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STRUCTURAL INVESTIGATION OF *Klebsiella* SEROTYPE K32 POLYSACCHARIDE

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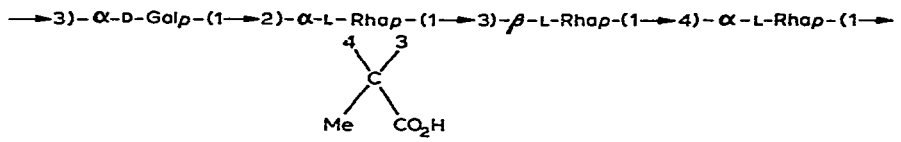
ABSTRACT

The capsular polysaccharide from *Klebsiella* K32 has been studied by using methylation, periodate oxidation, and partial-hydrolysis techniques. The polysaccharide is shown to comprise the four-sugar repeating unit below. Features of interest in this structure include the presence of a β-linked L-rhamnosyl residue, and the extreme lability of the 1-carboxyethylidene acetal towards acid. N.m.r. spectroscopy was used extensively to establish the nature of the anomeric linkages and to identify oligosaccharides obtained by the various degradative techniques used.



INTRODUCTION

In a qualitative analysis of the capsular polysaccharide from *Klebsiella* K32, Nimmich[1,2] found the presence of only galactose, rhamnose, and pyruvic acid. The absence of any uronic acid indicates that, as a capsular antigen, the polysaccharide relies on the 1-carboxyethylidene acetal for its overall negative charge and, hence, its virulence. The polysaccharides of *Klebsiella* K types 56 (ref. 3) and 72 (ref. 4) had been investigated previously, and the present study completes the structural analysis of this trio of polysaccharides of *Klebsiella* serotypes in which pyruvic acid is the only acidic component.

RESULTS AND DISCUSSION

Composition and n.m.r. spectra. — Isolation and purification of the polysaccharide were conducted as previously described[5]. One precipitation with Cetavlon was performed. The purified material had $[\alpha]_D$ +113°. ^{1}H-N.m.r. spectroscopy of the polysaccharide in deuterium oxide showed the presence of four anomeric protons,

and also indicated that three 6-deoxy sugars (rhamnose residues) and one 1-carboxyethylidene acetal group were present per repeating unit[6,7a,7b]. After acid hydrolysis of the polysaccharide, paper chromatography of the hydrolyzate indicated the presence of rhamnose and galactose. Quantitation as alditol acetates proved these two sugars to be present in the percentages of 72 and 28, respectively. By circular dichroism measurements on the derived alditol acetate[8], the rhamnose was shown to belong to the L series, and the galactose was confirmed as being in the D configuration by the positive action of D-galactostat on the free sugar.

Methylation of original and partially depyruvalated polysaccharide. — Complete methylation of K32 polysaccharide proved to be difficult. Owing to the lability of the 1-carboxyethylidene acetal (see later), the polysaccharide could not be converted into the free-acid form without the loss of a substantial proportion of the acetal groups. Methylation of the polysaccharide was hindered by the low solubility in dimethyl sulfoxide of the native material (in the sodium salt form). Two successive, Hakomori[9] treatments and a subsequent Purdie[10] methylation were necessary in order to achieve a product that showed no hydroxyl absorption in the infrared region. Methylation analysis[11,12] of the native polysaccharide and of material that had been converted into its free acid by passage through an ion-exchange resin (see Table I, columns I and II) indicated that *Klebsiella* K32 polysaccharide is composed of a linear, tetrasaccharide repeating-unit comprising three L-rhamnosyl residues (linked through O-2, -3, and -4, respectively) and one D-galactosyl residue (linked through O-3). The 1-carboxyethylidene group is present as an acetal spanning O-3 and O-4 of the L-rhamnosyl residue linked through O-2. Separation of the partially methylated alditol acetates corresponding to the 2,3-, 2,4-, and 3,4-di-*O*-methyl-L-rhamnose

