68° was added, with stirring. After being stirred for 10 min, the suspension was cooled, and centrifuged at 4000 r.p.m. for 40 min at 4°. The aqueous layer was collected, exhaustively dialyzed against distilled water, and freeze-dried; yield 1.8 g. The material was dissolved in 0.5M sodium chloride solution (100 mL), and the solution treated with a 2% solution of Cetavlon (45 mL). The nucleic acid–Cetavlon complex which precipated out on gradual dilution of the solution was removed at the centrifuge, and the supernatant liquor was dialyzed, and freeze-dried. The lipopolysaccharide (LPS), containing a trace of nucleic acid, was purified by gel filtration in a column (1 m × 2.5 cm) of Sephadex G-100, using pyridine–acetic acid buffer (pH 4.5); the elution curve is shown in Fig. 1. Fraction A (tubes 40–51) showed u.v. absorption, indicating the presence of nucleic acid. The LPS, showing a small u.v. absorption, was obtained

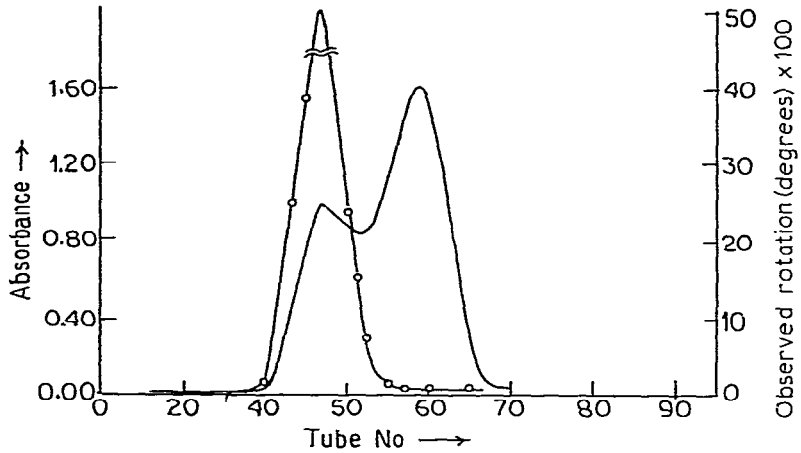


Fig. 1. Elution curve for purification of the lipopolysaccharide on a column of Sephadex G-100. [Key: (–O—O–) observed rotation (degrees) × 100; (———) absorbance at 260 nm.]

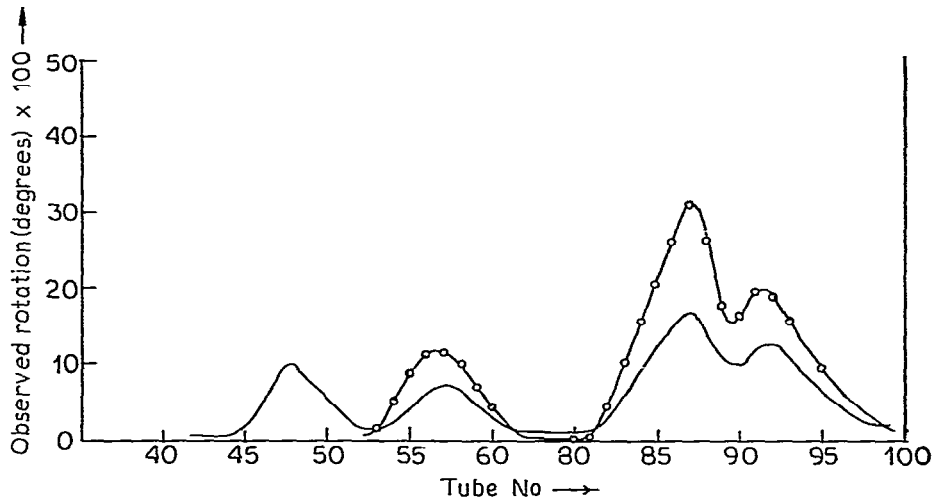


Fig. 2. Elution curve for purification of the polysaccharide on a column of Sephadex G-75. [Key: (–O—O–) observed rotation (degrees) × 100; (———) differential, refractive index monitored.]

from Fraction B (tubes 52–66) by freeze-drying; yield, 980 mg; $[\alpha]_D^{23}$ +105° (*c* 0.75, water).

