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STRUCTURAL INVESTIGATION OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* SEROTYPE K80

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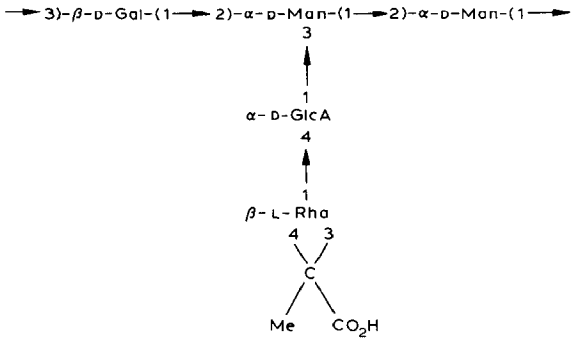
ABSTRACT

The use of methylation, periodate oxidation, β-elimination, and selective hydrolysis enabled the structure of the K80 polysaccharide to be determined. The nature of the anomeric linkages was established by ^{1}H-n.m.r. spectroscopy, and confirmed by the results of oxidation of the fully acetylated polysaccharide with chromic acid. The K80 polysaccharide is comprised of repeating units of the pentasaccharide shown, and contains a pyruvic acetal on each repeating unit. This pattern constitutes the first instance, in this series of polysaccharides, of a pyruvic acetal attached to a side-chain rhamnosyl group.



INTRODUCTION

Serotype K80 is one of three strains of *Klebsiella* whose capsular polysaccharides are composed of D-glucuronic acid, D-galactose, D-mannose, and L-rhamnose, but the other two strains, *Klebsiella* K40 (ref. 1) and K53 (ref. 2), do not contain a 1-carboxyethylidene substituent. The only other known *Klebsiella* polysaccharides containing a 1-carboxyethylidene substituent on a rhamnose residue are *Klebsiella* K32 (ref. 3), K70 (ref. 4), and K72 (ref. 5). We now report the structure of *Klebsiella* K80 polysaccharide.

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TABLE I

N M R DATA FOR *Klebsiella* K80 POLYSACCHARIDE AND THE DERIVED OLIGOSACCHARIDES

Compound[a]	^{1}*H-N m.r. data*			*Assignment*[c]	^{13}*C-N.m.r. data*	
	δ[b]	$J_{1,2}$ *(Hz)*	*Integral proton*		*(p.p.m.)*[d]	*Assignment*[e]
Galβ1→2Manα1→2Gly	5.21	s	1	→2)Manα→	103.1	Galβ→
P2	4.46	8	1	Galβ→	98.4	→2)Manα→
[→3)Galβ1→2Manα1→2Manα1→]$_n$	5.35	s	1	→4)GlcAα→	102.9	→3)Galβ→
α3 1 GlcA β4 1 Rha	5.23	s	1	→2)Manα→ 3 α	102.0	Rhaβ→
K80D	5.18	s	1	→2)Manα→	100.8	→4)GlcAα→, →2)Manα→ 3 α
	4.80	s	1	Rhaβ→	95.8	→2)Manα→
	4.53	8	1	→3)Galβ→	17.3	CH_3 of Rha
	1.26	7	3	CH_3 of Rha		
[→3)Galβ1→2Manα1→2Manα1→]$_n$	5.33	br	1	→4)GlcAα→	102.9	→3)Galβ→
α3 1 GlcA β4 1 Rha 4 3 \ / pyr	5.23	br	1	→2)Manα→ 3 α	102.2 101.9	Rhaβ→, Rhaβ→ 4 3 \ / pyr
	5.17	br	1	→2)Manα→	100.9	→4)GlcAα→

