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Structure of *Klebsiella aerogenes* Type 8 Polysaccharide*

I. W. Sutherland

ABSTRACT: The capsular polysaccharide has been prepared from a strain of *Klebsiella aerogenes* type 8. It contained D-glucose, D-galactose, and D-glucuronic acid in the molar proportions 1:2:1. Periodate oxidation destroyed the glucuronic acid but not the neutral sugars. Carboxyl-reduced polysaccharide containing equimolar amounts of glucose and galactose was prepared. The glucose formed from glucuronic acid was identified using sodium borotritide as reductant. Partial acid hydrolysis was used to obtain oligosaccharides from the original polysaccharide, the oxidized polymer, and the carboxyl-reduced polysaccharide. The structures of several of these oligosaccharides have been determined, the largest fragment from any preparation being a tetrasaccharide. On the basis of the oligosaccharide structures and other information, a tetrasaccharide repeating unit is proposed with the structure

$$\begin{array}{l}\alpha\text{-GlcUA} \\ \quad 1\downarrow\uparrow 4 \quad \beta \qquad\quad \alpha \qquad\quad \beta \\ \text{-(}\rightarrow\text{3-Gal-1}\rightarrow\text{3-Gal-1}\rightarrow\text{3-Glc-1}\rightarrow\text{)-}\end{array}$$

Despite improved analytical methods now available, the number of bacterial exopolysaccharides whose structure is known is exceedingly limited. This is particularly true of gram-negative bacteria such as *Klebsiella aerogenes* in which most strains secrete large amounts of capsular or slime material of differing chemotype. Such results as have been published on the structure of exopolysaccharides from *K. aerogenes* types 2 and 54 (Gahan *et al.*, 1967; Conrad *et al.*, 1966) and from various *Escherichia coli* serotypes (*e.g.*, Jann *et al.*, 1965, 1968) indicate that, as in the polysaccharides of gram-positive bacteria, a repeating unit of varying complexity is normally found. This unit may be as simple as the trisaccharide types found in the exopolysaccharides of *E. coli* K30 and K42 (Hungerer *et al.*, 1967; Jann *et al.*, 1965) or it may be rather more complex such as the acetylated and pyruvylated hexasaccharide composed of four different sugars postulated for "colanic acid" in several species of Enterobacteriaceae (Sutherland, 1969; Lawson *et al.*, 1969). The disadvantage of many of the earlier, purely chemical, studies has been that labile noncarbohydrate components such as acetyl or pyruvyl groups have frequently been overlooked. The importance of thorough examination for such substituents can be seen in the increasing number of bacterial exopolysaccharides now known to contain such substituents. These include the capsular polysaccharides of many *Rhizobium* strains and species (Dudman and Heidelberger, 1969) and of *K. aerogenes* types 1 to 6 (Luderitz *et al.*, 1968).

An examination of the literature for a suitable organism to use in biosynthetic studies indicated that *K. aerogenes* type 8 (strain A4) produced a capsular polysaccharide containing three sugars, glucose, glucuronic acid, and galactose, in the approximate molar ratio 1:1:2 (Dudman and Wilkinson, 1956). Thus all the component sugars are readily determinable and the presumed nucleotide sugar precursors are available. However, no data on the structure of this polymer appear to have been published. A reexamination of the polysaccharide and a possible structure are now reported.

Materials

The K. aerogenes type 8 strain A4 was obtained from the departmental collection. It produced an exopolysaccharide capsule as determined by the India Ink technique (Duguid,

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1951). Cultures were harvested after 2–3-days growth at 35° in a solid or liquid nitrogen deficient medium (Sutherland and Wilkinson, 1965). The polysaccharide was separated from the cells and purified by the methods of Dudman and Wilkinson (1956).

