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STRUCTURAL INVESTIGATION OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* SEROTYPE K44

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ABSTRACT

The capsular polysaccharide from *Klebsiella* K44 has been investigated by the techniques of methylation, base-catalyzed elimination, Smith degradation, and partial hydrolysis. The last-named yielded an oligosaccharide corresponding to one repeating unit. The anomeric configurations of the sugar residues were determined by ^{1}H- and ^{13}C-n.m.r. spectroscopy. The polysaccharide has a fractional acetyl content and is the first in this series to be based on a linear, pentasaccharide repeating unit.

→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→

INTRODUCTION

The capsular polysaccharides of the genus *Klebsiella* can be grouped into several chemotypes, one of which comprises the serologically distinct strains *Klebsiella* K17, K23, K44, and K45. The constituent sugars of the chemogroup are D-glucuronic acid, D-glucose, and L-rhamnose[1]. The structure of the polysaccharide from K23 has been published[2], that of K17 has been completed[3], K45 is being examined[4], and we now report our results on the polysaccharide from *Klebsiella* K44; a preliminary account has appeared[5]. Heidelberger and Nimmich predicted[6], on the basis of serological cross-reactions, that the capsular polysaccharide from K44 would contain two, or three, consecutive rhamnose residues; this is confirmed by our results.

RESULTS AND DISCUSSION

N.m.r. spectra and composition

Klebsiella K44 bacteria were grown on an agar medium, and the polysaccharide was purified by one precipitation with Cetavlon[2]. The material thus obtained, having a molecular weight of 2.6×10^5 (as determined by gel chromatography), was homogeneous by electrophoresis, and had $[\alpha]_D$ +4.0°.

The anomeric region of both the ^{1}H- and ^{13}C-n.m.r. spectra of the K44 polysaccharide, which had to be mildly depolymerized in order to lessen the viscosity,

TABLE I

N.M.R. DATA FOR *Klebsiella* K44 CAPSULAR POLYSACCHARIDE AND THE OLIGOSACCHARIDES ISOLATED[a]

Compound	δ^b	$J_{1,2}$ *(Hz)*[c]	^1H*-n.m.r. data*		^{13}C*-n.m.r. data*	
			Integral (H)	*Assignment*[d]	*p.p.m.*[e]	*Assignment*[f]
Rha $\overset{1\,3}{\underset{\alpha}{-}}$ Glc $\overset{1\,2}{\underset{\beta}{-}}$ erythritol (**1**)	5.18	s	1	α-Rha	103.3	β-Glc
	4.60	8	1	β-Glc	101.9	α-Rha
	1.28	6 ($J_{5,6}$)	3	CH_3 of Rha	63.2	C-6 of Glc
					62.0	C-1 of erythritol
					61.6	C-4 of erythritol
					17.3	CH_3 of Rha
GlcA-$\overset{1\,2}{\underset{\beta}{-}}$ Rha-OH (**2**)	5.34	b	0.6	α-Rha-OH	105.0	β-GlcA
	4.85	s	0.4	β-Rha-OH	93.8	α,β-Rha
	4.70	6	1	β-GlcA	17.6	CH_3 of Rha
	1.28	6 ($J_{5,6}$)	3	CH_3 of Rha		
GlcA-$\overset{1\,2}{\underset{\beta}{-}}$ Rha $\overset{1\,3}{\underset{\alpha}{-}}$ Rha-OH (**3a**[g])	5.32	s	1	α-Rha	105.1	β-GlcA
	5.11	b	0.4	α-Rha-OH	101.6	α-Rha
	4.88	s	0.6	β-Rha-OH	94.8	α-Rha-OH
	4.69	7	1	β-GlcA	94.2	β-Rha-OH
	1.29	6 ($J_{5,6}$)	6	CH_3 of Rha	17.5	CH_3 of Rha