Isolation of the polysaccharide[19]. — The LPS (960 mg) in 1 % acetic acid (100 mL) was heated for 2 h at 100°, the precipitated lipid removed at the centrifuge, the supernatant liquor freeze-dried, and an aqueous solution thereof passed through a column (1 m × 3 cm) of Sephadex G-75, using pyridine–acetic acid buffer (pH 4.5) as the eluant; the elution curve is shown in Fig. 2. Of the four fractions, the first (tubes 44–51) showed u.v. absorption at 260 nm, and contained nucleic acid. The second fraction (tubes 54–65) contained LPS, and the third (tubes 82–89) the O-

TABLE I

SUGAR COMPONENTS OF DIFFERENT MATERIALS

Material	*Sugar content*[a]									
	Glucose		*Galactose*		*2-Amino-2-deoxyglucose*		*Anhydrohexitol*		L-glycero-D-manno-*Heptose*	
	%	*Mole proportion*	%	*Mole proportion*	%	*Mole proportion*	%	*Mole proportion*	%	*Mole proportion*
Lipopolysaccharide	14.6	1.8	10.6	1.3	9.5	1.2			10.3	1.1
Polysaccharide	46.2	3.9	24.2	2.0	23.6	2.0				
IO_4^--oxidized polysaccharide	52.2	3.1			17.2	1.0				
Oligosaccharide V	61.0	3.0	20.5	1.0			18.0	1.0		
Oligosaccharide VI	35.0	1.1	34.5	1.1			30.0	1.0		

[a] *myo*-Inositol was used as the internal standard.

TABLE II

RESULTS OF METHYLATION ANALYSIS OF THE POLYSACCHARIDE

O-*Methyl sugars*	*Retention time*[a]		*Mole ratio*	*Structural unit deduced*
	Column 1	*Column 2*		
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.0	1.0	1.0	$\overset{1}{\text{Glc}}\rightarrow$
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.25	1.20	1.05	$\overset{1}{\text{Gal}}\rightarrow$
2,4,6-Tri-*O*-methyl-D-glucose	1.98	1.82	1.10	$\rightarrow\overset{3\ \ 1}{\text{Glc}}\rightarrow$
2,3,4-Tri-*O*-methyl-D-galactose	3.40	2.50	1.10	$\rightarrow\overset{6\ \ 1}{\text{Gal}}\rightarrow$
4,6-Di-*O*-methyl-D-glucose	4.00	3.41	2.30	$\rightarrow\overset{3\ \ 1}{\text{Glc}}\rightarrow$ $\uparrow 2$
2-Amino-2-deoxy-3,6-di-*O*-methyl-D-glucose	1.74[b]		1.09	$\rightarrow\overset{4\qquad 1}{\text{GlcNAc}}\rightarrow$
2-Amino-2-deoxy-4,6-di-*O*-methyl-D-glucose	2.32[b]		1.10	$\rightarrow\overset{3\qquad 1}{\text{GlcNAc}}\rightarrow$

[a]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on (*1*) ECNSS-M column at 270°, and (*2*) OV-225 column at 170°. [b]The same, but at 190°.

specific polysaccharide (PS), which was the major component; the fourth fraction, (tubes 92–96) corresponded to core oligosaccharide present in the LPS. The third fraction was freeze-dried, and an aqueous solution thereof was passed through a column of Sephadex G-100, affording the PS free from oligosaccharide; yield, 230 mg; $[\alpha]_D^{23}$ +122° (*c* 0.75, water); oligosaccharide, 180 mg; $[\alpha]_D^{23}$ +92° (*c* 1.0, water).

Test for homogeneity. — The LPS and PS were subjected to high-voltage paper electrophoresis for 60 min, using Whatman 3 MM strips and pyridine acetate buffer (pH 4.5) at a potential gradient of 40 V/cm. On developing the papers with the ninhydrin reagent, a single spot was detected in both cases. In Ouchterlony gel-diffusion[20], using rabbit antisera raised against whole cells, one band was detected with LPS and PS; these bands fused with each other.