Glucose oxidase and galactose oxidase reagents were obtained from Boehringer and Soehne G.m.b.H., Mannheim, Germany, and Worthington Biochemical Corp., Freehold, N. J., respectively. Three commercial glycosidase preparations were used: β-glucosidase (EC 3.2.1.21) from L. Light, Colnbrook, Bucks; β-galactosidase (EC 3.2.1.23) and β-glucuronidase (EC 2.2.1.31) from British Drug Houses, Poole, Dorset. α-Glucosidase was prepared from yeast by the method of Robbins and Uchida (1962). A crude α-galactosidase (EC 3.2.1.22) preparation from a strain of *Cytophaga* was also used.

Methods

D-Glucose and D-galactose were estimated in hydrolysates of polysaccharides or oligosaccharides (1 N H_2SO_4, 16 hr) using the respective oxidases. D-Glucuronic acid was determined by a modification of the carbazole–H_2SO_4 technique (Bitter and Muir, 1962), on unhydrolyzed material. The methods of Hestrin (1949) and Sloneker and Orentas (1962) were used to test for the possible presence of *O*-acyl or pyruvyl groups, respectively. To determine the configuration of the glycosidic linkages in the oligosaccharides, the appropriate enzymes were used under conditions giving total hydrolysis of approximately equimolar amounts of substrates such as cellobiose, maltose, lactose, or melibiose. All radioactivity measurements were made on samples in a dioxane-based scintillator (Nuclear Enterprises, Edinburgh) using a Beckman scintillation spectrometer.

Periodate oxidation was performed according to Conrad *et al.* (1966). Residual sugars after various time intervals were determined on the polysaccharide after borohydride reduction, dialysis, and hydrolysis.

Carboxyl Reduction. The polysaccharide (500 mg) in the H^+ form was obtained by passage through Amberlite IR120 ion-exchange resin. After lyophilization, it was dissolved in 50 ml of water and an equal volume of ethylene oxide at 0° was added. The mixture was left for 5 days at 20°, then dialyzed. The esterified product was lyophilized and redissolved in 50 ml of water containing 5 ml of glycerol. An equal volume of 10% (v/v) aqueous glycerol containing 0.6 g of sodium borohydride and 0.5 mCi of sodium borotritide was added. The mixture was stirred and left for 24 hr at 4°. The excess borohydride was then destroyed with 1 N acetic acid and the product was exhaustively dialyzed against distilled water. It was then freeze dried (yield 415 mg).

Paper Electrophoresis and Paper Chromatography. Paper electrophoresis was performed in pyridinium acetate buffer (pH 5.3) using a current of 80–100 mA at 3000 V. The equipment was a Locarte (London) type with 70 × 20 cm cooled plate area. The current was applied for 3–4 hr for oligosaccharide separation and for 30 min for removal of salts and enzyme protein. Oligosaccharides were separated and identified with three descending chromatographic systems: pyridine–butan-1-ol–water (4:6:3, v/v, solvent A; Whistler and Conrad, 1954), butan-1-ol–acetic acid–water (4:1:5, v/v, solvent B; Partridge, 1946), and ethyl acetate–acetic acid–formic acid–water (18:3:1:4, v/v, solvent C Feather and Whistler, 1962).

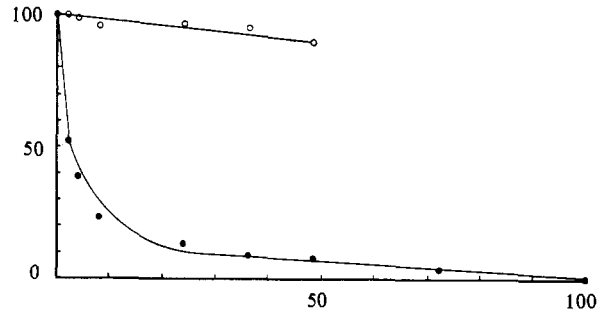


FIGURE 1: Destruction of glucuronic acid on oxidation. Vertical axis, recovery per cent. Horizontal axis, time (hours). (○—○) Glucose and (●—●) glucuronic acid.