Glc-(1→4)-β Glc-(1→4)-α GlcA-(1→2)-β Rha-OH (**4a**[g])	5.42	3	1	α-Glc	105.1	β-GlcA
	5.30	b	0.6	α-Rha-OH	103.3	β-Glc
	4.83	s	0.4	β-Rha-OH	99.3	α-Glc
	4.69	8	1	β-GlcA	93.8	α,β-Rha
	4.53	7	1	β-Glc	61.4	C-6 of α-Glc
	1.28	6 ($J_{5,6}$)	3	CH_3 of Rha	60.2	C-6 of β-Glc
					17.6	CH_3 of Rha
Glc-(1→4)-β Glc-(1→4)-α GlcA-(1→2)-β Rha-(1→3)-α Rha-OH (**5**)	5.41	3	1	α-Glc	105.2	β-GlcA
	5.29	b	1	α-Rha	103.2	β-Glc
	5.09	b	0.5	α-Rha-OH	101.7	α-Rha
	4.86	s	0.5	β-Rha-OH	99.4	α-Glc
	4.69	8	1	β-GlcA	94.8	α-Rha-OH
	4.53	7	1	β-Glc	94.2	β-Rha-OH
	1.27	6 ($J_{5,6}$)	6	CH_3 of Rha	61.5	C-6 of α-Glc
					60.2	C-6 of β-Glc
					17.4	CH_3 of Rha
-3)-Glc-(1→4)-β Glc-(1→4)-α GlcA-(1→2)-β Rha-(1→3)-α Rha-(1→	5.41	4.5	1	α-Glc	105.2	β-GlcA
	5.28	~1	1	α-Rha	103.2	β-Glc
	5.15	1.5	1	α-Rha	101.8	α-Rha(2)
(Native K44 polysaccharide)	4.71	8	1	β-GlcA	99.8	α-Glc
	4.54	8	1	β-Glc	61.6	C-6 of β-Glc
					60.2	C-6 of α-Glc
	1.28	6.5	6	CH_3 of Rha	17.4	CH_3 of Rha

[a]For the origin of oligosaccharides **1**–**5**, see text. [b]Chemical shift relative to internal acetone; δ 2.23 downfield from sodium 4,4-dimethyl-4-silapentene-1-sulfonate (D.S.S.). [c]b = broad, unable to assign accurate coupling constant; s = singlet. [d]For example, α-Rha = proton on C-1 of α-linked L-Rha residue. [e]Chemical shift, in p.p.m. downfield from Me_4Si, relative to internal acetone; 31.07 p.p.m. downfield from D.S.S. [f]As for [d], but for anomeric, ^{13}C nuclei. [g]Oligosaccharides **3** and **4** were not completely pure, but **3a** and **4a** constituted the major component of each fraction; for clarity, minor, n.m.r. signals are omitted (see text for details).

showed that the repeating unit was a pentasaccharide[7,8]. The p.m.r. spectrum indicated that (*a*) three of the sugar units were α-linked and two β-linked, (*b*) two 6-deoxy sugar units were present, and (*c*) some hydroxyl groups were acetylated. Integration showed that the last-named were present to the extent of only one acetate group to approximately three repeating units; pyruvate was absent. The ^{13}C-n.m.r. spectrum also demonstrated that there were two hexose units unsubstituted at O-6 (see Table I).

The ratio of rhamnose to glucose (after reduction of the uronic acid) was found to be 9:11 and the configurations were determined to be L and D, respectively, by measurement of the c.d. spectra of the alditol acetates[9].

Methylation analysis

Methylation[10] of K44 polysaccharide, followed by reduction of the uronic ester and analysis of the hydrolysis products by g.l.c.–m.s.[11,12], gave the results shown in Table II, columns I and II. Despite the low yield of 2,3-di-*O*-methylglucose, presumably because of incomplete reduction, the data are consistent with the concept of a linear, pentasaccharide, repeating unit.

Uronic acid degradation

A portion of the methylated polysaccharide was degraded with base[13,14], and the product, which was not polymeric, was directly alkylated with ethyl iodide. Table II, column III, shows the formation of 2-*O*-ethyl-3,4-di-*O*-methylrhamnose

TABLE II

METHYLATION ANALYSES OF ORIGINAL, AND DEGRADED, *Klebsiella* K44 CAPSULAR POLYSACCHARIDE

Methylated sugars[a] *(as alditol acetates)*	T[b]		*Mole %*[c]			
	Column B[d]	*Column C*[e]	*I*[f]	*II*	*III*	*IV*
2,3,4-Rha	0.48					49.8[g]
2-Et-3,4-Rha[h]		0.50			26.4	
3,4-Rha	0.77	0.89	19.8	19.9		
2,4-Rha	0.90	0.95	23.7	22.4	31.9	
2,4,6-Glc	1.56	1.51	46.4	22.0	34.0	33.5
2,3,6-Glc	1.56	1.72		21.8	7.7	
2,3-Glc (from D-GlcA)	2.14	2.41	10.2	13.9		

