

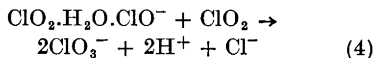
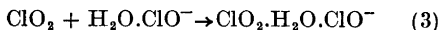
Fig. 1. An example of the effect of pH and the presence of "chlorine" ( $\text{Cl}_2 + \text{HOCl} + \text{OCl}^-$ ) on the rate of decomposition of aqueous chlorine dioxide solutions. The logarithm of the reaction rate in mol/s is plotted versus pH. Temp.  $78^\circ\text{C}$ .  $[\text{ClO}_2] = 0.0028$  mol/l. The initial rate of decomposition  $r_0$ , at different "chlorine" concentrations: A ( $\blacktriangle$ )  $< 1 \times 10^{-4}$ , B ( $\triangle$ )  $2 \times 10^{-4}$ , C ( $\circ$ )  $1.5 \times 10^{-3}$  mol/l; as well as the rate  $r_s$ , of the rapid reaction R ( $\bullet$ ), that occurs after an induction period, are given.

of  $1.59 \times 10^{-7}$  mol/l according to Flis.<sup>10</sup> Both rate constants have activation energies of about 45 kJ/mol. If the temperature dependence of the equilibrium  $\text{ClO}^-/\text{HClO}$  is neglected,  $k_2$  has an apparent activation energy of 70 kJ/mol. The rate equation proposed here, for conditions prevailing during chlorine dioxide bleaching, is different from those previously proposed for chlorine dioxide degradation under other conditions.<sup>3-7</sup>

The first term in the rate equation found can be described by the reactions



while the second term is described by



The reactions (1) and (3) are assumed to be slow and rate determining. This hypothesis is supported by the observation that the entropy of activation,  $\Delta S^\ddagger$ , for the chlorine dioxide decomposition is negative  $-200$  J/K, mol in the absence and  $-100$  J/K, mol in the presence of "chlorine". The entropy values observed

are typical for association reactions of the type (1) and (3).<sup>11</sup>

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## Structural Studies of the *Klebsiella* O Group 8 Lipopolysaccharide

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Five of the twelve different *Klebsiella* O groups contain D-galactose as the only sugar in the O-specific side chains of their lipopolysaccharides (LPS).<sup>1</sup> We have reported structural studies of three of these, O groups 1 and 6<sup>2</sup> (identical) and O group 9.<sup>3</sup> We now report similar studies of the O group 8 LPS.

The LPS was isolated from *Klebsiella* O8:K69 as previously described<sup>1</sup> and showed  $[\alpha]_{D}^{25} + 59^\circ$ . Analysis of a hydrolysate, using D-arabinose as an internal standard, showed that the LPS contained 36 % galactose, 2 % glucose, and 2 % heptose residues. The galactose has the D-configuration, as it reacts with galactose oxidase.<sup>1</sup> Assuming that the sugar residues account for the greater part of the optical rotation, most or all of the D-galactose residues should be  $\alpha$ -linked. The LPS showed strong absorption in the IR at  $1730\text{ cm}^{-1}$ , indicating the presence of O-acyl groups. These were transferred into benzyl esters and identified as acetyl groups by GLC-MS.<sup>4</sup>

A methylation analysis (Table 1, column A) showed that the O-specific side chains

and the products were reduced (NaBD<sub>4</sub>) and subjected to methylation analysis. The results (Table 1, column B) demonstrate that about half of the furanosidic linkages have been hydrolysed and, as 2,3,4,6- but no 2,3,5,6-tetra-O-methyl-D-galactose was found, that no galactofuranose residues are adjacent. The O-specific side chains in bacterial LPS are generally composed of oligosaccharide repeating units. The simplest unit for the present LPS should be a (1 $\rightarrow$ 3)-linked trisaccharide with one D-galactofuranose and two D-galactopyranose residues.

O-Acetyl groups were located by the method of de Belder and Norrman,<sup>3,5</sup> wherein hydroxyl groups are protected as acetals by treatment with methyl vinyl ether, and the product subjected to methylation analysis. The formation of 2-, 4-, and 6-O-methyl-D-galactose (15, 2, and 8 %, respectively) demonstrated the presence of O-acetyl groups in these positions in the original polysaccharide. The degree of substitution was 0.25.

Part of the acetalated polysaccharide was trideuteriomethylated, hydrolysed under mild conditions during which only the protecting acetal groups should be removed and remethylated (CH<sub>3</sub>I). Analysis of this product (Table 1, column C) revealed that some furanosidic linkages had been cleaved, since a small portion of 2,3,4,6-tetra-O-methyl-D-galactose was obtained. MS of the derived alditol acetates showed the presence of trideuteriomethyl groups at C-2 in both furanosidic and pyranosidic residues and at C-6 in pyranosidic residues. The small percentage of O-acetyl groups in the 4-positions was not revealed by this procedure.

From the combined evidence presented above, structure I is proposed for the trisaccharide repeating unit in the O-specific side chains of the *Klebsiella* O group 8 LPS. A dotted line indicates a substituent that is not present in all repeating units. As no sugar deriving from the non-reducing terminal was observed in the methylation analysis it is inferred that the O-specific side chains are at least thirty repeating units long. The structure has structural features in common with the pentasaccharide repeating unit (II) proposed for the *Klebsiella* O group 9 LPS.<sup>3</sup> (Most sugar residues in I and II should be  $\alpha$ -linked but not necessarily all, as indicated in the formulae.) Immunological cross-reactions between these two O groups may therefore be expected. The observation

Table 1. Methylation analyses of original and modified *Klebsiella* O group 8 LPS.