Results

Composition of the Polysaccharide. Hydrolysates of several preparations of the polysaccharide all contained D-glucose, D-galactose, and glucuronic acid. The sugars were identified by paper chromatography and by the reaction with their respective oxidases in the case of glucose and galactose. Traces of mannose reported in the earlier work of Dudman and Wilkinson (1956) were not detected. After periodate oxidation, the yield of neutral sugars was unchanged, but the uronic acid was almost completely destroyed. Figure 1 shows the destruction of uronic acid during periodate oxidation. Recovery of glucose is also included for comparison. After carboxyl reduction the polysaccharide contained only a negligible amount of uronic acid, while the content of D-glucose was increased almost twofold. This confirms that the uronic acid was D-glucuronic acid. The results of analyses on the capsular polysaccharide before and after periodate treatment and after carboxyl reduction are shown in Table I. Tests for acetyl or other *O*-acyl groups by the alkaline hydroxylamine technique and for pyruvate were negative.

Hydrolysis of the Acid Polysaccharide. Preliminary experiments indicated that mild acid hydrolysis rapidly released galactose along with the formation of several charged fragments. Glucose was also released after a short period of such hydrolysis. No neutral material moving more slowly than glucose or galactose was detected despite use of a wide range of hydrolysis conditions. The optimal yield of charged

TABLE I: Composition of A4 Polysaccharide.

	Glucose[a]	Galactose	Glucuronic Acid
Acid polysaccharide	22.7	47.1	20.6
Oxidized polysaccharide	22.4	48.5	0.9
Carboxyl-reduced polysaccharide	40.9	46.3	2.9

[a] All figures are expressed as per cent dry weight.

TABLE II: Acid Oligosaccharide.

Fraction	Yield (μmoles/g of Polysaccharide)	Components	Molar Ratio	M_{GlcUA}[a]	R_{Glc}[b] Solvent B	R_{Glc}[b] Solvent C
E4	40	GlcUA, Gal	1:1	0.71	0.26	0.33
E3	48	GlcUA, Gal, Glc	1:1:1	0.55	0.08	0.11
E2	5	GlcUA, Gal	1:2	0.54	0.05	0.06
E1	14	GlcUA, Gal, Glc	1:2:1	0.44	0.03	0.04

[a] M_{GlcUA} = mobility relative to GlcUA. [b] R_{Glc} = mobility relative to glucose.

oligosaccharides was obtained after hydrolysis with 0.5 N H_2SO_4 at 100° for 45 min. The hydrolysate was neutralized with saturated barium hydroxide solution, filtered, and subjected to preparative paper electrophoresis. The fractions obtained in this way were checked for purity by paper chromatography in several acid solvent systems, and, if impure, rerun in solvent C. In this way, four fragments named in order of increasing electrophoretic and chromatographic mobility E1–4 were isolated. The neutral material remaining at the origin on paper electrophoresis was eluted and run in chromatograms in several solvent systems. Only glucose and galactose were detected. The complete absence of neutral oligosaccharides, although unusual, has been observed on similar treatment of other bacterial exopolysaccharides (*e.g.*, Hungerer *et al.*, 1967).

Oligosaccharide E4. The fastest moving charged oligosaccharide proved on hydrolysis and chromatography in solvents A and C to contain two sugars identified as galactose and glucuronic acid. Analysis showed that equimolar proportions were present and reduction with sodium borohydride converted all the galactose into galactitol. The aldobiouronic acid was resistant to hydrolysis with β-glucuronidase and differed from a number of β-glucuronosylgalactose disaccharides in its chromatographic mobilities. It is therefore probably an *O*-α-D-glucuronosyl-D-galactose.