[a]2,3,4-Rha = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol, etc. [b]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [c]Values corrected by use of effective, carbon response-factors given by Albersheim *et al.*[30]. [d]OV-17 column, programmed at 175° for 8 min, and then 2°/min to 210°. [e]OV-225 column, programmed at 180° for 4 min, and then at 2°/min to 230°. [f]I, original polysaccharide, column *B*; II, original polysaccharide, column *C*; III, polysaccharide after uronic acid degradation, column *C*; IV, polysaccharide after periodate oxidation–Smith degradation, compound **1**, column *B*. [g]Remainder of the total is made up of 2-*O*-acetyl-1,3,4-tri-*O*-methyl-D-erythritol, 16.7%. [h]1,5-Di-*O*-acetyl-2-*O*-ethyl-3,4-di-*O*-methyl-L-rhamnitol.

and the almost total removal, by peeling, of the 4-linked D-glucose unit. Thus, the structure of the region surrounding the uronic acid may be written as

→4)-D-Glc*p*-(1→4)-D-Glc*p*A-(1→2)-L-Rha*p*-(1→.

Smith degradation

The polysaccharide consumed 3.6 molecules of periodate per repeating unit (theoretical, 3.0) in 24 h. Smith degradation[15], followed by gel chromatography, yielded oligomer **1**, having $[\alpha]_D$ −44°. The p.m.r. spectrum (see Table I) demonstrated the presence of two anomeric protons, one corresponding to a β-linked glucosyl residue and the other attributable to an α-linked rhamnosyl residue. The number of signals in the ^{13}C-n.m.r. spectrum, including three in the range 61–63 p.p.m., indicated the existence of a tetritol (erythritol) as the nonreducing aglycon (see Table I). Methylation analysis of **1** (see Table II, column IV), in conjunction with the spectral data, showed that **1** is

α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→2)-D-erythritol.

1

The nature of **1**, together with the result of the β-elimination experiment, established the sequence of the sugar units in the polysaccharide, but left unresolved the configuration of certain glycosidic linkages; this was solved by partial hydrolysis.

Partial hydrolysis

The polysaccharide from *Klebsiella* K44 was partially hydrolyzed under two sets of conditions, namely, with 0.1 and 0.5M trifluoroacetic acid. Neutral and acidic oligosaccharides were separated on an ion-exchange resin, and the latter were then subjected to gel chromatography. Oligosaccharides **2–5** were obtained, of which

TABLE III

METHYLATION ANALYSES OF ACIDIC OLIGOSACCHARIDES ISOLATED AFTER PARTIAL, ACID HYDROLYSIS OF *Klebsiella* K44 CAPSULAR POLYSACCHARIDE

Methylated sugars[a] *(as alditol acetates)*	T[b]	*Mole* %[c]			
		I[d]	*II*	*III*	*IV*
3,4-Rha	0.77	24.8	27.5	36.2	61.3
2,4-Rha	0.90	19.9	7.5	22.9	
2,3,4,6-Glc	1.00	22.6	28.0	6.7	
2,3,6-Glc	1.56	20.4	18.1		
2,3,4-Glc	1.62			27.0	38.7
2,3-Glc (from D-GlcA)	2.14	12.3	18.9	7.2	

[a]3,4-Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol, etc. [b]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an OV-17 column, programmed at 175° for 8 min, and then at 2°/min to 210°. [c]Values corrected by use of effective, carbon response-factors given by Albersheim *et al.*[30]. [d]I, pentasaccharide, compound **5**; II, tetrasaccharide, component **4**; III, trisaccharide, component **3**; IV, aldobiouronic acid, compound **2**.

2 and **5** were single compounds, and **3** and **4** were mixtures. Oligosaccharide **5** was the main component isolated under each set of conditions.

The fractions were examined by n.m.r. spectroscopy and by methylation analyses; details are given in the Experimental section and in Tables I and III. Oligosaccharide **2** was shown to be β-D-Glc*p*A-(1→2)-L-Rha, thus confirming the result obtained by β-elimination; this is the aldobiouronic acid found in the polysaccharides from K18 (ref. 16), K36 (ref. 17), and K81 (ref. 18). Oligosaccharide **5** was a pentasaccharide corresponding to one repeating unit having the structure

β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→2)-α-L-Rha*p*-(1→3)-L-Rha.

