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# Structure of the capsular polysaccharide from the *Klebsiella* K8 reference strain 1015

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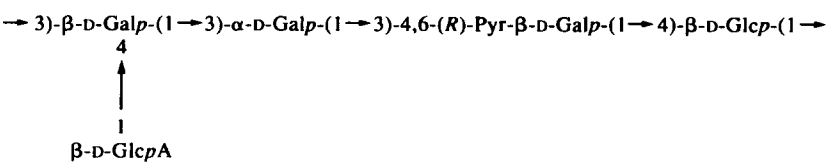
**Abstract**

The structure of the capsular polysaccharide from the *Klebsiella* K8 reference strain 1015 has been elucidated. The structure was deduced from sugar analysis, different methylation analyses, a uronic acid degradation, and NMR spectroscopy. It is concluded that the polysaccharide is composed of pentasaccharide repeating units with the structure:




The structure differs from that of the previously published structure of the capsular polysaccharide from *Klebsiella* K8, which originates from another strain and has the following structure:

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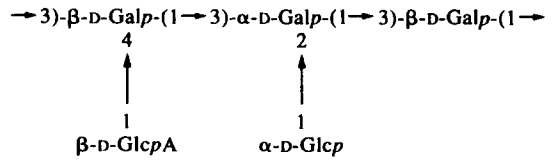



The serological similarity between the two strains is most likely derived from a common tetrasaccharide which is substituted in different ways in the two strains. Since the strain in the present investigation originates from the *Klebsiella* K reference strain collection of the International Escherichia and Klebsiella Centre, Copenhagen, Denmark, it is suggested that it should keep the designation K8. The other polysaccharide with *Klebsiella* K8 specificity should be renamed as K8,52,59 based on the cross-reactivity of the strain (I. Ørskov, unpublished).

*Keywords: Klebsiella*; Capsular polysaccharide

## 1. Introduction

The K-antigens of *Klebsiella* bacteria have been divided into some 80 different types [1] and most of these capsular polysaccharides have been characterised. One of them (serotype 8, strain A4) has been investigated and re-investigated [2,3] and the structure for the repeating unit is as shown in the Abstract. The same repeating unit but with additional *O*-acetyl groups was suggested for a polysaccharide produced by *Klebsiella* SK1 [4]. A third polysaccharide, deriving from the Danish type culture collection (serotype 8, strain 1015), had only been chemotyped and the presence of galactose, glucose, glucuronic acid, and pyruvic acid was demonstrated [1]. The fact that the polysaccharide from serotype 8 (K8) contains pyruvic acid had been overlooked in the previous investigations [2,3] of strain A4. From the $^1$H NMR spectrum of the capsular polysaccharide from the *Klebsiella* K8 strain 1015, it became apparent, however, that there were two different polysaccharides that had K8 specificity. We now report studies of the strain 1015 capsular polysaccharide, hereafter referred to as K8. The renaming of the polysaccharide strain from A4 to K8,52,59 has now been made because of the serological cross-reactivity with K52 and K59 antisera.

## 2. Results and discussion

Hydrolysis of the capsular polysaccharide from *Klebsiella* type 8 strain 1015 (K8) with trifluoroacetic acid yielded galactose and glucose in the ratio 2.2:1.0. A sample that had been treated with methanolic hydrogen chloride also indicated the presence of glucuronic acid. The absolute configurations were determined by GLC of the acetylated (+)-2-butyl glycosides or (+)-2-butyl glycoside (+)-2-butyl esters essentially as described [5], and were D for all the sugars. Methylation analysis of K8 revealed the presence of 4-substituted D-glucose, 3-substituted D-galactose, 3,4-substituted D-galac-

Table 1
Methylation analysis data for K8 and modifications thereof

| Sugar residue [a] | Detector response (%) | | |
|---|---|---|---|
| | A | B | C |
| 2,3,6-Glc [b] | 26 | 20 | 26 |
| 2,4,6-Gal | 25 | 18 | 46 [c] |
| 2,3,4-Glc | | 18 | |
| 2,6-Gal | 27 | 22 | 3 |
| 2-Gal | 22 | 22 | 25 |

[a] Key: A, methylated native K8; B, methylated and carboxyl-reduced K8; C, methylated K8 subjected to uronic acid degradation.
[b] 2,3,6-Glc = 2,3,6-tri-*O*-methyl-D-glucose, etc.
[c] 50% labelled with a trideuteriomethyl group at O-4.

tose, and 3,4,6-substituted D-galactose (Table 1, column A). Methylation analysis with carboxyl-reduction of the methylated polysaccharide further yielded 2,3,4-tri-*O*-methyl-D-glucose deriving from terminal D-glucuronic acid (Table 1, column B).