Monosaccharide composition. — The LPS and PS were hydrolyzed with 3M hydrochloric acid for 8 h at 100°. The acid was evaporated under vacuum (over P_2O_5 and sodium hydroxide), and the last traces of the acid were removed by co-distillation with methanol. The hydrolyzate of the LPS gave spots corresponding to

TABLE III

RESULTS OF METHYLATION ANALYSIS OF THE OLIGOMERS

Methyl sugars	*Retention time*[a]		*Mole proportion of O-methyl sugars in oligosaccharides*						
	Column 1	*Column 2*	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	1.00		1.0	2.2	1.1	2.1		
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.25	1.19	1.0	1.1		1.0		1.0	
3,4,6-Tri-*O*-methyl-D-glucose	1.94	1.83	1.0			1.0		1.0	
2,4,6-Tri-*O*-methyl-D-glucose	1.98	1.82						1.1	1.1
4,6-Di-*O*-methyl-D-glucose	4.00	3.40		1.0	1.1	1.0	0.9		2.0
2,3,4-Tri-*O*-methyl-D-galactose	3.40	2.50			1.1		1.0		
2,5-Anhydro-1,3,6-tri-*O*-methyl-D-mannitol		0.70						0.9	
2,5-Anhydro-1,4,6-tri-*O*-methyl-D-mannitol		0.60					1.0		
2-Amino-2-deoxy-3,6-di-*O*-methyl-D-glucose	1.74[b]		1.0			1.1			1.0
2-Amino-2-deoxy-4,6-di-*O*-methyl-D-glucose	2.32[b]			1.0	1.0	1.0			1.0

[a]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on (*1*) ECNSS-M column at 170°, and (*2*) OV-225 column at 170°. [b]The same, but at 190°.

glucose, galactose, 2-amino-2-deoxyglucose, and a heptose, and that of the PS gave spots corresponding to glucose, galactose, and 2-amino-2-deoxyglucose. Their quantities were determined by g.l.c. (columns *1* and *3*), and the results are given in Table I. The hydrolyzate obtained by using 0.5M sulfuric acid gave the same results.

Methylation analysis. — The PS (14 mg) was methylated by the Hakomori method[21]. The product was dialyzed against distilled water, freeze-dried, and remethylated by the Purdie method[22]. The product showed no absorption band at 3600–3300 cm^{-1} in its i.r. spectrum, and had $[\alpha]_D^{23}$ +35° (*c* 0.5, $CHCl_3$). The methylation product was hydrolyzed with 0.5M hydrochloric acid for 10 h at 100°, and the methylated sugars were converted into their alditol acetates, and these identified, and estimated, by g.l.c. and g.l.c.–m.s. The results are given in Table II.

Graded hydrolysis. — The PS (100 mg) was heated with M hydrochloric acid (20 mL) for 1 h at 100°, and the acid was removed *in vacuo*. In p.c., the hydrolyzate gave seven spots. The mixture was separated into its components on 3 MM paper, using solvent *B*. Besides a mixture containing glucose, galactose, and 2-amino-2-deoxyglucose, four oligomers were isolated, and designated Oligomer I, II, III, and IV in the order of decreasing mobility. Each oligomer was purified by passing a solution

thereof through a column of Sephadex G-25, and was found to be homogeneous by high-voltage electrophoresis.

The sugar compositions of the oligomers were determined in the usual way. The carbohydrates were fully methylated by the Hakomori method[21], and then by the Purdie method[22]. Each methyl derivative was hydrolyzed, and the methylated sugars were identified, and estimated, by g.l.c. The results are given in Table III, columns I–IV.

To identify the sugar residues at the reducing end of oligomers I and II, the oligomers were reduced with $NaBH_4$, the products hydrolyzed, and the sugars identified by p.c., using aniline oxalate as the spray reagent. In both cases, spots corresponding to galactose and glucose were identified, indicating that a 2-amino-2-deoxyglucose residue constituted the reducing end of each of the oligomers.

Deamination of the polysaccharide. — The dried PS (10 mg) was *N*-deacetylated[23] by heating with anhydrous hydrazine (1.5 mL) and hydrazine sulfate (150 mg) in a sealed tube for 10 h at 100°. The hydrazine was evaporated, and the salts were removed by passing through a column (1 m × 2.5 cm) of Sephadex G-50, with water as the eluant. The material was freeze-dried, and then dried *in vacuo* over P_2O_5. The product was deaminated by dissolving it in cold water (2 mL) at 0°, and adding ice-cold sodium nitrite solution (5%, 2 mL), with stirring, followed by cold acetic acid (33%, 2 mL) dropwise. After 2 h, the solution was warmed to room temperature, and the excess of nitrous acid was removed by bubbling nitrogen through it. The solution was passed through a column of Dowex 50 (H^+) ion-exchange resin, the eluate freeze-dried, the residue dissolved in water (1 mL), and the solution fractionated in a column (1 m × 3 cm) of Sephadex G-75. From the eluate, two fractions, designated *A* and *B*, were isolated as solids. Fraction *A* (3 mg) had $[\alpha]_D^{23}$ +14° (*c* 0.5, water) and fraction *B* (6 mg) had $[\alpha]_D^{23}$ +32° (*c* 0.75, water). The sugar constituents in them were estimated by g.l.c. in the usual way, and the results are given in Table I. Both fractions (2 mg each) were methylated by the Hakomori method[21], and the constituent methylated sugars were identified and estimated in the usual way. The results are given in Table III, columns V and VI.