	4.85 4.80	br	0.4 0.6	Rha-β, Rha-β 4 3 \ / pyr	100.6	2-Man-α 3 |
	4.53	br	1	3-Gal-β	96.0	2-Man-α
	1.57	s	1.2	CH_3 of pyr	30.8	CH_3 of pyr
	1.33	d	0.4	CH_3 of Rha 4 3 \ / pyr	17.4	CH_3 of Rha, CH_3 of Rha 4 3 \ / pyr
	1.27	d	0.6	CH_3 of Rha		
$\left[\text{3-Gal-}\beta\text{-1,2-Man-}\alpha\text{-1,2-Man-}\alpha\text{-1}\right]_n$ α 3-1	5.35	s	1	4-GlcA-α	102.9	3-Gal-β
GlcA β 4-1	5.21	s	1	2-Man-α 3 |	102.1	Rha-β
Rha 50%	5.18	s	1	2-Man-α	100.9	4-GlcA-α
K80SH						
	4.80	s	0.2	Rha-β	100.7	2-Man-α 3 |
	4.53	br	1	3-Gal-β	95.8	2-Man-α
	1.27	br	0.9	CH_3 of Rha	17.3	CH_3 of Rha

[a]For the sources of **P2**, **K80D**, and **K80SH**, see text. [b]Chemical shift relative to internal acetone; Δ2.23 downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). [c]The numerical prefix indicates the position at which the sugar is substituted; α or β, the configuration at the glycosidic bond, or the anomer of a (terminal) reducing, sugar residue. Thus 2-Man-α refers to the anomeric proton of a 2-linked mannosyl residue in the α-anomeric configuration. The absence of a numerical prefix indicates a (terminal) nonreducing group. [d]Chemical shift in p.p.m. downfield from Me_4Si, relative to internal acetone; 31.07 p.p.m. downfield from DSS. [e]As in *c*, but for ^{13}C nuclei.

RESULTS AND DISCUSSION

Composition and n.m.r. spectra. — The isolation and purification of the polysaccharide were achieved as previously described[6–8]. The purified product obtained after one Cetavlon precipitation was shown by gel-permeation chromatography to be homogeneous ($\bar{M}_w = 0.9 \times 10^6$).

The ^{1}H-n.m.r. spectrum indicated the presence of five anomeric protons, corresponding to three α and two β linkages. One β signal showed splitting, which was also reflected in the doubling of the signal due to the protons on C-6 of the rhamnose unit, and was shown to have been caused by the removal of 25% of the 1-carboxyethylidene groups during harvesting of the native polysaccharide. Both the anomeric and the high-field signals collapsed to a single resonance when the native polymer was treated with mild acid, which removed all of the 1-carboxyethylidene acetal groups. This indicated that the rhamnose residue carries the acetal substituent. These observations were verified by the ^{13}C-n.m.r. data (see Table I).

Paper chromatography of an acid hydrolyzate of the polysaccharide showed galactose, glucuronic acid, mannose, and rhamnose. Determination of the neutral sugars as the alditol acetates showed rhamnose, mannose, and galactose in the ratios of 1.00:1.45:1.11. The carboxyl-reduced polysaccharide gave rhamnose, mannose, galactose, and glucose in the ratios of 0.90:1.80:1.11:1.00, indicating that the uronic acid is glucuronic acid. The glucose, and hence, glucuronic acid, and mannose were proved to be of the D configuration, and rhamnose of the L configuration, by circular dichroism measurements[9] made on the alditol acetates.

TABLE II

METHYLATION ANALYSES OF K80 POLYSACCHARIDE AND DERIVED PRODUCTS

Methylated sugars[a] *as alditol acetates*	*T*[b]			*Mol%*[c]			
	Column A[d] *(OV225)*	*Column B*[e] *(ECNSS M)*	*Column C*[f] *(SP 1000)*	*I*[g]	*II*	*III*	*IV*
2,3,4-Rha	0.50	0.47	0.54	21.15	14.84	13.92	9.40
2-Rha	1.38	1.53	1.37	6.34	4.56	4.18	16.97
3,4,6-Man	1.80	1.91	1.60	30.21	22 83	23.20	22.93
2,4,6-Gal	1.98	2.20	1.87	27.19	22.37	22.04	18.58
2,3,6-Glc	2.24	2.37	1.87	—	—	13.92	15.37
4.6-Man	2.85	3.18	2.54	15.10	21.69	22.73	16.74
2,3-Glc	4.26	5.75	3.33	—	13.70	—	—

[a]2,3,4-Rha = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, etc. [b]Relative retention-time, referred to 2,3,4,6-Glc as 1.00. [c]Values are corrected by use of the effective, carbon-response factors given by Albersheim *et al* [14]. [d]Isothermal; 180°. [e]Isothermal; 175°. [f]Isothermal; 220°. [g]I, Original capsular polysaccharide; II, compounds from $LiAlH_4$ reduction of methylated K80; III, compounds from remethylation of the reduced, methylated polymer; IV, compounds from carboxyl-reduced, methylated polysaccharide.