The $^{1}H$ NMR spectrum of K8 showed a signal at $\delta$ 1.48 in agreement with the presence of a pyruvic acid acetal. The anomeric region (Fig. 1) showed signals for five anomeric protons at $\delta$ 5.30, 4.86, 4.75, 4.73, and 4.55. This is significantly different from the spectrum of K8,52,59 which showed resonances for anomeric protons at $\delta$ 5.63, 5.26, 4.91, 4.72, and 4.70. Furthermore, the latter strain did not contain any signal for pyruvic acetal. The presence of a pyruvic group in K8 was evident from a signal in the $^{13}C$ NMR spectrum at $\delta$ 25.8. The anomeric region (Fig. 2) contained signals for five anomeric carbons at $\delta$ 104.5, 104.5, 103.4, 102.8, and 96.1. A signal from a quaternary carbon is observed at $\delta_C$ 101.8 and is thus assigned to C-2 of the pyruvic group. The above data are indicative of a pentasaccharide repeating unit for the polysaccharide. Residues are designated **A–E** based on decreasing chemical shift of the anomeric protons. Residue **A** has the $\alpha$ configuration since $^{1}J_{C,H}$ for the anomeric proton is 171 Hz. Residues **B–D** are $\beta$-linked since $^{3}J_{H\text{-}1,H\text{-}2}$ are 8 Hz. Residue **E** shows

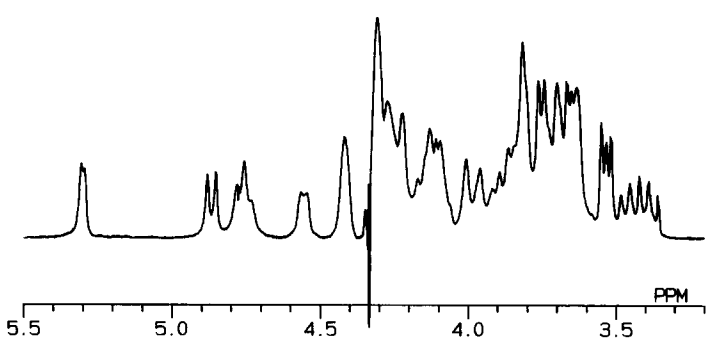



Fig. 1. The 3.2–5.5 ppm region in the $^{1}H$ NMR spectrum of K8.

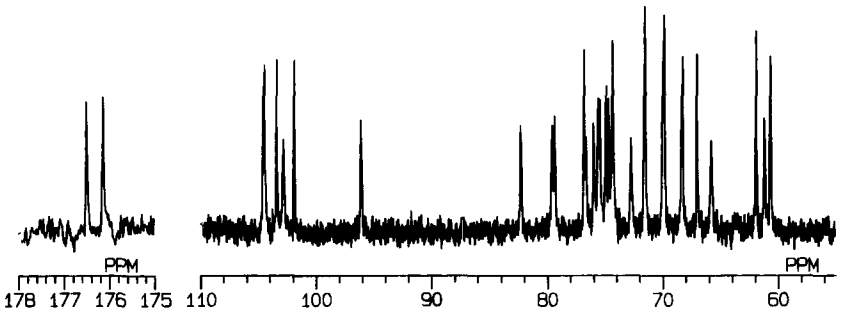
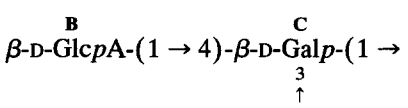



Fig. 2. The $^{13}C$ NMR spectrum of K8. The signal from the pyruvic group at $\delta_C$ 25.8 is not shown.

a $^1J_{C,H}$-value of 160 Hz and is thus also β-linked. From methylation analysis and NMR data (vide infra) all residues are demonstrated to be pyranoid.