Oligosaccharide E3. This compound contained glucose, galactose, and glucuronic acid in equal amounts. As all the galactose was reduced on borohydride treatment, it is the terminal reducing sugar. Treatment with β-glucosidase released all the available glucose. The products of enzymic hydrolysis were equal amounts of glucose and the aldobiouronic acid. The same products were obtained following mild acid hydrolysis (0.5 N H_2SO_4 at 100° for 30 min). This fraction appears to be a trisaccharide: *O*-β-D-glucosyl(glucuronosylgalactose).

Oligosaccharide E2. This oligosaccharide was only with difficulty separated from the trisaccharide E3. It contained glucuronic acid and galactose in the approximate molar ratio 1:2. None of the galactose was released by treatment with α- or β-galactosidase nor had β-glucuronidase any effect. Mild acid hydrolysis (as for E3) released almost equal amounts of galactose and aldobiouronic acid. Treatment with sodium borohydride converted 50% of the galactose to galactitol. When the product of borohydride reduction was submitted to mild acid hydrolysis, galactitol and the aldobiouronic acid glucuronosylgalactose were recovered. Thus the terminal galactose moiety does not form part of the aldobiouronic acid. It is concluded that the oligosaccharide E2 is a trisaccharide: *O*-α-D-glucuronosyl-D-galactosyl-D-galactose.

Oligosaccharide E1. The largest fragment obtained from partial acid hydrolysates of the acid polysaccharide contained glucose, galactose, and glucuronic acid in the approximate molar proportions 1:2:1. The terminal reducing sugar was galactose. All the glucose could be released by hydrolysis with β-glucosidase. No other glycosidases tested had any effect. Partial acid hydrolysis yielded glucose, galactose, and the aldobiouronic acid in almost equal amounts, together with traces of material indistinguishable from the two trisaccharides E2 and E3. As with the trisaccharide E2, the terminal reducing galactose did not form part of the aldobiouronic acid. The tetrasaccharide E1 thus seems to be: *O*-β-D-glucosyl(-D-glucuronosyl-D-galactosyl-D-galactose). The properties and yield of these oligosaccharides are shown in Table II.

Hydrolysis of Periodate-Oxidized Polysaccharide. As glucuronic acid was the only sugar destroyed by periodate oxidation, it seemed possible that destruction of the acid stable aldobiouronic acid might permit isolation of fragments with more labile linkages. An aliquot (500 mg) of polysaccharide was oxidized with periodate for 144 hr and excess periodate was destroyed with ethylene glycol. After exhaustive dialysis against distilled water, the product was reduced with sodium borohydride, redialyzed, and lyophilized. The product was dissolved in 1 N H_2SO_4 and hydrolyzed at 100° for 30 min. After neutralization with Amberlite IR410 resin (HCO_3form), any charged material was removed by preparative paper electrophoresis. The neutral material was eluted and separated by preparative paper chromatography in solvent A. Preliminary experiments showed the presence of three fragments running slower than galactose. These were eluted and checked for purity by paper chromatography in three solvent systems. All proved to be pure. They were named in order of increasing chromatographic mobility C1–3.

Oligosaccharide C3. Hydrolysis and analysis showed that this compound contained glucose and galactose in equimolar proportions. On borohydride treatment, all the galactose was converted into galactitol. Treatment with β-glucosidase released 30% of the available glucose, while α-glucosidase had no effect. The oligosaccharide is thus a *O*-β-D-glucosyl-

D-galactose. The configuration of this fragment was identified by degradation with lead tetraacetate according to Hungerer *et al.* (1967). After removal of ions, the degraded material was hydrolyzed and the components were identified by paper chromatography in solvents A and C. The only material detected was glucose and a spot identical with the pentose, lyxose. It gave the characteristic red color of a pentose on staining with aniline oxalate. The lyxose is thus derived from the galactose moiety which is substituted in the 3 position.