Periodate oxidation. — The PS (4.0 mg) was dissolved in water (2.0 mL), and 0.04M sodium metaperiodate (2.0 mL) and water (2.0 mL) were added. The reaction was allowed to proceed in the dark at 4°, the consumption of the oxidant being monitored spectrophotometrically[24]. The uptake of periodate became constant in 23 h, corresponding to 0.66 mol per mol of hexosyl residue.

In a separate experiment, the PS (20 mg) was treated with 0.02M sodium metaperiodate solution (10 mL) for 23 h at 4°, the excess of periodate was reduced with ethylene glycol, and the solution dialyzed against distilled water, and freeze-dried. The periodate-oxidized product (1 mg) was hydrolyzed with 2M hydrochloric acid for 6 h at 100°, and *myo*-inositol was added as the internal standard. The sugars present in the hydrolyzate were estimated by g.l.c. in the usual way, and the results are given in Table I. The periodate-oxidized material (5 mg) was reduced with $NaBH_4$, and the solution was dialyzed, and freeze-dried. The product was methylated by the

TABLE IV

SUGARS (PERCENT)[a] IN POLYSACCHARIDE OXIDIZED WITH Cr_2O_3

Time of oxidation (hours)	*Galactose*	*Glucose*	*2-Amino-2-deoxy-glucose*
0	24.9	49.4	25.4
1	10.0	42.9	20.0
2	6.5	38.0	18.1
2.5	5.0	30.1	15.0

[a]*myo*-Inositol was used as the internal standard.

Hakomori method[21]; the ethers were hydrolyzed, and the methylated sugars were analyzed in the usual way. The results are given in Table III, column VII.

Oxidation with chromium trioxide[25]. — To a mixture containing PS (11.0 mg) and *myo*-inositol (9.0 mg) in formamide (1.5 mL) were added acetic anhydride (3 mL) and pyridine (3.5 mL), with stirring. After 16 h at room temperature, the mixture was dissolved in chloroform (20 mL), and the solution washed with water (3 × 25 mL), dried (anhydrous Na_2SO_4), and evaporated to dryness. The material was reacetylated in the same way. The acetylation product was dissolved in glacial acetic acid (5 mL), and the solution was treated with Cr_2O_3 (300 mg) at 50°. Aliquots were removed at intervals, and immediately diluted with water (to stop oxidation). The mixture was extracted with chloroform and the extract was dried (anhydrous Na_2SO_4), and evaporated to dryness. The resulting material was hydrolyzed with 3M HCl for 8 h at 100°. The sugars in the hydrolyzate were converted into their alditol acetates, and estimated by g.l.c. The results are given in Table IV.