Assignments of the $^1H$ and the $^{13}C$ NMR spectra of K8 were obtained by 2D NMR techniques and are given in Table 2. The glycosylation shifts obtained by comparison with the monomers are also given in Table 2. From the relayed COSY spectrum it was evident that the cross-peaks between H-1 and H-3 for residues **B** and **D** contained only large couplings and thus that these residues should be assigned to the glucuronic acid and the glucose residues, and that the remaining residues should be assigned to the galactose residues. The assignment of the sets of chemical shifts for **B** and **D** could be performed using, inter alia, a $^1H$-HOHAHA experiment, and the terminal D-glucuronic acid was assigned to residue **B**. Consequently, **D** was assigned to the 4-substituted D-glucose residue. The single large glycosylation shift of the C-3 signal from residue **A** showed that it could be assigned to the 3-substituted α-linked galactose residue. The $^1H$ and the $^{13}C$ NMR chemical shifts for signals from atoms H-4 to H-6, C-5, and C-6 in residue **E** are in good agreement with those from a 4,6-pyruvated β-D-galactose residue [6], and hence it is assigned to the pyruvated 3-substituted β-linked galactose residue. The remaining residue **C** must thus be assigned to the 3,4-substituted β-D-galactose residue. Glycosylation shifts of the C-3 and C-4 signals of $\Delta\delta$ 8.5 and 5.7, respectively, further corroborated substitution at C-3 and C-4.

A uronic acid degradation, i.e., methylation, treatment with strong base, and subsequent trideuteriomethylation, in turn degraded the glucuronic acid, liberated the hydroxyl group at position 4 of the branch-point galactose residue and trideuteriomethylated it. Hydrolysis of the resulting material gave 2,4,6-tri-*O*-methylgalactose with a trideuteriomethyl group at O-4 in place of a 2,6-di-*O*-methylgalactose (Table 1, column C). This demonstrates disaccharide element **1**.




**1**

NOESY experiments were employed in order to obtain further information on the sequence. The cross-peaks of the anomeric protons were examined and, in addition to

Table 2
$^{1}H$ and $^{13}C$ NMR data for K8

| Sugar residue | Chemical shift [a] ($\delta$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H/C | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6a | 6b | Me–C– | O–C–O | C=O |
| → 3)-α-D-Gal*p*-(1 → | 5.30 [8] [b] | 4.08 | 4.15 | 4.22 | 4.14 [c] | ~ 3.75 [c] | ~ 3.75 [c] | | | |
| **A** | (0.08) | (0.30) | (0.34) | (0.27) | (0.11) | (0.06) | (0.06) | | | |
| | 96.1 {171} | 68.3 | 79.3 | 70.0 | 71.5 | 61.9 | | | | |
| | (2.8) | (−1.1) | (9.2) | (−0.3) | (0.2) | (−0.2) | | | | |
| β-D-Glc*p*A-(1 → | 4.86 [8] | 3.39 | 3.53 | 3.52 [c] | 3.67 [c] | | | | | |
| **B** | (0.21) | (0.09) | (0.01) | (−0.02) | (−0.05) | | | | | |
| | 102.8 {166} | 74.3 | 76.6 | 72.7 | 75.9 | 176.5 [c] | | | | |
| | (6.0) | (−0.7) | (0.1) | (0.0) | (−1.0) | (0.0) | | | | |
| → 3)-β-D-Gal*p*-(1 → | 4.75 [8] | 3.86 | 3.93 | 4.42 | 3.72 [c] | n.d. | n.d. | | | |
| **C** 4 | (0.22) | (0.41) | (0.34) | (0.53) | (0.07) | | | | | |
| ↑ | 104.5 {n.d.} [d] | 71.5 | 82.3 | 75.4 | 74.6 | 60.6 [c] | | | | |
| | (7.1) | (−1.4) | (8.5) | (5.7) | (−1.3) | (−1.2) | | | | |
| → 4)-β-D-Glc*p*-(1 → | 4.73 [8] | 3.45 | 3.72 | 3.66 [c] | 3.62 [c] | ~ 3.83 [c] | ~ 3.99 [c] | | | |
| **D** | (0.09) | (0.20) | (0.22) | (0.24) | (0.16) | (0.11) | (0.09) | | | |
| | 104.5 {n.d.} | 74.3 | 74.9 [c] | 79.5 | 75.5 | 61.2 [c] | | | | |
| | (7.6) | (−0.9) | (−1.9) | (8.8) | (−1.2) | (−0.7) | | | | |
| → 3)-4,6-(*R*)-Pyr-β-D-Gal*p*-(1 → | 4.55 [12] [b] | 3.79 | 3.81 | 4.40 | 3.62 | 3.98 | 4.10 | 1.48 | | |
| **E** | (0.22) [e] | (0.18) | (0.14) | (0.23) | (0.07) | (0.04) | (0.05) | (0.02) | | |
| | 103.4 {160} | 69.8 | 76.8 | 68.2 | 67.0 | 65.8 | | 25.8 | 101.8 | 176.1 [c] |
| | (−0.8) [e] | (−1.4) | (4.2) | (−3.5) | (0.1) | (0.3) | | (−0.2) | (0.3) | (0.2) |