TABLE I

METHYLATION ANALYSES OF NATIVE AND DEACETALATED[a] *Klebsiella* K32 CAPSULAR POLYSACCHARIDE

Methylated sugar[b]	T[c]	*I*	*II*
		Mole %[d,e]	
3,4-Rha	0.88	—	15.1
2,3-Rha	0.91	26.8	25.6
2,4-Rha	1.00	27.0	23.3
Rha	1.53	21.6	8.2
2,4,6-Gal	1.61	24.7	28.0

[a]Conversion of the native polysaccharide into the free acid form by passage through IR-120 (H^+) ion-exchange resin resulted in removal of ~ 50% of the 1-carboxyethylidene acetal groups. [b]3,4-Rha = 3,4-di-*O*-methyl-L-rhamnose, etc. [c]Retention time of corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity. Column used was 3% of HIEFF-1B on Gas Chrom Q (100–120 mesh), programmed from 160 to 190° at 1°/min. Column dimensions: 1.83 m × 3.2 mm. [d]Figures are corrected by use of the molar response factors given by Albersheim *et al.*[13]. [e]I, native polysaccharide; II, deacetalated polysaccharide.

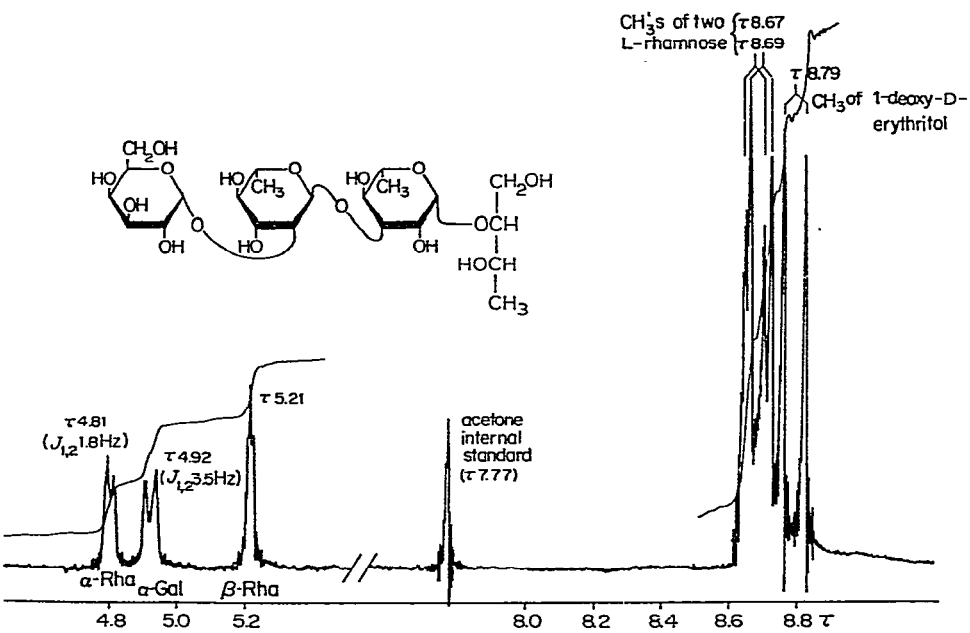


Fig. 1. ^{1}H-N.m.r. spectrum (D_2O, 90°) of oligosaccharide **1** (obtained by periodate oxidation of *Klebsiella* K32 capsular polysaccharide; see text for details).

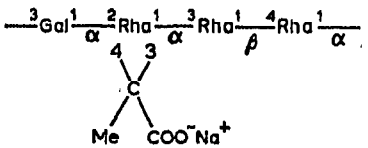
derivatives was achieved by gas–liquid chromatography using a polar, liquid phase of HIEFF-1B (see Table I for details).

Periodate oxidation[14]. — Periodate oxidation of native K32 polysaccharide proceeded rapidly and, after 40 h, analysis of the oxidized polymer (see Experimental section) indicated complete oxidation. The oxidation was performed in 0.05M sodium periodate, and was buffered at pH 6.5 to minimize hydrolysis of the 1-carboxyethylidene acetal. However, analysis of the polymer after 40 h of oxidation at this pH indicated that some 15% of the acetal groups had, in fact, been removed. The derived polyol was subjected to a Smith degradation[15], and the resulting mixture of oligosaccharides was separated by gel chromatography. Three chromatographically pure components, **1**, **2**, and **3**, were isolated.