RESULTS AND DISCUSSION

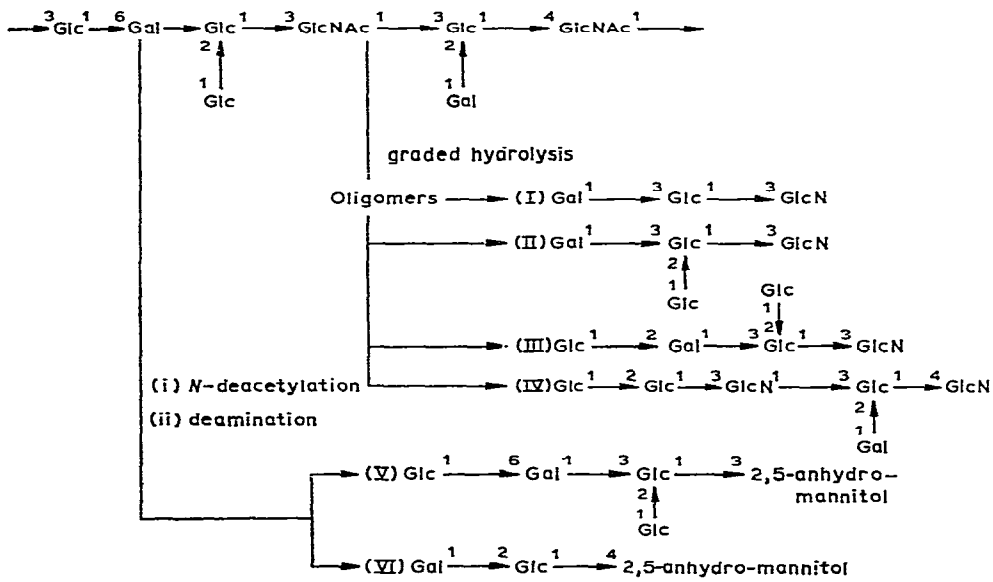
The cells of *Sh. dysenteriae* type 7 were extracted with 45% phenol at 65–68°, and on cooling and centrifuging, the extract gave three layers. The aqueous layer (containing the O-somatic antigen and nucleic acid) was treated with Cetavlon to form with the latter a complex which was separated in a centrifuge. From the supernatant liquor, lipopolysaccharide (LPS) containing some nucleic acid was isolated. The LPS was purified by fractionating the mixture on a column of Sephadex G-100. The material had $[\alpha]_D^{23}$ +65°, and showed a small absorption at 260 nm, indicating the presence of nucleic acid. It contained 45.0% of carbohydrate, and was homogeneous in paper electrophoresis. The LPS was found to contain D-galactose (10.6%), D-glucose (14.6%), 2-acetamido-2-deoxy-D-glucose (9.5%), and L-*glycero*-D-*manno*-heptose (10.3%). To remove lipid components, the LPS was heated with 1% acetic acid and the material in the aqueous layer was fractionated on a column of Sephadex G-75. Two major fractions were obtained, one containing the polysaccharide (PS), and the other, the core oligosaccharide (see Fig. 2). The PS had $[\alpha]_D^{23}$ +122°.

The LPS and PS were homogeneous in paper electrophoresis, and gave a single band in Ouchterlony gel-diffusion against the homologous antiserum, whereas the oligosaccharide did not give any band. The precipitin bands with LPS and PS fused, indicating the presence of common, antigenic determinants in them.

The PS contained D-galactose (24.2%), D-glucose (46.2%), and 2-acetamido-2-deoxy-D-glucose (23.6%). It showed absorption bands at 1650 and 1570–1560 cm^{-1} in the i.r. spectrum, characteristic of an amide grouping. In the n.m.r. spectrum, a signal at 2.08 p.p.m. confirmed the presence of *N*-acetyl groups in the material.

On hydrolysis, the fully methylated PS gave methylated sugars that were converted into their alditol acetates, and these were analyzed by g.l.c. and g.l.c.–m.s. The results are given in Table II. Isolation and characterization of 1 mol proportion each of 2,3,4,6-tetra-*O*-methyl-D-glucose and -D-galactose indicated that the average repeating-unit of the PS contains one nonreducing end each of D-glucopyranosyl and D-galactopyranosyl groups; it also contains (1→3)-linked D-glucopyranosyl, (1→6)-linked D-galactopyranosyl, and two (1→3)-linked 2-amino-2-deoxy-D-glucosyl residues. The branches originate from the D-glucopyranosyl residues in the chain, and the hexosyl residues at the branch points are (1→2,3-)linked.

To determine the sequence of the sugar residues, the PS was subjected to graded hydrolysis, to yield a mixture containing monosaccharides and four oligosaccharides which were isolated as homogeneous fractions. The oligomers were characterized by methylation studies. The results are given in Table III. Oligomer I, having $[\alpha]_D^{23}$ +5°, was found to contain galactose, glucose, and 2-amino-2-deoxy-glucose in the molar ratios of 1.0:1.0:1.1. On methylation and hydrolysis, it gave 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol), 3,4,6-tri-*O*-methyl-D-glucose (1.1 mol),



Scheme 1. *Shigella dysenteriae* type 7 polysaccharide, and oligomers isolated from it.