Establishment of the structure of **5**, taken in conjunction with the results of the previous experiments, made it unnecessary to examine the neutral oligosaccharides.

The structures shown in Table I for the major components **3a** and **4a** in the mixed fractions may be readily rationalized when the nature of **5** is known. The trisaccharide **3a**, which amounted to ~80% of the fraction, had been isolated in studies on the capsular polysaccharides from *Klebsiella* K36 (ref. 17) and K81 (ref. 18).

It therefore follows that the structure of the capsular polysaccharide from *Klebsiella* K44 is based on the following pentasaccharide repeating unit,

→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→,

to which must be added, on average, one *O*-acetyl group for each three repeating units. The presence of a non-integral proportion of acetate is not uncommon in these capsular polysaccharides, and has been found, for example, in those of strains K20 (ref. 19) and K59 (ref. 20).

Other *Klebsiella* capsular polysaccharides containing uronic acid have been shown to have linear structures, but these have been based on a trisaccharide repeating unit [K1 (ref. 21), K5 (ref. 22), and K63 (ref. 23)], a tetrasaccharide [K4 (ref. 24) and K6 (ref. 25)], or a hexasaccharide [K70 (ref. 26) and K81 (ref. 18)]. The polysaccharide from K44 appears to be the first example of such a pentasaccharidic structural pattern.

EXPERIMENTAL

General methods. — Instrumentation used has been described[2], and photocopies of the n.m.r. spectra recorded in Table I are available[3]. For descending paper-chromatography, the following solvent systems (v/v) were used: (*1*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, and (*2*) 8:2:1 ethyl acetate–pyridine–water. Analytical, g.l.c. separations were performed in stainless-steel columns (1.8 m × 3 mm), with a carrier-gas flow-rate of 20 mL/min. Columns used were (*A*) 3% of SP-2340 on Supelcoport (100–120 mesh), (*B*) 3% of OV-17 on Gas Chrom Q (100–120 mesh), and (*C*) 3% of OV-225 on Supelcoport (100–120 mesh). Preparative g.l.c. separations were performed in a column (1.8 m × 6.3 mm) of (*D*) 5% of Silar 10C on Gas Chrom Q (100–120 mesh).

Preparation and properties of Klebsiella *K44 capsular polysaccharide.* — A

culture of *Klebsiella* K44 (7730), obtained by courtesy of Dr. I. Ørskov, was grown and isolated as described previously for *Klebsiella* K23 (ref. 2). The isolated polysaccharide, in the sodium salt form, had $[\alpha]_D$ +4.0° (*c* 1.1, water). The purity of the polysaccharide was checked by electrophoresis, using a 1% solution on cellulose acetate strips (Sepraphore III; 15 × 2.5 cm) in veronal buffer, pH 8.6 (LKB-Produkter AB, Stockholm 12, Sweden) at 300 V for 90 min and then development in either Alcian Blue in citrate-buffered ethanol (pH 4) or periodate–Schiff reagent. Homogeneity was also confirmed by gel chromatography, by courtesy of Dr. S. C. Churms, University of Cape Town, South Africa, who determined the molecular weight of K44 polysaccharide to be 2.6×10^5.

The ^{1}H-n.m.r. spectrum of partially depolymerized K44 polysaccharide (0.4M trifluoroacetic acetic acid for 15 min at 95°), in D_2O at 90°, revealed signals corresponding to five anomeric protons at δ 5.41 (1 H, $J_{1,2}$ 4.5 Hz), 5.28 (1 H, $J_{1,2} \sim 1$ Hz), 5.15 (1 H, $J_{1,2}$ 1.5 Hz), 4.71 (1 H, $J_{1,2}$ 8 Hz), and 4.54 (1 H, $J_{1,2}$ 8 Hz). Two unresolved doublets, centered at δ 1.28 (6 H, $J_{5,6}$ 6.5 Hz), were signals attributable to the methyl groups of two 6-deoxyhexose residues (see Table I). A sharp singlet at δ 2.17 integrated to approximately one proton (by comparison with the methyl signal of rhamnose), and was assigned to the methyl group of an acetate. Migration of this signal to δ 1.91 upon addition of sodium hydroxide to the contents of the sample tube confirmed this assignment.