Galactose was assigned the D configuration from circular dichroism measurements made on the partially methylated alditol acetate derivative.

Methylation analysis[10]. — Methylation of the K80 polysaccharide, followed by hydrolysis, conversion of the neutral sugars into alditol acetates, and g.l.c.–m.s. analysis thereof gave the values shown in Table II, column I. These results indicated that the polysaccharide consists of a pentasaccharide repeating-unit with a branch on mannose, and a terminal rhamnose unit. Part of the 1-carboxyethylidene groups had been removed under the conditions of methylation [exchanging with Amberlite IR-120 (H^+) resin], but from those that survived, it could be deduced that this group is linked to O-3 and O-4 of the terminal rhamnosyl group. On reduction of the methylated polysaccharide with $LiAlH_4$ (see Table II, column II), the proportion of 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methylmannitol was increased, and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylglucitol was formed, demonstrating that glucuronic acid is linked at O-4 and that it is joined to the branch point mannose. Remethylation of the lithium aluminum hydride-reduced polymer, and analysis of the product, gave the values shown in Table II, column III. These results imply the presence of one branch point on the D-mannosyl residue and a 1-carboxyethylidene group linked to the terminal rhamnosyl group.

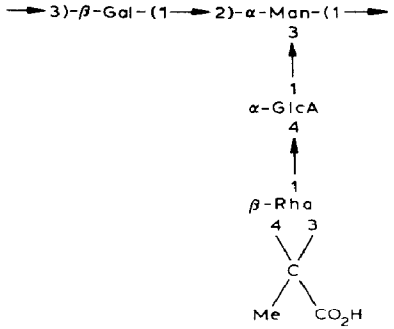
Methylation of the carboxyl-reduced polysaccharide[11] (see Table II, column IV) showed an increase in the proportion of 2-*O*-methylrhamnose, as this sample was not de-ionized by treatment with a cation-exchange resin. This observation confirmed the presence of a 3,4-linked 1-carboxyethylidene group on the terminal rhamnosyl group. The appearance of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol provided further proof of the presence of a 4-linked glucosyluronic acid residue.

Base-catalyzed alduronic acid degradation. — A sample of the methylated K80 polysaccharide was treated overnight with dimethylsulfinyl anion, and the product was treated with methyl iodide, and the methylated material extracted from water with chloroform. Analysis as the partially methylated, alditol acetate derivatives showed the presence of 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylmannitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol in the ratio of 2.19:1.00. Small proportions of terminal rhamnose and the branch point di-*O*-methylmannose were also obtained. This proved that the alduronic acid is linked at O-3 of the branch-point mannose.

Oxidation of the carboxyl-reduced[11] *polysaccharide with chromic acid*[12]. — The carboxyl-reduced, fully acetylated polysaccharide was subjected to oxidation with chromic acid. The product was analyzed for the presence of neutral sugars (as their alditol acetates). Galactose was completely degraded, but only part of the rhamnosyl residues was oxidized. The ratios of rhamnose, mannose, and glucose obtained were 0.42:2.00:0.71. The survival of the glucosyl and both mannosyl residues is proof of their α linkages. The galactose is β-linked, and so undergoes oxidation. Rhamnose was assigned a β linkage, as part of the sugar was oxidized and the anomeric signal in the ^{1}H-n.m.r. spectrum had a low value (δ 4.8).

On methylation, and analysis as the partially methylated alditol acetates,

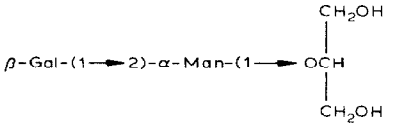
2,3,4-tri-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylglucose, 3,4,6-tri-*O*-methylmannose, 2,4,6-tri-*O*-methylmannose, and 2,3,6-tri-*O*-methylglucose were found in the ratios of 0.25:0.80:1.00:1.05:0.50. The methylated glucose units arise from the reduced alduronic acid substituted at O-4 by rhamnose, and from that which had part of the rhamnose degraded, thus proving that rhamnose is linked to O-4 of glucuronic acid. The 2,4,6-tri-*O*-methylmannose is obtained from the original,



branch-point mannose on degradation of the β-linked galactose, thus showing that galactose is linked to O-2 of the mannose residue. The results obtained thus far led to the partial structure and showed the presence of a 2-linked α-mannosyl residue.