[a] Chemical shift differences compared to the monomer are given in parentheses. $^{3}J_{H\text{-}1,H\text{-}2}$ values in square brackets, and $^{3}J_{H\text{-}1,C\text{-}1}$ values in braces.
[b] $v_{1/2}$ value in Hz.
[c] Tentative assignment.
[d] n.d. = Not determined.
[e] Chemical shift differences compared to Me 4,6-(*R*)-Pyr-β-D-Gal*p* [6].

Table 3
Observed NOE from anomeric protons of K8

| Residue | Anomeric proton | NOE to | |
|---|---|---|---|
| | $\delta$ | $\delta$ | Residue, atom |
| → 3)-α-D-Gal*p*-(1 → | 5.30 | 4.40 | **E**, H-4 |
| **A** | | 4.08 | **A**, H-2 |
| | | 3.81 | **E**, H-3 |
| β-D-Glc*p*A-(1 → | 4.86 | 4.42 | **C**, H-4 |
| **B** | | 3.67 | **B**, H-5 |
| | | 3.53 | **B**, H-3 |
| | | 3.45 | **D**, H-2 |
| → 3)-β-D-Gal*p*-(1 → | 4.75 | 4.22 | **A**, H-4 |
| **C** 4 ↑ | | 4.15 | **A**, H-3 |
| → 4)-β-D-Glc*p*-(1 → | 4.73 | 3.93 | **C**, H-3 |
| **D** | | 3.86 | **C**, H-2 |
| | | 3.72 | **D**, H-3 |
| → 3)-4,6-(*R*)-Pyr-β-D-Gal*p*-(1 → | 4.55 | 3.81 | **E**, H-3 |
| **E** | | 3.62 | **E**, H-5 |

intra-residual connectivities, inter-residual connectivities were found (Table 3). Thus, NOEs between H-1 of the 3-substituted galactose (**A**, $\delta$ 5.30) and H-3 and H-4 of the 3-substituted 4,6-pyruvated residue (**E**, $\delta$ 4.40 and 3.81) were observed. This establishes structural element **2**. In addition, an intra-residue correlation to H-2 was observed. The pyruvic group has the *R*-configuration, i.e., the methyl group is equatorial, as is evident from the chemical shift of the methyl signal, $\delta_H$ 1.48 and $\delta_C$ 25.8 [6].

$$\overset{\mathbf{A}}{\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}4,6\text{-}(R)\text{-Pyr-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow}\overset{\mathbf{E}}{}$$

**2**

That residue **A** is linked to the 3-position of a galactosyl residue is also evident from the chemical shift of the C-1 signal from **A**, as it appears at a very low value, $\delta$ 96.1 [7]. This is typical of a glycosidic linkage with a γ-gauche interaction, i.e., a five-bond proton–proton interaction, in this case between H-1 in **A** and H-4 in **E**. There are, however, two 3-substituted galactosyl residues; C-4 in **E** has an upfield shift displacement of −3.5 ppm, in accord with structural element **2**. The anomeric proton of the glucuronic acid group (**B**, $\delta$ 4.86) showed an NOE to H-4 in the 3,4-substituted galactose residue (**C**, $\delta$ 4.42) in agreement with structural element **1**. Furthermore, the anomeric proton of the branch-point galactose (**C**, $\delta$ 4.75) showed NOEs to H-3 and H-4 of the 3-substituted D-galactose residue (**A**, $\delta$ 4.15 and 4.22). Structural element **3** is thus demonstrated.