Component **1** (120 mg) showed $[\alpha]_D$ +102°. ^{1}H- and ^{13}C-N.m.r. studies indicated **1** to be a trisaccharide glycoside consisting of one galactosyl residue (α-linked), two rhamnosyl residues (one α-linked and the other β-linked), and a 1-deoxyerythritol residue (see Table II for data). The ^{1}H-n.m.r. spectrum of **1** is shown in Fig. 1; it clearly shows the presence of three signals for protons on nonreducing, anomeric carbon atoms. The signal at τ 5.21, with $J_{1,2}$ 1 Hz, is from the anomeric proton of the β-linked L-rhamnosyl residue, and is readily distinguishable from the signal (τ 4.81, $J_{1,2}$ 1.8 Hz) of the anomeric proton of the α-linked L-rhamnosyl residue. The full assignment of the spectrum is given in Table II. Field-desorption mass-spectrometry[16,17] (f.d.m.s.) of **1** gave peaks at *m/e* 561, 583, and 599, corre-

TABLE II

N.M.R. DATA FOR *Klebsiella* K32 CAPSULAR POLYSACCHARIDE, AND OLIGOSACCHARIDES ISOLATED[a]

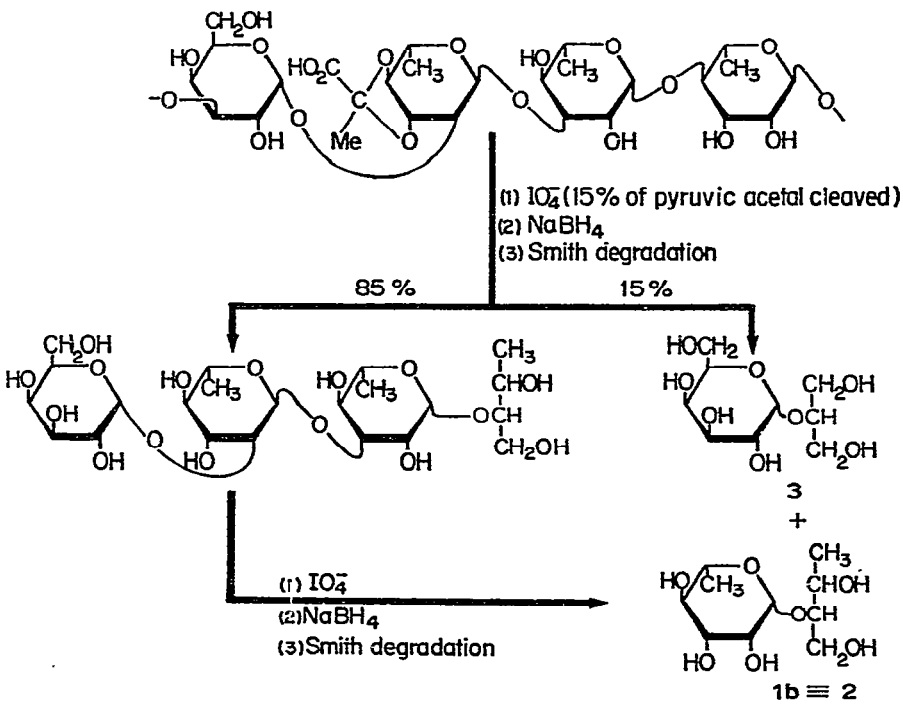
Compound	τ[b]	$J_{1,2}$[c] *(Hz)*	^1H-*N.m.r. Integral*	*Assignment*[d]	^{13}C-*N.m.r. p.p.m.*[e]	*Assignment*[f]
Gal$\overset{1\,2}{\underset{\alpha}{-}}Rha\overset{1\,3}{\underset{\alpha}{-}}Rha\overset{1\,3}{\underset{\beta}{-}}$1-deoxy-D-erythritol (**1**)	4.81	1.8	1 H	α-Rha	(22 signals overall)	
	4.92	3.5	1 H	α-Gal	100.41 }	{ α-Rha
	5.21	1	1 H	β-Rha	100.35 }	{ β-Rha
	8.69	6 ($J_{5,6}$)	6 H	CH_3 of Rha's	98.42	α-Gal
	8.79	6	3 H	CH_3 of 1-deoxy-D-erythritol	62.06 }	{ C-6 of Gal
					61.76 }	{ C-4 of 1-deoxy-D-erythritol
					17.95 }	{ CH_3 of Rha's
					17.55 }	{
					17.36 }	{ CH_3 of 1-deoxy-D-erythritol
Rha$\overset{1\,3}{\underset{\beta}{-}}$1-deoxy-D-erythritol (**2** or **1b**)	5.24	1	1 H	β-Rha	(10 signals overall)	
	8.69	6 ($J_{5,6}$)	3 H	CH_3 of Rha	100.69	β-Rha
	8.79	6	3 H	CH_3 of 1-deoxy-D-erythritol	62.10	C-4 of 1-deoxy-D-erythritol
					17.54	CH_3 of Rha
					18.05	CH_3 of 1-deoxy-D-erythritol
Gal$\overset{1\,2}{\underset{\alpha}{-}}$glycerol (**3**)	4.83	3		α-Gal	(9 signals overall)	
					98.89	α-Gal
					61.28	C-6 of Gal
					62.01	
					62.28	C-1, C-3 of glycerol