The ^{13}C-n.m.r. spectrum of partially depolymerized K44 polysaccharide showed four signals in the anomeric region, at 105.2, 103.2, 101.8, and 99.8 p.p.m., with the signal at 101.8 p.p.m. being approximately twice the height of the other three signals. Signals at 61.6 and 60.2 p.p.m. due to C-6 of two hexose units, and at 17.4 p.p.m., due to C-6 of 6-deoxyhexose units also appeared. No signal from the CH_3 of acetate was distinguishable (see Table I).

Analysis of sugar constituents. — Sugar analysis was performed as previously described[2]. The alditol acetates of rhamnose and glucose were identified by g.l.c. (column *A*; programmed at 195° for 4 min, and then at 2°/min to 260°) and found to be present in the ratio of 9:11. Preparative g.l.c. (column *D*; programmed at 210°, and then at 4°/min to 250°) to isolate the derivatives, and then c.d. measurement, showed $\Delta\varepsilon_{215}^{MeCN}$ −1.8 for the rhamnitol pentaacetate, and $\Delta\varepsilon_{215}^{MeCN}$ +0.30 for the glucitol hexaacetate.

Methylation analysis of native polysaccharide. — A sample of K44 polysaccharide was passed through a column of Amberlite IR-120 (H^+) resin, and then methylated by the Hakomori[10] procedure. Methylation was incomplete, and treatment with the Purdie reagents[27] was needed in order to give a product that showed negligible absorption in the hydroxyl-group region (~3500 cm^{-1}) of the infrared spectrum. Carboxyl reduction of the methylated polysaccharide with sodium borohydride in 1:1 oxolane–ethanol, hydrolysis with 2M trifluoroacetic acid, and reduction with sodium borohydride were followed by acetylation with 1:1 acetic anhydride–pyridine. The mixture of acetates of partially methylated alditols was analyzed by g.l.c.–m.s.[11,12]. The alditol acetates of 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2,4,6-

tri-*O*-methylglucose, 2,3,6,-tri-*O*-methylglucose, and 2,3-di-*O*-methylglucose were identified (see Table II, columns I and II).

Uronic acid degradation[13,14]. Methylated K44 polysaccharide (37 mg) was carefully dried and then, together with a trace of *p*-toluenesulfonic acid, dissolved in 19:1 dimethyl sulfoxide–2,2-dimethoxypropane (20 mL) under nitrogen in a sealed flask; dimethylsulfinyl anion (10 mL) was now added, and allowed to react at room temperature. After 18 h, the degraded material was alkylated directly[14] with ethyl iodide. Following neutralization of the base with 50% acetic acid, and addition of water, the ethylated, degraded product was isolated by partition between chloroform and the aqueous solution. Hydrolysis of the isolated product was performed with 2M trifluoroacetic acid: g.l.c.–m.s. analysis of the alditol acetate derivatives yielded peaks corresponding to 2-*O*-ethyl-3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2,4,6-tri-*O*-methylglucose, and a minor peak due to 2,3,6-tri-*O*-methylglucose (see Table II, column III).

Periodate oxidation of K44 polysaccharide[15]. — A solution of K44 polysaccharide (300 mg) in water (50 mL) was mixed with 0.1M sodium periodate (50 mL). The reaction was allowed to proceed at 4° in the dark, and the periodate consumption was monitored by analyzing 1-mL aliquots by the Fleury–Lange method[28]. Periodate consumption was rapid, and reached a level of 3.6 molecules per repeating unit of *Klebsiella* K44 (theoretical, 3 molecules) within 24 h. Following the addition of ethylene glycol, dialysis, reduction with sodium borohydride, and re-dialysis, the polyalcohol (192 mg) was obtained by freeze-drying.

Smith degradation[15] of the polyol (105 mg) with 0.5M trifluoroacetic acid during 17 h at room temperature yielded a mixture of products which was separated by gel-filtration chromatography in a column (100 × 3 cm) of Bio-Gel P-2. Several fractions (total weight 82 mg) were obtained, but only one was found to be a pure compound, as judged by ^{1}H-n.m.r. spectroscopy.