Periodate oxidation. — Periodate oxidation of the native polymer and analysis, as the peracetylated alditols, of the sugars after hydrolysis showed the presence of glycerol, erythritol, mannose, galactose, and glucose in the ratios of 0.44:0.71:1.00:1.00:0.25. These results showed that the 1-carboxyethylidene groups do not survive under the reaction conditions employed, and that one mannosyl, the rhamnosyl, and part of the glucosyluronic acid residues were oxidized. As expected, the 3-linked galactosyl and the branched mannosyl residues survived oxidation.

Smith degradation of the original polysaccharide enabled the isolation of an oligosaccharide which, by sugar analysis and methylation analysis, was shown to be as follows.



P2

This fragment can only arise if the original 2-linked mannose was linked at O-2 to the branch-point mannose, and was itself linked at O-3 to galactose, and it is therefore in accord with the partial structure previously predicted.

The ^{1}H-n.m.r. spectrum of **P2** showed the presence of one α and one β signal. From the coupling constants, it was obvious that galactose was β-linked and that

TABLE III

METHYLATION ANALYSES OF SELECTIVELY HYDROLYZED K80 POLYSACCHARIDE

Methylated sugars[a] *as alditol acetates*	*T*[b]		*Mol%*[c]	
	Column A[d] *(OV225)*	*Column B*[e] *(ECNSS-M)*	*I*[f]	*II*
2,3,4-Rha	0.55	0.46	24.05	3.07
3,4,6-Man	1.47	1.92	25.31	27.93
2,3,6-Gal	1.56	2.21	25.31	24.58
2,3,4-Glc	1.64	—	—	14.52
4,6-Man	1.91	3.19	25.31	24.86
2,3-Glc	2.22	—	—	5.02

[a,b,c]As in Table II. [d]Programmed for 4 min at 180° and then at 2°/min to 260°. [e]Isothermal; 170°. [f]I, Selectively hydrolyzed K80 polysaccharide (**K80SH**); II, compounds from $LiAlH_4$ reduction of the methylated **K80SH**.

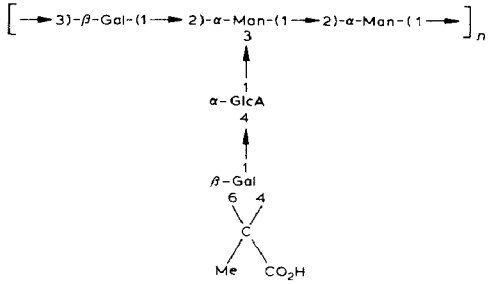
mannose was α-linked. The mass spectrum of the per-*O*-methylated oligosaccharide **P2** provided further proof of its structure. Because ions of the B fragmentation series are obtained only for (1→2)- or (1→4)-linked oligosaccharides[13], it may be seen that the branch-point mannosyl residue is linked at O-2 (not O-3) to the galactosyl residue.

Selective, partial hydrolysis. — Treatment of the K80 polysaccharide with very dilute acid, and dialysis of the products against distilled water, afforded a nondialyzable, polymeric material and a dialyzate. The dialyzate contained rhamnose, as shown by paper chromatography, and g.l.c. as the alditol acetate revealed the sole presence of rhamnose. The ^{1}H-n.m.r. spectrum of the nondialyzable material (K80SH) showed the depression of one signal (δ 4.80), and the ^{13}C-n.m.r. spectrum, of the signal at 102.0 p.p.m. Only 50% of the rhamnose had been removed, as was indicated both by ^{1}H-n.m.r. spectroscopy and sugar analysis. The per-*O*-acetylated alditols from rhamnose, mannose, and galactose were in the ratios of 0.50:1.50:1.15. Carboxyl reduction[11] of the nondialyzable material, and analysis as the per-*O*-acetylated alditols, gave rhamnose, mannose, galactose, and glucose in the ratios of 0.22:2.07:1.00:0.77. Methylation analysis of the nondialyzable fraction as the partially methylated alditol acetates, and analysis after reduction of the permethylated polysaccharide by $LiAlH_4$ (see Table III, columns I and II), showed that the terminal rhamnosyl group is linked to O-4 of the glucosyluronic acid residue.