Gal$\overset{1\,2}{\underset{\alpha}{-}}Rha\overset{1\,3}{\underset{\alpha}{-}}Rha\overset{1\,4}{\underset{\beta}{-}}$Rha-OH (**4**)	4.80	1.8	1 H	α-Rha		
	4.87	1.8	0.6 H	α-Rha-OH	insufficient material	
	4.94	3	1 H	α-Gal		
	5.12	1	0.4 H	β-Rha-OH		
	5.25	1	1 H	β-Rha		
	8.69	6 ($J_{5,6}$	6 H	CH_3 of Rha's		
	8.79	6	3 H	CH_3 of 1-deoxy-D-erythritol		
	4.80	2	1 H	α-Rha	incomplete	
	4.93	b	2 H	α-Gal and α-Rha		
	5.25	s	1 H	β-Rha		
	8.41	s	3 H	CH_3 of pyruvic acetal		
Native K32 polysaccharide	8.70	6 ($J_{5,6}$)	9 H	CH_3 of Rha's		

[a]For the origin of oligosaccharides **1**, **2**, **3**, and **4**, see text. [b]Chemical shift relative to internal acetone; τ 7.77 downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate. [c]b, broad; s, singlet. [d]For example, α-Rha = proton on C-1 of α-linked L-Rha residue; (Gal = D-Gal). [e]Chemical shift in p.p.m. downfield from Me_4Si relative to internal acetone; 31.07 p.p.m. from Me_4Si. [f]As for footnote *d*, but for anomeric ^{13}C nuclei.

sponding to $(M + 1)^+$, $(M + Na)^+$, and $(M + K)^+$, respectively. Periodate oxidation of **1**, and subsequent reduction, Smith degradation, and gel filtration yielded a component **1b** having $[\alpha]_D$ +104°. ^{1}H- and ^{13}C-N.m.r. spectroscopy indicated that **1b** is composed of one rhamnosyl residue (β-linked) and a deoxyerythritol residue (see Table II for n.m.r. data). After hydrolysis of a small portion of **1b**, paper chromatography of the hydrolyzate revealed two components, indistinguishable from rhamnose and 1-deoxyerythritol. F.d.m.s. of **1b** gave peaks at *m/e* 253, 275, and 291, corresponding to $(M + 1)^+$, $(M + Na)^+$, and $(M + K)^+$, respectively. The survival of the deoxyerythritol (that initially results from periodate attack at a four-linked L-rhamnosyl residue) during the periodate oxidation of oligosaccharide **1** indicates that the linkage to this terminating glycoside must be to O-3. Consequently, the structure of component **1b** is β-L-Rha*p*-(1→3)-1-deoxy-D-erythritol. The structure of oligosaccharide **1** is, therefore, α-D-Gal*p*-(1→2)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→3)-1-deoxy-D-erythritol.