Oligosaccharide C2. Paper chromatography of hydrolysates of this fraction showed only galactose. On borohydride treatment, hydrolysis, and analysis, 50% of the galactose was found to have been converted into galactitol. The enzyme β-galactosidase released 86% of the available galactose. α-Galactosidase was without effect. The oligosaccharide C2 thus appears to be a disaccharide. The configuration of this material was confirmed by lead acetate degradation when lyxose was the sole product of oxidation. The disaccharide is therefore *O*-β-D-galactosyl-(1→3)-D-galactose.

Oligosaccharide C1. The third oligosaccharide in this series (C1) contained glucose and galactose in the molar ratio of 1:2. As determined by borohydride reduction, the terminal reducing sugar was galactose, 50% of that available being reduced to galactitol. Neither α- or β-galactosidase nor α-glucosidase had any effect on the oligosaccharide. However β-glucosidase released 65% of the available glucose. The products of enzymic hydrolysis were identified as glucose and material identified with the disaccharide *O*-β-D-galactosyl-D-galactose (C2) in equimolar amounts. Partial acid hydrolysis (0.5 N H_2SO_4 at 100° for 30 min) yielded small amounts of the free sugars together with material indistinguishable from the two disaccharides *O*-β-D-glucosyl-D-galactose (C3) and *O*-β-D-galactosyl-D-galactose (C2). From these results, it is concluded that the oligosaccharide C1 is a trisaccharide, *O*-β-D-glucosyl-β-D-galactosyl-D-galactose. As this fragment is, in the intact polysaccharide, periodate resistant the linkages may be 1→3 or the sugars may originally have had further substituents.

The properties of these three oligosaccharides are listed in Table III.

Carboxyl-Reduced Polysaccharide. A small quantity of the original glucuronic acid was not converted into glucose. To eliminate this and associated charged oligosaccharides, the following protocol was adopted. The carboxyl-reduced polysaccharide was dissolved in 1 N H_2SO_4 and hydrolyzed for 20 min at 100°. The hydrolysate was neutralized with Amberlite IR410 resin and subjected to paper electrophoresis. The neutral material was separated by preparative paper chromatography in solvent A. The glucose and material moving more slowly than galactose were eluted. The glucose was found to have a specific activity of 1449 cpm/μmole. On checking the purity of the oligosaccharides, two were resolved into two components. They were therefore rerun in solvent C. The fragments obtained are listed in order of decreasing chromatographic mobility N5 to N1.

Oligosaccharide N5b. This fraction proved on hydrolysis to contain equimolar amounts of glucose and galactose. Galactose was the terminal reducing sugar. The enzyme β-glucosidase had no effect but α-glucosidase released 30% of the available glucose. The oligosaccharide N5b is therefore a disaccharide, *O*-α-D-glucosyl-D-galactose. It was radioactive, with twice the specific activity per μmole of free glucose. The glucose moiety is thus entirely derived from glucuronic acid and the oligosaccharide corresponds to the charged fragment E3 obtained from the original polysaccharide. The confirmation of the α configuration accounts for the lack of activity of β-glucuronidase on E3. When tested by the diphenylamine reagent (Bailey and Bourne, 1960) this fraction gave a blue color similar to that obtained with maltose and cellobiose. This indicates that the disaccharide is in fact *O*-α-D-glucosyl-1→4-galactose.

TABLE III: Oligosaccharides from Oxidized Polysaccharide.

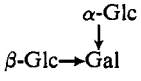
Fraction	Components	Molar Ratio	R_{Glc}[a] Solvent A	Solvent B	Solvent C
C3	Glc, Gal	1:1	0.61	0.32	0.34
C2	Gal	2	0.53	0.26	0.31
C1	Glc, Gal	1:2	0.28	0.11	0.09

[a] R_{Glc} = mobility relative to glucose.

Oligosaccharide N5a. This oligosaccharide resembled N5b in that it also contained equal amounts of glucose and galactose. It proved to be identical in all respects tested with the oligosaccharide C3 from periodate-oxidized polysaccharide. No radioactivity was associated with the fraction. It is a *O*-β-D-glucosyl-D-galactose.