$$\overset{\mathbf{C}}{\underset{\overset{4}{\uparrow}}{\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}}}\overset{\mathbf{A}}{\alpha\text{-D-Gal}p\text{-}(1 \rightarrow}$$

**3**

Table 4
Observed $^{2}J_{C,H}$ and $^{3}J_{C,H}$ connectivities in a $^{1}$H-detected HMBC experiment from anomeric protons and carbons of the capsular polysaccharide from K8

| Residue | Anomeric atom | | $J_{H,C}$ connectvities to | | Residue, atom |
|---|---|---|---|---|---|
| | $\delta(^{1}H)$ | $\delta(^{13}C)$ | $\delta(^{1}H)$ | $\delta(^{13}C)$ | |
| → 3)-α-D-Gal*p*-(1 → | 5.30 | | | 71.5 | **A**, C-5 |
| **A** | | | | 76.8 | **E**, C-3 |
| | | | | 79.3 | **A**, C-3 |
| β-D-Glc*p*A-(1 → | 4.86 | | | 75.4 | **C**, C-4 |
| **B** | | 102.8 | 4.42 | | **C**, H-4 |
| | | | 3.39 | | **B**, H-2 |
| → 3)-β-D-Gal*p*-(1 → | 4.75 | | | ~ 79.4 | **A**, C-3 or **D**, C-4 |
| **C** 4 ↑ | | 104.5 | 3.86 | | **C**, H-2 |
| → 4)-β-D-Glc*p*-(1 → | 4.73 | | | 82.3 | **C**, C-3 |
| **D** | | 104.5 | 3.45 | | **D**, H-2 |
| → 3)-4,6-(*R*)-Pyr-β-D-Gal*p*-(1 → | 4.55 | | | ~ 79.4 | **D**, C-4 or **A**, C-3 |
| **E** | | 103.4 | 3.79 | | **E**, H-2 |

Finally, for residue **D**, inter-residual NOEs are observed from the anomeric proton in **D** to H-2 and H-3 in **C**, demonstrating structural element **4**.

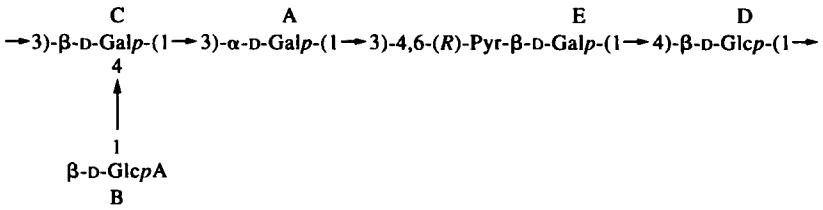
**D** **C**
→ 4)-β-D-Glc*p*-(1 → 3)-β-D-Gal*p*-(1 →
4
↑

**4**

For residue **E** no conclusive inter-residue NOEs were observed. Additionally, an NOE between H-1 in **B** and H-2 in **D** was also observed which is in agreement with the close proximity between protons in a geometrical arrangement of this type [8], i.e., β-D-Glc*p*-(1 → 4)[ β-D-Glc*p*-(1 → 3)]-D-Gal*p*. For this type of substitution pattern in which the galactose residue is vicinally disubstituted, a downfield $^{1}$H shift of ~ 0.2 ppm should be obtained for the signal of the anomeric proton of the residue that substitutes position 4 whereas no such change should be observed for the residue that substitutes position 3 in the galactose residue. The anomeric proton signal of the terminal glucuronic acid group in K8 shows such a downfield displacement.