Oligosaccharide **2** (20 mg), obtained from the periodate oxidation of the native polysaccharide, was indistinguishable in every respect from component **1b**.

Oligosaccharide **3** (20 mg), also obtained from the periodate oxidation of the native polysaccharide, had $[\alpha]_D$ +118°. Hydrolysis and paper chromatography revealed the presence of only galactose and glycerol. The results of ^{1}H- and ^{13}C-n.m.r.



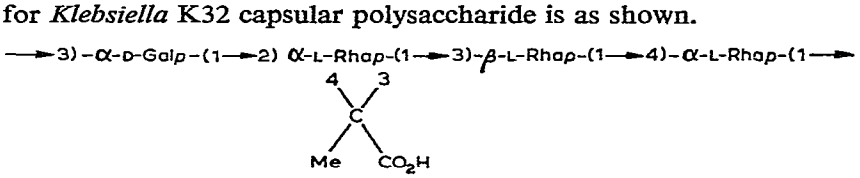
Scheme 1. Scheme for the periodate oxidation of *Klebsiella* K32 capsular polysaccharide (see text for details).

studies (see Table II for data) were in agreement with formulation of **3** as comprising one D-galactosyl group α-linked to a glycerol residue. F.d.m.s. of **3** gave peaks at *m/e* 255, 277, and 293, corresponding to $(M + 1)^+$, $(M + Na)^+$, and $(M + K)^+$, respectively. The structure of oligosaccharide **3**, is, therefore, α-D-Gal*p*-(1→2)-glycerol.

The results of the periodate-oxidation studies on *Klebsiella* K32 polysaccharide are summarized in Scheme 1. Identification and characterization of components **1**, **1b**, **2**, and **3** established the tetrasaccharide repeating structure of the polysaccharide, and also located the 1-carboxyethylidene acetal group.

Partial hydrolysis. — Although the structure of K32 capsular polysaccharide can be deduced from the periodate-oxidation data already discussed, supporting evidence was sought by use of the technique of partial hydrolysis. Mild, acid hydrolysis of K32 native polysaccharide resulted in very nonspecific cleavage of the glycosidic linkages. As a result, many (very similar) oligosaccharides were simultaneously released. Monitoring by paper chromatography of a progressive, acid hydrolysis of a small amount of native polysaccharide revealed that maximal production of oligosaccharide occurred after treatment for 9 h at 95° with 0.03M trifluoroacetic acid. Alternatively, on using the polysaccharide in its free acid form (pH 3.0), maximal production of oligosaccharide occurred after 18 h of autohydrolysis at 95°. By use of the latter conditions, a larger sample of K32 polysaccharide was hydrolyzed; the product was then dialyzed against a fixed volume of water. The dialyzable material was examined by gel chromatography, and a pure oligosaccharide (**4**, 4 mg) was obtained. ^{1}H-N.m.r. spectroscopy indicated that **4** is a tetrasaccharide containing three L-rhamnose residues, one of which is reducing (see Table II for ^{1}H-n.m.r. data, and their interpretation). Reduction of **4** with sodium borodeuteride and subsequent methylation analysis yielded the acetylated derivatives of 2,3,4,6-tetra-*O*-methylgalactitol, 3,4-di-*O*-methylrhamnitol, 2,4-di-*O*-methylrhamnitol and, from the reducing terminus of **4**, 1,2,3,5-tetra-*O*-methylrhamnitol. These components were obtained in approximately equal amounts, but some of the last-named component, monodeuterated at C-1, was lost under diminished pressure during derivatization. In light of the sequence of oligosaccharide **1** revealed by periodate oxidation, the structure of the reducing tetrasaccharide **4** may be written as α-D-Gal*p*-(1→2)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→4)-L-Rha*p*. During the isolation of **4** by gel filtration, difficulties were encountered in separating this oligosaccharide from many other, chemically similar, components; this resulted in an extremely low yield of purified **4**. The structure of this tetrasaccharide does, however, agree with the data obtained from periodate-oxidation studies.