Oligosaccharide N4. This fraction contained galactose only and was identical in all respects tested with the oligosaccharide C2 obtained from partial acid hydrolysates of the periodate-oxidized polysaccharide. It is a *O*-β-D-galactosyl-D-galactose.

Oligosaccharide N3. This fraction also contained both glucose and galactose, the molar proportions being 2:1. On borohydride treatment all the galactose was reduced to galactitol. No hydrolysis was obtained with β-galactosidase but α-glucosidase and β-glucosidase released 15 and 10%, respectively, of the available glucose. Only the glucose released by α-glucosidase was radioactive. Partial acid hydrolysis (0.5 N H_2SO_4 at 100° for 30 min) followed by chromatography in solvent A revealed free glucose and galactose together with two spots equidistant with oligosaccharides N5a and N5b, respectively. The glucose in the oligosaccharide N3 had the same specific activity as that from the carboxyl-reduced polysaccharide. It thus consists of equimolar amounts derived from glucose and glucuronic acid, respectively, in the original polysaccharide. From all these results it is proposed that the fraction N3 has the structure



and corresponds to a portion of the original polysaccharide with the structure

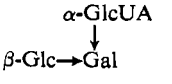


TABLE IV: Oligosaccharides from Carboxyl-Reduced Polysaccharide.

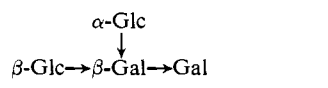
Fraction	Components	Molar Ratio	Radioactivity (cpm/μmole)	R_{Glc}[a]		
				Solvent A	Solvent B	Solvent C
N5b	Glc, Gal	1:1	2858	0.68	0.30	0.41
N5a	Glc, Gal	1:1	0	0.61	0.28	0.33
N4	Gal	2	0	0.53	0.26	0.31
N3	Glc, Gal	2:1	1501	0.43	0.10	0.15
N2b	Glc, Gal	1:2	0	0.29	0.09	0.09
N2a	Glc, Gal	1:2	2472	0.28	0.04	0.08
N1	Glc, Gal	2:2	1343	0.18	0.03	0.04

[a] R_{Glc} = mobility relative to glucose.

Oligosaccharide N2b. This oligosaccharide was also identical with a fraction isolated from the periodate-oxidized material. It contained 1 mole of glucose and 2 moles of galactose and was devoid of radioactivity. It could not be distinguished from the trisaccharide C1, *O*-β-D-glucosyl-β-D-galactosyl-D-galactose.

Oligosaccharide N2a. Like the previous fragment N2b, this material contained glucose and galactose in the molar ratio 1:2. In it, 50% of the galactose was reduced by sodium borohydride. Of the enzymes tested, β-glucosidase and β-galactosidase had no effect but a small amount of glucose was liberated by α-glucosidase. The specific activity of the glucose in the oligosaccharide and that released by enzyme treatment indicated that it was derived from glucuronic acid. Partial acid hydrolysis produced material identical with the disaccharides, *O*-α-D-glucosyl-D-galactose (N5b) and β-*O*-D-galactosyl-D-galactose (N4). The trisaccharide N2a is thus *O*-α-D-glucosyl-β-D-galactosyl-D-galactose.

Oligosaccharide N1. The slowest moving fragment, N1, obtained from the carboxyl-reduced polysaccharide contained glucose and galactose in equimolar amounts. The terminal reducing sugar determined by borohydride treatment was galactose, 50% of which was reduced. There was no release of galactose by α- or β-galactosidases. However both β-glucosidase and α-glucosidase released some glucose. Each enzyme liberated 10–15% of the total glucose available. The specific activity of the glucose in the oligosaccharide was equal to that from the polysaccharide, indicating equal derivation from glucose and glucuronic acid. The glucose released by α-glucosidase was radioactive but not that from β-glucosidase action. Partial acid hydrolysis (0.5 N H_2SO_4 for 30 min at 100°) released glucose and galactose together with all the oligosaccharides already listed from the carboxyl-reduced polysaccharide. Some of these fragments were only released in very small amounts. From these results it seems that the oligosaccharide N1 is a tetrasaccharide for which the proposed structure is



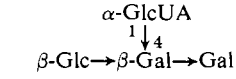
In this structure, the α-glucosyl residue is that derived from glucuronic acid.