Compound **1** (R_{Glc} 0.44, solvent *2*) had $[\alpha]_D$ −44° (*c* 0.87, water). In the ^{1}H-n.m.r. spectrum, signals attributable to anomeric protons were observed at δ 5.18 (s, 1 H) and 4.60 (1 H, $J_{1,2}$ 8 Hz), as well as δ 1.28 (3 H, $J_{5,6}$ 6 Hz) due to the methyl group of rhamnose (see Table I). The ^{13}C-n.m.r. spectrum showed signals at 103.3 and 101.9 p.p.m. due to anomeric carbon atoms, at 63.2, 62.0, and 61.6 p.p.m. due to C-6 of glucose and C-1 and C-4 of erythritol, and at 17.3 p.p.m. from C-6 of rhamnose (see Table I). Hakomori methylation of **1**, followed by hydrolysis, reduction, and acetylation, yielded the alditol acetates from 2,3,4-tri-*O*-methylrhamnose and 2,4,6-tri-*O*-methylglucose, as analyzed by g.l.c.–m.s. Only a minor proportion of 2-*O*-acetyl-1,3,4-tri-*O*-methylerythritol was detected, because of its volatility (see Table III, column IV).

Partial hydrolysis of K44 polysaccharide with acid. — A portion of K44 polysaccharide (666 mg) was partially hydrolyzed in 0.1M trifluoroacetic acid at 95° in an apparatus similar to that described by Galanos and co-workers[29]. After 38 h, most of the material had been dialyzed, and only a small amount (40 mg) of polysaccharide remained in the dialysis sac. All of the dialyzed material in as small a

volume as possible (5–10 mL) was then applied to a column (30 × 1.5 cm) of Bio-Rad AG1-X2(Cl^-) resin. Neutral compounds were eluted with water (1.600 L) and the eluate was freeze-dried (yield 340 mg), but the residue was not further examined. Acidic compounds were eluted with 10% formic acid (200 mL), and the eluate was several times evaporated to dryness under diminished pressure with water, in order to eliminate formic acid, and then freeze-dried (yield 220 mg).

The acidic fraction, free from neutral compounds as judged by paper chromatography (solvent *2*), was separated by gel-filtration chromatography on a column (100 × 3 cm) of Bio-Gel P-2, using 500:5:2 water–pyridine–acetic acid for irrigation at a flow rate of 10 mL/h. No material was present in the void volume (160 mL; blue dextran) after collection and freeze-drying. Fractions (2–2.5 mL) were collected in tared tubes, freeze-dried, and weighed. Every third tube was analyzed by paper chromatography (solvent *1*).

A major component (**5**, 32 mg) having R_{Glc} 0.14 (solvent *1*) and $[\alpha]_D$ +25° (*c* 1.95, water) was obtained. The ^{1}H-n.m.r. spectrum of **5** showed signals attributable to anomeric protons at δ 5.41 (1 H, $J_{1,2}$ 3 Hz), 5.29 (b, 1 H), 5.09 (b, 0.5 H), 4.86 (s, 0.5 H), 4.69 (1 H, $J_{1,2}$ 8 Hz), and 4.53 (1 H, $J_{1,2}$ 7 Hz). A doublet ($J_{5,6}$ 6 Hz) at δ 1.27 (6 H) was also apparent (see Table I). In the ^{13}C-n.m.r. spectrum, four signals for nonreducing, anomeric atoms, at 105.2, 103.2, 101.7, and 99.4 p.p.m., and two signals for reducing, anomeric atoms, at 94.8 and 94.2 p.p.m., were observed. Two signals at 61.5 and 60.2 p.p.m. due to C-6 of two glucosyl residues, and a large one at 17.4 p.p.m. due to C-6 of two rhamnosyl residues, were also evident (see Table I). A portion (16 mg) of compound **5** was methylated under Hakomori conditions. The permethylated product was then reduced with lithium aluminum hydride in refluxing oxolane overnight, the product hydrolyzed with 2M trifluoroacetic acid, the hydrolyzate reduced with sodium borohydride, and the product acetylated with 1:1 acetic anhydride–pyridine. G.l.c.–m.s. analysis indicated the presence of 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylglucose, 2,3,6-tri-*O*-methylglucose, and 2,3-di-*O*-methylglucose as their alditol acetate derivatives (see Table II, column I).