From the results reported here, it is deduced that the repeating-unit structure for *Klebsiella* K32 capsular polysaccharide is as shown.



The presence of a β-linked L-rhamnose residue in Pneumococcus type II polysaccharide[18,19] and Pneumococcus type XXVII polysaccharide[20] has been proposed, but we believe our finding to be the first, unequivocal evidence for the existence of such a linkage. If L-rhamnose is assumed to exist in the $^4C_1$(L) conformation of the pyranoid form, it might be assumed that a β-linked L-rhamnosyl residue would be more susceptible to acid hydrolysis than the comparable α-linked structure; however, the isolation, after partial hydrolysis, of an oligosaccharide (*e.g.*, **4**) in which the β-L-rhamnose linkage is intact suggests that this may not always be so.

The 1-carboxyethylidene acetal group in K32 polysaccharide is, perhaps, the most acid-sensitive acetal in the *Klebsiella* polysaccharides examined to date. Part of the reason for this susceptibility to acid hydrolysis is that the acetal spans *trans*-diequatorial, vicinal hydroxyl groups. Only as a result of a large degree of distortion of the sugar ring can these two hydroxyl groups be brought into the reasonably planar orientation that is necessary for the formation of this type of acetal. The existence of a 1-carboxethylidene acetal group attached to O-3 and O-4 of an L-rhamnopyranosyl residue had been noted[4,21]. 1-Carboxyethylidene acetals spanning vicinal, *trans*-diequatorial hydroxyl groups (O-2 and O-3) of a 4-linked D-glucosyluronic residue[22], and the same positions of a D-galactosyl residue[23], have been demonstrated. In all these cases, the acetal has been found to be readily removable under mild, acid conditions.

EXPERIMENTAL

General methods. — The equipment for m.s., n.m.r. spectroscopy, g.l.c., and g.l.c.–m.s. was the same as that used in the investigation of *Klebsiella* K36 polysaccharide[5]. F.d.m.s. was performed with an A.E.I. M.S. 902 mass spectrometer equipped with an e.i./f.i./f.d. source. The columns used for g.l.c. separations were (*1*) 3% of HIEFF-1B on Gas Chrom Q (100–120 mesh), and (*2*) 0.2% of poly(ethylene glycol succinate), 0.2% of poly(ethylene glycol adipate), and 0.4% of XF-1150 on the same support. For descending, paper chromatography, the following solvent systems (v/v) were used: (*A*) freshly prepared 2:1:1 1-butanol–acetic acid–water, and (*B*) 4:1:1 ethyl acetate–pyridine–water.

Preparation and properties of K32 capsular polysaccharide. — The preparation was performed as previously described[5]. The isolated polysaccharide showed $[\alpha]_D$ +113° (*c* 3.8, water). ^{1}H-N.m.r. spectroscopy was performed on the polysaccharide in the sodium salt form, and revealed signals in the anomeric region that integrated as four protons relative to the signals at τ 8.41 (3 H, s) and 8.70 (9 H, $J_{5,6}$ ~6 Hz). For the assignment of the anomeric-proton signals, see Table II.

Sugar and methylation analysis of native and partially deacetalated polysaccharides. — Hydrolysis of a sample of native K32 polysaccharide with 2M trifluoroacetic acid for 6 h at 95°, and subsequent derivatization of the liberated monosaccharides as their alditol acetates gave, in g.l.c., peaks corresponding to rhamnitol pentaacetate and galactitol hexaacetate in the ratio of 18:7 (column *2*; programmed at 120° for

8 min, and then at 1°/min to 200°). Preparative g.l.c. gave galactitol hexaacetate (m.p. 168°) and rhamnitol pentaacetate. Circular dichroism of the latter component showed ε_{213}^{MeCN} −1.20 and, by comparison with authentic standards, confirmed that the sugar had the L configuration. The configuration of the galactose was shown to be D by the positive action of D-galactostat on a portion of the free sugar isolated by gel filtration of a sample of the hydrolyzed polysaccharide.