Table IV lists the properties of the fragments obtained from carboxyl-reduced polysaccharide.

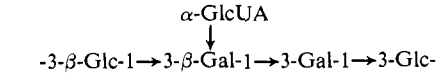
Discussion

One of the problems in selecting a system for the study of bacterial exopolysaccharide synthesis is the lack of knowledge of the structure of such polymers. The observation by Dudman and Wilkinson (1956) that *K. aerogenes* type 8 secreted a capsule containing glucose, galactose, and glucuronic acid indicated that it might provide a suitable system. The molar ratio of the component sugars has been confirmed as glucose:galactose:glucuronic acid 1:2:1. This indicates the possibility of a tetrasaccharide repeating unit, whose structure is simplified by the absence of acyl or pyruvyl groups.

The largest fragment isolated from the original polysaccharide or from the carboxyl-reduced polymer was a tetrasaccharide. From all the data obtained, the structure of this tetrasaccharide in the native polysaccharide is postulated as

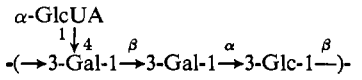


If this is in fact the repeating unit, some reconciliation with the results is required. The position of the glucuronic acid as a side chain renders it susceptible to periodate oxidation as was confirmed. However, in a repeating tetrasaccharide structure, the periodate-oxidized material should yield fragments larger than the tetrasaccharide β-glucosyl-β-galactosylgalactose, as all the neutral sugar residues were periodate resistant. The observation that the terminal reducing galactose in the above structure and the terminal glucose moiety were not oxidized by periodate indicates that both are substituted in the 3 position. Thus if there is a repeating structure of the tetrasaccharide type, the polymer has the general form



This should therefore yield a number of possible trisaccha-

rides and larger fragments on partial hydrolysis of the oxidized polysaccharide. One possibility, albeit unlikely, is the presence of some other periodate-sensitive constituent between the galactosyl and glucosyl residues to give the sequence -3-galactosyl-1-X-3-glucose. The alternative is that the linkage between galactose and glucose is extremely labile. Studies on the structure of lipopolysaccharides from *Salmonella* species (Osborn *et al.*, 1964; Sutherland *et al.*, 1965) indicated that the linkage in the disaccharide α-galactosyl-1→3-glucose is indeed extremely acid labile. No disaccharide of this structure was obtained in partial acid hydrolysates of the lipopolysaccharides and its configuration was only proved by other means (Osborn *et al.*, 1964). It is not known whether the corresponding β-linked disaccharide is equally acid labile. Although both types of this disaccharide have been synthesized, no record of their isolation from polysaccharides of which they form part has been given (Bailey, 1965). The presence of this highly acid-labile linkage in the polysaccharide of *K. aerogenes* type 8 would lead to the preferential production of the tetrasaccharide isolated. The corresponding neutral tetrasaccharide would be obtained from the carboxyl-reduced polymer and the trisaccharide from the oxidized polysaccharide. It is therefore postulated that the repeating unit of this exopolysaccharide is a tetrasaccharide with the structure



It is interesting that, along with the exopolysaccharides of *K. aerogenes* types 2 and 54 (Gahan *et al.*, 1967; Conrad *et al.*, 1966), the basic structure of *K. aerogenes* type 8 capsular polysaccharide should be a tetrasaccharide in which one of the sugars forms a short side chain. The only other similarity between these three polymers is that each tetrasaccharide contains three different sugars and that two moles of glucose are present in each.

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