A minor fraction (**3**, 15 mg), a mixture inseparable by the gel chromatography described, having R_{Glc} 0.77 (solvent *1*) and $[\alpha]_D$ −19° (*c* 1.44, water), was also obtained. The major signals in the ^{1}H-n.m.r. spectrum occurred at δ 5.32, 5.11, 4.88, and 4.69 in the anomeric region, and at δ 1.29 due to CH_3 of rhamnose (see Table I). A small signal at δ 5.43 that integrated to ~0.2 proton indicated the presence of an α-D-glucosyl component also. In the ^{13}C-n.m.r. spectrum, two signals for nonreducing, anomeric atoms, at 105.1 and 101.6 p.p.m., and two signals for reducing, anomeric atoms, at 94.8 and 94.2 p.p.m., as well as a signal at 17.5 p.p.m. for a rhamnose methyl group, were observed (see Table I). Other, less intense signals were present at 99.6 (nonreducing α-D-glucose), 93.7 (reducing rhamnose), and 60.9 p.p.m. (C-6 of glucose). Hakomori methylation of **3**, followed by carboxyl-reduction, hydrolysis, and derivatization were conducted under the same conditions as for compound **5**. G.l.c.–m.s. analysis identified a mixture of alditol acetates corre-

sponding to 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylglucose, 2,3,4-tri-*O*-methylglucose, and 2,3-di-*O*-methylglucose in the approximate ratios of 5:3:1:4:1 (see Table III, column III).

Another partial-hydrolysis experiment was performed on K44 polysaccharide (240 mg) with 0.5M trifluoroacetic acid for 1 h at 95°. After removal of the acid by several successive evaporations with water, and neutralization with 0.1M sodium hydroxide, the hydrolyzed material was separated into neutral and acidic fractions by use of Amberlite AG-1X-2(Cl^-) ion-exchange resin. When separated by gel chromatography as already described, the acidic components (200 mg) provided an aldobiouronic acid (**2**, 7 mg), a trisaccharide mixture (**3**, 17 mg), a tetrasaccharide mixture (**4**, 17 mg), and an acidic pentamer (**5**, 39 mg).

The aldobiouronic acid **2** (refs. 16 and 17) had $[\alpha]_D$ −7.0° (*c* 0.46, water). The ^{1}H-n.m.r. spectrum contained signals at δ 5.34 (b, 0.6 H), 4.85 (s, 0.4 H), and 4.70 (1 H, $J_{1,2}$ 6 Hz) in the anomeric region, as well as at δ 1.28 (3 H, $J_{5,6}$ 6 Hz) due to the methyl group of rhamnose (see Table I). In the ^{13}C-n.m.r. spectrum, signals occurred in the anomeric region at 105.0 (nonreducing) and 93.8 p.p.m. (reducing), and at 17.6 p.p.m. for C-6 of rhamnose (see Table I). Hakomori methylation of **2**, followed by carboxyl-reduction, hydrolysis, and derivatization in the usual way, produced the alditol acetates of 3,4-di-*O*-methylrhamnose and 2,3,4-tri-*O*-methylglucose (g.l.c.–m.s.; Table III, column IV).

Fraction **3** was the same mixture as that obtained from the first partial hydrolysis, as judged by the identical, respective ^{1}H- and ^{13}C-n.m.r. spectra.

Fraction **4**, a tetrasaccharide mixture not completely separated by gel chromatography, had $[\alpha]_D$ +41° (*c* 1.53, water). The ^{1}H-n.m.r. spectrum contained major, anomeric signals at δ 5.42, 5.30, 4.83, 4.69, and 4.53, and two sets of unresolved doublets ($J_{5,6}$ 6 Hz) centered at δ 1.28 (see Table I). An additional signal (~0.2 H) was apparent at δ 5.10, suggesting the presence of a minor, α-L-rhamnosyl component as an impurity. Four major, anomeric signals were present in the ^{13}C-n.m.r. spectrum at 105.1, 103.3, 99.3, and 93.8 p.p.m., along with signals at 61.4 (C-6 of glucose), 60.2 (C-6 of glucose), and 17.6 p.p.m. (C-6 of rhamnose) as listed in Table I. A signal at 101.7 p.p.m. confirmed the presence of an α-L-rhamnosyl residue. Derivatization of **4** to afford partially methylated alditol acetates (in the usual way) produced a mixture, identified by g.l.c.–m.s., corresponding to 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylglucose, 2,3,6-tri-*O*-methylglucose, and 2,3-di-*O*-methylglucose (see Table III, column II).

Compound **5**, the acidic pentasaccharide, gave a ^{1}H-n.m.r. spectrum identical to that from the pentamer isolated after the first, partial-hydrolysis experiment.

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