Methylation of K32 capsular polysaccharide in the sodium salt form was performed by using the Hakomori procedure[9]. Difficulty was encountered in dissolving the polysaccharide in dimethyl sulfoxide, and agitation in a sonicator for two days at room temperature was needed in order to achieve complete dissolution. Two successive Hakomori methylations were performed, and these were followed by a Purdie[10] treatment (silver oxide in methyl iodide). The final product showed no absorption at 3600 cm^{-1} in the i.r. spectrum, and had $[\alpha]_D$ +10° (*c* 2.2, chloroform). Hydrolysis of this material with 2M trifluoroacetic acid for 16 h at 95°, and subsequent derivatization as the alditol acetates, gave a mixture of components that was analyzed by g.l.c.–m.s. as detailed in Table I, column 1.

A sample of K32 capsular polysaccharide that had been passed through a column of Amberlite IR-120 (H^+) ion-exchange resin was shown by ^{1}H-n.m.r. spectroscopy to have lost ~70% of the 1-carboxyethylidene acetal groups. Methylation of this partially deacetalated material in the free acid form proceeded without complication; the subsequent, methylation analysis results are shown in Table I, column 2.

Periodate oxidation. — A sample (616 mg) of *Klebsiella* K32 capsular polysaccharide in the sodium salt form (with a full complement of acetal) was dissolved in one l of phosphate buffer, pH 6.5, that was 0.05M in $NaIO_4$. The solution was stirred in the dark for 40 h at 4°, and then ethylene glycol (10 ml) was added. Dialysis of the solution against tap-water overnight was followed by reduction with sodium borohydride (1 g). The solution was then made neutral with glacial acetic acid, dialyzed overnight, and lyophilized, to yield 610 mg of the derived polyol. A 10-mg portion of this polyol was hydrolyzed with 2M trifluoroacetic acid; paper chromatography (solvent *B*) revealed the presence of 1-deoxyerythritol, galactose, and rhamnose. Reduction of this hydrolyzate and acetylation of the products gave a mixture of alditol acetates that was shown by g.l.c.–m.s. (column *2*) to contain components corresponding to rhamnose and galactose in the ratio of 63:37 (theoretical, 66:33); this result indicated that oxidation was complete.

Smith hydrolysis of the polyol with 0.5M trifluoroacetic acid overnight at room temperature was followed by removal of the acid by evaporation with successive portions of water, and reduction of the residue with sodium borohydride in water overnight. After the usual processing, lyophilization yielded 520 mg of material that was investigated by gel-filtration chromatography. The material (3 × 150 mg) was applied to a column (160 × 2.5 cm) of Bio-Gel P-4 which was irrigated with distilled water at a flow rate of ~7 ml/h. Fractions (2 ml) were collected, lyophilized individually, and examined by paper chromatography (solvent *A*). Three pure com-

ponents, **1**, **2**, and **3**, with R_{Glc} values (solvent *A*) of 1.15, 1.75, and 1.14, respectively, were isolated.

Oligosaccharide **1** (120 mg) had $[\alpha]_D$ +102° (*c* 3.2, water) and was examined by ^{1}H- and ^{13}C-n.m.r. spectroscopy (see Table II for details). After hydrolysis of **1** with 2M trifluoroacetic acid for 6 h at 95°, paper chromatography (solvent *A*) revealed the presence of rhamnose, galactose, and 1-deoxyerythritol. The mass spectrum (f.d.) of **1** gave, *inter alia*, peaks at *m/e* 561(36), 562(12), 583(100), 584(34), 585(17), 599(21), and 600(22). The major peaks at *m/e* 561, 573, and 599 correspond to $(M + 1)^+$, $(M + Na)^+$, and $(M + K)^+$, respectively. Methylation of a small portion of **1** yielded a product that, after hydrolysis with 2M trifluoroacetic acid for 16 h at 95° and subsequent derivatization, was shown by g.l.c.–m.s. (column *I*) to contain the alditol acetates from 2,3,4,6-tetra-*O*-methylgalactose, 3,4-di-*O*-methyrhamnose, and 2,4-di-*O*-methyrhamnose in equal proportions. The volatile 1-deoxy-tri-*O*-methyl-erythritol component was lost under diminished pressure during processing.