and 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose (1.0 mol). The reducing end was occupied by a 2-amino-2-deoxy-D-glucose residue. The structure assigned to oligomer I is given in Scheme 1. Oligomer II, having $[\alpha]_D^{23}$ +25°, is composed of galactose, glucose, and 2-amino-2-deoxyglucose in the molar ratios of 1.0:1.95:1.0. On hydrolysis, the fully methylated derivative yielded 2,3,4,6-tetra-*O*-methyl-D-glucose (1.0 mol), 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol), and 2-amino-2-deoxy-4,6-di-*O*-methyl-D-glucose (1.0 mol). The 2-amino-2-deoxy-D-glucose unit forms the reducing end of the oligosaccharide. The structure of oligomer II is given in Scheme 1. Oligomer III had $[\alpha]_D^{23}$ +11.4°, and on hydrolysis it gave galactose, glucose, and 2-amino-2-deoxyglucose in the molar ratios of 1.0:1.0:..1, whereas its permethylated derivative yielded 2,3,4,6-tetra-*O*-methyl-D-glucose (2.2 mol), 2,3,4-tri-*O*-methyl-D-galactose (1.1 mol), 4,6-di-*O*-methyl-D-glucose (1.1 mol), and 2-amino-2-deoxy-4,6-di-*O*-methyl-D-glucose (1.0 mol). The structure assigned to III is given in Scheme 1. Oligomer IV, $[\alpha]_D^{23}$ +37°, is composed of galactose, glucose, and 2-amino-2-deoxyglucose in the molar ratios of 1:3:2. The hydrolyzate of fully methylated oligomer IV contained 2,3,4,6-tetra-*O*-methyl-D-glucose (1.1 mol), 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol), 3,4,6-tri-*O*-methyl-D-glucose (1.0 mol), 4,6-di-*O*-methyl-D-glucose (1.0 mol), 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose (1.1 mol), and 2-amino-2-deoxy-4,6-di-*O*-methyl-D-glucose (1.0 mol). From these results, a structure was assigned to this fragment (see Scheme 1).

Selective cleavage, based on *N*-deacetylation followed by deamination, was performed in order to identify the position of the amino sugar units in the repeating unit. The material obtained on *N*-deacetylation with anhydrous hydrazine in the presence of hydrazine sulfate was passed through a column of Sephadex G-75, and a fraction having a high molecular weight was eluted; this was deaminated with nitrous acid, reduced ($NaBH_4$), de-ionized, and freeze-dried. Hydrolysis of a small portion of it, followed by g.l.c. examination, gave only a small quantity of 2-amino-2-deoxyglucose indicating >95% deamination. The remaining material was fractionated in a column of Sephadex G-75. Two fractions, designated oligosaccharide V and VI, were collected. Oligosaccharide V contained glucose, galactose, and 2,5-anhydro-D-mannitol in the molar ratios of 3.0:1.0:1.0, whereas oligomer VI had glucose, galactose, and 2,5-anhydro-D-mannitol in the molar ratios of 1.1:1.1:1.0 (see Table I). The hydrolyzate of the fully methylated oligosaccharide V was found to contain 2,3,4,6-tetra-*O*-methyl-D-glucose (2.1 mol), 2,3,4-tri-*O*-methyl-D-galactose (1.0 mol), 4,6-di-*O*-methyl-D-glucose (0.9 mol), and 2,5-anhydro-1,4,6-tri-*O*-methyl-D-mannitol (1.0 mol) (see Table III). On methylation analysis, oligosaccharide VI gave 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol), 3,4,6-tri-*O*-methyl-D-glucose (1.0 mol), and 2,5-anhydro-1,3,6-tri-*O*-methyl-D-mannitol (0.9 mol) (see Table III).

From the results of methylation studies on PS and its degradation products, a structure was assigned to the repeating unit of *Sh. dysenteriae* type 7 polysaccharide; this is given in Scheme 1. On periodate oxidation, the PS consumed 0.66 mol of oxidant per mol of hexosyl residue in 23 h, the theoretical value, calculated on the basis of the structure assigned, being 0.75 mol. The sugar residues surviving oxidation

were those of glucose (52.2%) and 2-amino-2-deoxyglucose (17.2%) (see Table I), the calculated values being 43 and 28%, respectively. On methylation and hydrolysis, the periodate-oxidized, reduced PS gave 2,3,4,6-tetra-*O*-methyl-D-glucose (1.1 mol), 4,6-di-*O*-methyl-D-glucose (2.0 mol), and 2-amino-2-deoxy-4,6-di-*O*-methyl-D-glucose (1.0 mol).

The results of oxidation of the permethylated PS with Cr_2O_3 are shown in Table IV. The rate of oxidation of galactose and amino sugar units was appreciably higher than that of glucose residues. From these results, it was concluded that the PS contains β-linked galactose and α-linked glucose residues[26]. The high optical rotation of the polysaccharide, together with the fact that the glycosidic bonds of the amino sugars are susceptible to hydrolysis under mild conditions, indicated that the glycosidic linkages of the majority of the amino sugars units are also α.

The polysaccharide from the O-somatic antigen of *Sh. dysenteriae* type 7, strain NCTC 519/66, is composed of an octasaccharide repeating-unit having a sugar composition different from that reported earlier[14].

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