A $^{1}$H-detected HMBC experiment (Heteronuclear Multiple Bond Connectivity) was employed in order to obtain corroborating sequential information. The $^{3}J_{C,H}$ connectivities of the anomeric protons as well as the anomeric carbons were examined and, in addition to intra-residual connectivities, inter-residual connectivities were observed (Table 4). A correlation from the proton with a signal at δ 5.30, i.e., H-1 in residue **A**, to a carbon resonance at δ 76.8 is observed. The latter signal was assigned to C-3 of residue **E**, thus confirming the structural element **2**. The anomeric proton of **B** (δ 4.86) correlates with a carbon signal at δ 75.4, which was assigned to C-4 of **C** in agreement with earlier observations, i.e., with structural element **1**. The same structural element is also given by the long-range correlation between C-1 in **B** and H-4 in **C**. A correlation between a signal at δ 4.73, H-1 of the 4-substituted glucosyl residue, and a carbon

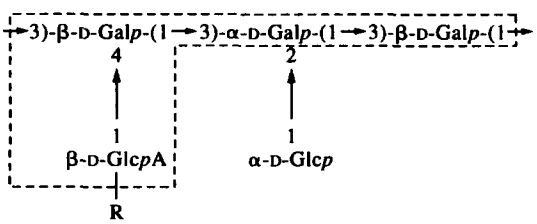
signal at $\delta$ 82.3, C-3 in **C**, gives the sequence **D** to **C**, i.e., structural element **4**. Long-range correlations to signals ~ 79.4 ppm are observed from H-1 in **C** as well as from H-1 in **E**. These observations are in agreement with structural element **3** defined above and with the sequence **E** to **D**. Furthermore, in the HMBC spectrum the H-6 proton resonating at $\delta_H$ 3.98 shows a long-range correlation to C-2 in the pyruvic group. From the combined evidence of the uronic acid degradation, the NOE, and the $^3J_{C,H}$ correlations, the following repeating unit, **5**, can be postulated for K8:




**5**

Since K8 from strain 1015, i.e., the present investigation, originates from the type culture collection in Copenhagen, Denmark, it is suggested that it should keep the designation K8. The other polysaccharide with *Klebsiella* K8 specificity should be renamed as K8,52,59 since it shows serological cross-reactivity with K52 and K59 antisera. This type of cross-reactivity has been observed before (I. Ørskov, unpublished).

The difference between K8,52,59 and SK-1 is one-third of a mole of an *O*-acetyl group located at either O-2, O-3, or O-4 of the terminal GlcA group. The common factor for all three strains is a tetrasaccharide element shown within the dashed line in the structure below.




K8,52,59 R = H
SK1 R = OAc

## 3. Experimental

*Bacterial strain.*—Strain 1015 used in the isolation of the K8 capsular polysaccharide was obtained from the *Klebsiella* K reference strain collection of the International Escherichia and Klebsiella Centre, Copenhagen, Denmark.

*General methods.*—Concentrations were performed under diminished pressure at $< 40°C$ under a stream of air or $N_2$. For GLC, a Hewlett–Packard 5890 instrument fitted with a flame-ionisation detector was used. GLC-MS (EI) was performed on a Hewlett–Packard 5970 MSD instrument. Alditol acetates and partially methylated alditol acetates were analysed on an HP-5 capillary column (25 m × 0.20 mm) using the temperature program 180°C (1 min) → 250°C at 3°C/min.

*NMR spectroscopy.*—NMR spectra of solutions in $D_2O$ were recorded at 70°C using either a Jeol GSX-270, a Jeol $\alpha$-400, or a Varian Unity 500 instrument. Chemical shifts are reported in ppm, using sodium 3-trimethylsilylpropanoate-$d_4$ (TSP, $\delta_H$ 0.00) or acetone ($\delta_C$ 31.00), as internal references. $^1H,^1H$-COSY, relayed and double relayed $^1H,^1H$-COSY, NOESY, $^1H$-HOHAHA, and $^{13}C,^1H$-COSY were performed using Jeol standard pulse-sequences. The mixing time in the NOESY experiment was 300 ms. The $^1J_{C\text{-}1,H\text{-}1}$ values were determined from a coupled HMQC inverse detected spectrum, and the $^1H-^{13}C$ long-range couplings were investigated with an HMBC inverse detected experiment using a delay time of 60 ms.

*Sugar and methylation analysis.*—Hydrolysis of native and methylated K8 was performed by treatment with 2 M trifluoroacetic acid at 120°C for 2 h. The sugars in the hydrolysates were converted into alditol acetates and partially methylated alditol acetates. Carboxyl-reduction of methylated polysaccharide was performed as described [9]. The uronic acid degradation was performed essentially as described [10]. The absolute configurations of the sugars in a hydrolysate were determined essentially as described [5], but using (+)-2-butanol.

## Acknowledgements

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