Periodate oxidation of **1** (30 mg) with 0.05M sodium periodate for 24 h, and reduction with sodium borohydride in the usual way, gave a product that was hydrolyzed with 0.5M trifluoroacetic acid for 16 h at room temperature (Smith hydrolysis). The hydrolyzate was applied to a column (160 × 2.5 cm) of Bio-Gel P-4 which was then eluted with distilled water. A pure component, **1b** (7 mg), was isolated that had $[\alpha]_D$ +104° (*c* 0,78, water). ^{1}H- and ^{13}C-N.m.r. data for **1b** are recorded in Table I. After hydrolysis of **1b**, paper chromatography (solvent *B*) of the hydrolyzate gave components indistinguishable from authentic samples of rhamnose and 1-deoxyerythritol. The f.d. mass spectrum of **1b** had, *inter alia*, peaks at *m/e* 253(100), 254(24), 275(30), and 291(11). The peaks at *m/e* 253, 275, and 291 correspond to $(M + 1)^+$, $(M + Na)^+$, and $(M + K)^+$, respectively. Oligosaccharide **1b** is, therefore, β-L-Rha*p*-(1→3)-1-deoxy-D-erythritol, and **1** is α-D-Gal*p*-(1→2)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→3)-1-deoxy-D-erythritol.

Oligosaccharide **2** (20 mg) was identical to component **1b** in every respect.

Component **3** (20 mg) showed $[\alpha]_D$ +117° (*c* 0.4, water). The f.d. mass spectrum of **3** had, *inter alia*, peaks at *m/e* 255(29), 277(17), and 293(100). These peaks correspond to $(M + 1)^+$, $(M + Na)^+$, and $(M + K)^+$, respectively. The ^{1}H- and ^{13}C-n.m.r. spectra of **3** are detailed in Table II. After hydrolysis of a small portion of **3**, paper chromatography (solvent *A*) of the hydrolyzate showed two components indistinguishable from authentic samples of galactose and glycerol. Component **3** was chromatographically identical to an authentic sample of α-D-Gal*p*-(1→2)-glycerol.

Partial hydrolysis. — *Klebsiella* K 32 polysaccharide (0.5 g) was autohydrolyzed at pH 3.0 for 18 h on a steam bath, and the hydrolyzate was dialyzed against a fixed volume of water; lyophilization then yielded 175 mg of dialyzable oligosaccharides containing at least six different components, as indicated by paper chromatography (solvent *A*). Gel chromatography on a column (170 × 2.5 cm) of Sephadex G10 with a flow rate of 10 ml/h, and subsequent lyophilization of fractions and examination by paper chromatography revealed that the separation of the mixture was poor.

However, careful examination did allow the isolation of a small amount of pure oligomer **4** (4 mg). ^{1}H-N.m.r. spectroscopy of **4** (see Table II for details) indicated the presence of one galactose residue (α-linked) and three rhamnose residues (one α-linked, one β-linked, and one reducing). Oligosaccharide **4** was reduced with $NaBD_4$, and the product methylated. The permethylated derivative was hydrolyzed with 2M trifluoroacetic acid for 8 h at 95°, the mixture of ethers reduced, the alditols acetylated, and the acetates analyzed by g.l.c.–m.s. on column *1*. Partially methylated alditol acetates corresponding to 2,3,4,6-tetra-*O*-methylgalactose, 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, and 1,2,3,5-tetra-*O*-methylrhamnitol were identified, the last component being deuterated at C-1.

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