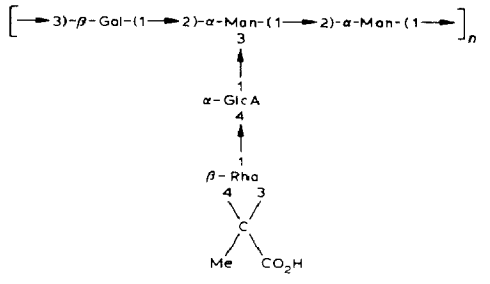
CONCLUSION

These experiments demonstrated that the structure of the capsular polysaccharide of *Klebsiella* K80 is as shown. This pattern is novel, in that the 1-carboxyethylidene group is attached to O-3 and O-4 of a terminal L-rhamnopyranosyl

group, whereas, in *Klebsiella* serotypes K32 (ref. 3), 70 (ref. 4), and 72 (ref. 5), the *O*-(1-carboxyethylidene)-L-rhamnose unit is a constituent of the main chain. The similarity of the structure of K74 polysaccharide[15] to that of K80 is noteworthy.



K74



K80

EXPERIMENTAL

General methods. — Paper chromatography was conducted by the descending method, using Whatman No. 1 paper and the following solvent systems (v/v): (*1*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, (*2*) 8:2:1 ethyl acetate–pyridine–water, (*3*) 2:1:1 1-butanol–acetic acid–water, and (*4*) 4:1:5 1-butanol–ethanol–water (upper layer). Chromatograms were developed with silver nitrate, or by spraying with *p*-anisidine hydrochloride in aqueous 1-butanol and heating the paper for 5–10 min at 110°. Preparative paper chromatography was performed by the descending method, using Whatman No. 1 paper and solvents *1* and *3*.

The instrumentation used for infrared and n.m.r. spectroscopy, g.l.c., preparative g.l.c., and g.l.c.–m.s., and circular dichroism and optical rotation measurements was as described previously[16]. The mass spectrum of the per-*O*-methylated oligosaccharide was recorded with a Kratos, MS-50 model, mass spectrometer, using a 70-eV beam. Analytical g.l.c. separations were achieved in stainless-steel columns (1.8 m × 3 mm), with nitrogen as the carrier gas at a flow rate of 20 mL/min. The columns used were (*A*) 3% of OV225 on Gas Chrom Q (100–120 mesh), (*B*) 5% of ECNSS-M on Gas Chrom Q (100–120 mesh), (*C*) 5% of SP 1000 on Gas Chrom Q (100–120 mesh), and (*D*) 3% of SP 2340 on Supelcoport (100–120 mesh). The columns (1.8 m × 6.3 mm) used for preparative g.l.c. with a helium flow-rate of 60 mL/min were (*a*) 5% of SP2340 on Gas Chrom Q (100–120 mesh) and (*b*) 5% of OV225 on Supelcoport (100–120 mesh).

Preparation and properties of K80 capsular polysaccharide. — A culture of *Klebsiella* K80, obtained from Dr. Ida Ørskov, Copenhagen, was grown as previously described[6–8], and the polysaccharide was purified by one precipitation with Cetavlon. The isolated polysaccharide (1.5 g) was shown by gel chromatography on a Sephadex 4B column (courtesy of Dr. S. C. Churms, Unversity of Cape Town, South Africa) to have an average molecular weight of 0.9×10^6. N.m.r. spectros-

copy (^1H and ^{13}C) was performed on the original K80 polysaccharide. All ^1H-n.m.r. spectra were recorded at high temperature in order to obtain better resolution. The principal signals and their assignments, for both the ^1H- and ^{13}C-n.m.r. spectra, are recorded in Table I.