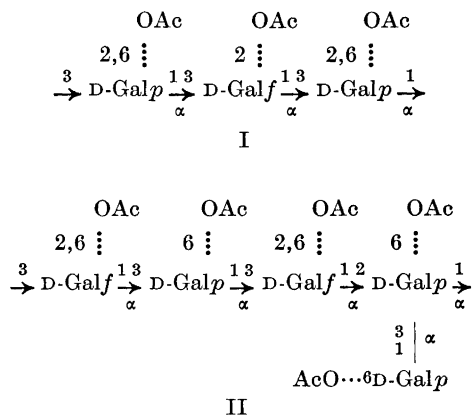
Sugars <sup>a</sup>	T <sup>b</sup>	Mol % <sup>c</sup>		
		A	B	C
1,2,4,5,6-Gal <sup>d</sup>	0.46	—	20	—
2,3,4,6-G	1.00	5	4	7
2,3,4,6-Gal	1.17	—	28	5
2,5,6-Gal	1.80	33	15	32 <sup>e</sup>
2,4,6-Gal	1.99	63	34	56 <sup>f</sup>

<sup>a</sup> 1,2,4,5,6-Gal = 1,2,4,5,6-penta-O-methyl-D-galactitol; 2,3,4,6-G = 2,3,4,6-tetra-O-methyl-D-glucose, etc. <sup>b</sup> Retention time of the corresponding alditol acetate relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol on a SP-1000 glass capillary column at 220°. <sup>c</sup> A: original LPS; B: partially acid degraded LPS (see text); C: acetalated, trideuteriomethylated, hydrolysed and methylated LPS (see text). <sup>d</sup> Monodeuterated at C-1. <sup>e</sup>  $\approx 18\%$  OCD<sub>2</sub>-groups at position 2. <sup>f</sup>  $\approx 12\%$  OCD<sub>2</sub>-groups at position 2 and  $\approx 12\%$  at position 6.

are linear and composed of (1 $\rightarrow$ 3)-linked furanosidic and pyranosidic D-galactose residues in the proportion 1:2. This proportion, for five different analyses, was  $1:2 \pm 0.2$ . Arabinose and galactose (1:1.9) were identified after periodate oxidation, borohydride reduction and acid hydrolysis of the LPS.

A sample of the LPS was subjected to mild acid hydrolysis, whereby only the furanosidic linkages should be hydrolysed,

by Kaluzewski<sup>8</sup> that they are serologically identical is, however, difficult to understand in the light of the present results.



*Experimental.* General methods and methods for sugar analysis, methylation analysis, and location of *O*-acetyl groups were essentially the same as used in studies on the related *Klebsiella* O group 9 LPS.<sup>3</sup> GLC of the partially methylated alditol acetates was performed at 220° on a glass capillary column (0.25 mm × 25 m) containing SP-1000 as the stationary phase. For GLC-MS an OV-225 SCOT column was used. The LPS was isolated<sup>1</sup> from strain *Klebsiella* O8:K69 (889), and showed  $[\alpha]_{578} + 59^\circ$  (*c* 0.25, water). In the IR (KBr) a strong absorption at 1730 cm<sup>-1</sup> was observed. The LPS contained 1.8 % P.

*Analysis of partially hydrolysed LPS.* The LPS (5 mg) in 0.025 M sulphuric acid (5 ml) was kept at 100° for 2 h and neutralised (BaCO<sub>3</sub>). Sodium borodeuteride (20 mg) was added and the solution kept for 3 h at room temperature. The solution was treated with Dowex 50 (H<sup>+</sup>), concentrated and boric acid removed by distillations with methanol. The product was dissolved in water, lyophilised and subjected to methylation analysis.

*Periodate oxidation of the LPS.* The LPS (5 mg) was dissolved in 0.1 M sodium acetate buffer of pH 3.9 (5 ml), 0.08 M sodium metaperiodate (2 ml) was added and the solution kept in the dark at 4° for 48 h. Excess periodate was reduced with ethylene glycol (0.5 ml) and the solution was dialysed against tap-water overnight. Sodium borohydride (60 mg) was added, the solution kept for 7 h at room temperature, excess borohydride decomposed by addition of 50 % acetic acid and the solution dialysed overnight. Sugar analysis of this

product showed arabinose and galactose in the molar proportion 1:1.9. D-Mannose (1 mg) was added as an internal standard, and the two sugars accounted for all the galactose in the original LPS.

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## On the Calibration of Circular Dichroism Spectrometers

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Due to uncertainties about CD standards<sup>1-3</sup> we want to suggest two methods of calibrating a CD spectrometer. The first determines whether the instrument is actually measuring  $CD = A_1 - A_r$ . It may also be used for the calibration over a wavelength range, in which the instrumental accuracy depends upon the wavelength program for the excitation voltage of the light modulation.<sup>4</sup> The second is appropriate for regular checking.