Hydrolysis of the polysaccharide. — Hydrolysis of a sample (10 mg) of K80 polysaccharide with 2M trifluoroacetic acid (TFA) for 24 h at 95°, removal of the acid by repeated coevaporation with water, followed by paper chromatography (solvents *1* and *2*) showed rhamnose, mannose, galactose, glucuronic acid, and an aldobiouronic acid. The neutral sugars were quantitatively determined by g.l.c. (column *D*, programmed for 4 min at 195° and then at 2°/min to 260°) as their alditol acetates, and the presence of rhamnose, mannose, and galactose was confirmed. The quantitative, sugar analysis of the carboxyl-reduced polysaccharide[11] revealed, in addition, the presence of the alditol acetate from glucose.

Circular dichroism measurements. — Glucitol hexaacetate, mannitol hexaacetate, and rhamnitol pentaacetate were separated on column *a*, programmed from 190 to 260° at 4°/min. A positive curve was obtained for the first two compounds and, hence, the D configuration was assigned to mannose and glucuronic acid; a negative curve for the last-mentioned compound was proof of its L configuration. Column *b* programmed from 195° at 4°/min to 260° was used to separate 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, which gave a positive curve for its circular dichroism spectrum, and this permitted assignment of the D configuration to galactose.

Methylation analysis. — The capsular polysaccharide (140 mg) in the free-acid form, obtained by passing the sodium salt through a column of Amberlite IR 120 (H^+) resin, was dissolved in dry dimethyl sulfoxide (12 mL), and methylated by the Hakomori[10] procedure. The product (150.3 mg), recovered after dialysis against running tap-water, showed complete methylation (no hydroxyl absorption in its i.r. spectrum). A portion (15 mg) of this product was hydrolyzed with 2M TFA, the sugars were reduced with sodium borohydride, and the alditols acetylated with 1:1 acetic anhydride–pyridine, and analyzed by g.l.c. in columns *A*, *B*, and *C* (see Table II, column I). G.l.c.–m.s. was conducted on column *B*. Another portion (30 mg) of the fully methylated polysaccharide was subjected to carboxylic ester reduction with lithium aluminum hydride in anhydrous oxolane. Half of the product was hydrolyzed with 2M TFA, and converted into the alditol acetates, and these were analyzed by g.l.c. in columns *A*, *B*, and *C* (see Table II, column II); column *B* was used for g.l.c.–m.s. The other half was remethylated by one Hakomori methylation[10], and the product converted into the alditol acetates as before. G.l.c. analysis was conducted in columns *A*, *B*, and *C* (see Table II, column III), and g.l.c.–m.s., in column *B*.

Carboxyl-reduced K80 polysaccharide (22.5 mg), obtained by using the carbodiimide-reduction method[11], was dissolved in dimethyl sulfoxide (3 mL) and methylated by the Hakomori procedure[10]. The product showed complete methylation (no hydroxyl absorptions in the i.r. spectrum). The partially methylated alditol

acetates were prepared in the usual way, and analyzed by g.l.c. in column *B* (see Table II, column IV). G.l.c.–m.s. was conducted with column *B*. All g.l.c. analyses on columns *A*, *B*, and *C* were performed isothermally, at 180, 175, and 220°, respectively.

Uronic acid degradation[17]. — A sample (20 mg) of methylated K80 polysaccharide was dried, and then, together with a trace of *p*-toluenesulfonic acid, was dissolved in 19:1 dimethyl sulfoxide–2,3-dimethoxypropane (12 mL), and the flask sealed under nitrogen. Dimethylsulfinyl anion (5 mL) was added, and allowed to react for 18 h at room temperature. To the cooled solution was added methyl iodide (3 mL), and the mixture was stirred for 1 h. The methylated, degraded product was isolated by partition between chloroform and water. The product was hydrolyzed with 2M TFA for 4 h at 95°, and the partially methylated alditol acetates were prepared as described earlier. G.l.c. analysis and g.l.c.–m.s. were conducted in Column *B* at 175° (isothermal).

Chromic acid oxidation[12]. — To a solution of the carboxyl-reduced K80 polysaccharide[11] (20 mg) in formamide (5 mL) were added acetic anhydride (2 mL) and pyridine (2 mL). The mixture was stirred overnight at room temperature, dialyzed for 2 d, and freeze-dried. The product was dissolved in acetic acid (5 mL), CrO_3 (150 mg) was added, and the solution was heated for 1 h at 50°. The product was isolated by partition between chloroform and water. A third of the product was hydrolyzed with 2M TFA, the sugars were converted into the alditol acetates, and these analyzed by g.l.c. in column *D*, programmed at 195° for 4 min and then 2°/min to 260°. The rest was methylated by the Hakomori procedure[10], and the product converted into the partially methylated alditol acetates. G.l.c. analysis and g.l.c.–m.s. were conducted in column *B* at 175° (isothermal).

Periodate oxidation. — To the K80 polysaccharide (42 mg) in water (10.0 mL) was aded 0.03M $NaIO_4$ (10.0 mL), and the solution was kept for 120 h at room temperature. Following addition of ethylene glycol (0.2 mL) and, after 1 h, sodium borohydride (100 mg), the product was isolated by dialysis and lyophilization. G.l.c. analysis was performed in column *D* at 195° for 4 min, and then 2°/min to 260°.

Smith degradation. — A solution of K80 polysaccharide (428.1 mg) in water (88.8 mL) was mixed with a solution of 0.2M $NaClO_4$ in 0.03M $NaIO_4$ (88.8 mL), and kept in the dark for 120 h at room temperature. Ethylene glycol (0.2 mL) was added, the mixture stirred for 1 h, and the polyaldehyde formed dialyzed overnight, and reduced with $NaBH_4$ (1 g). The base was neutralized with 50% HOAc, and the solution was dialyzed and freeze-dried. Smith hydrolysis was effected by treating with 0.5M TFA (100 mL), and stirring for 48 h at room temperature. Two compounds were isolated by preparative chromatography for 30 h, using solvent *3*. The faster-moving compound **P1**, was shown to be mainly erythritol, with a small proportion of glycerol. The slower-moving compound **P2**, contained galactose and mannose in equimolar proportions. Methylation of **P2** by the Hakomori procedure[10], and analysis of part of the product as the partially methylated alditol ace-

tates on Column *B* at 175° (isothermal) and on column *A*, programmed for 4 min at 180° and then 2°/min to 230°, revealed the presence of 2,3,4,6-tetra-*O*-methylgalactose and 3,4,6-tri-*O*-methylmannose in the ratio of 1.00:1.06. The rest of the permethylated **P2** oligosaccharide was purified on a Sephadex LH-20 column, with 1:1 methanol–chloroform as the eluant, and examined by mass spectrometry. All alditol acetates were analyzed by g.l.c. and g.l.c.–m.s. in column *D*, programmed for 8 min at 195°, and then at 4°/min to 260°.

Selective, partial hydrolysis. — Treatment of K80 polysaccharide (91 mg) with 0.1M TFA (25 mL) for 20 min at 95°, and dialysis for 2 d, gave a nondialyzable material that had all of the sugars intact, and only the 1-carboxyethylidene group removed (see Table I, K80D). Analysis as the alditol acetates by g.l.c. in column *D*, programmed for 4 min at 195° and then at 2°/min to 260°, showed the same molar ratios as for the native polysaccharide.

K80 polysaccharide (103 mg) and 0.1M TFA (25 mL) were heated for 90 min at 95°, and dialyzed after removal of TFA by coevaporation with water. The dialyzate was shown by paper chromatography (solvent *1*) to contain rhamnose. Analysis by g.l.c. revealed the presence of rhamnose only. Analysis of the nondialyzable fraction as the alditol acetates was conducted by g.l.c. The carboxyl-reduced, nondialyzable fraction was also analyzed by g.l.c. as the alditol acetates. Column *D*, programmed for 4 min at 195° and then at 2°/min to 260°, was used in all of these experiments.

The nondialyzable fraction (17 mg) was methylated by one Hakomori methylation[10]. One third was hydrolyzed with 2M TFA, and the sugars converted into the alditol acetates (see Table III, column I). G.l.c. analysis was performed in column *B* at 170° (isothermal). The remaining two-thirds was reduced with $LiAlH_4$ in anhydrous oxolane, the product hydrolyzed with 2M TFA, and the compounds in the hydrolyzate converted into alditol acetates (see Table III, column II) and analyzed by g.l.c. on column *A*, programmed for 4 min at 180°, and then at 2°/min to 260°.

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