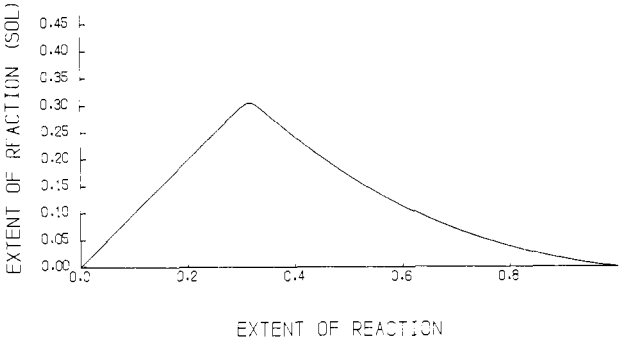

Figure 3. Graph of the extent of reaction in the sol against the overall extent of reaction. They are equal starting from 0 until a gel forms at α_c, when there is a sharp peak (rounded by computer error). The sol extent of reaction then declines monotonously to 0, at which point the gel contains every unit. (The values were computed for the equireactive A_2RB_3 model.)

The *conditional probability generating function for the sol* is

$$\hat{P}(a,b) \equiv \sum_{(k,l)} \hat{p}_{kl} a^k b^l = \frac{(1 - \alpha + \alpha\hat{a}\cdot a)^g (1 - \beta + \beta\hat{b}\cdot b)^{f-g}}{(1 - \alpha + \alpha\hat{a})^g (1 - \beta + \beta\hat{b})^{f-g}} = \left\{\frac{1-\alpha}{1-\alpha+\alpha\hat{a}} + \frac{\alpha\hat{a}}{1-\alpha+\alpha\hat{a}}a\right\}^g \left\{\frac{1-\beta}{1-\beta+\beta\hat{b}} + \frac{\beta\hat{b}}{1-\beta+\beta\hat{b}}b\right\}^{f-g} \quad (42)$$

(See (28) and (2)). Hence *the extents of reaction in the postgelation sol* are

$$\hat{\alpha} = \alpha\hat{a}/(1 - \alpha + \alpha\hat{a}) \qquad \hat{\beta} = \beta\hat{b}/(1 - \beta + \beta\hat{b})$$

As in Flory's RA_f model (Flory[5]), the postgelation sol at increasing extents of reaction mimics the pregelation sol at decreasing extents of reaction (see Figure 3).

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Appendix

Good's[10] generalization of Lagrange's expansion (stated for 2 variables) is to let $a = yG(a,b)$ and $b = zH(a,b)$, where G and H are analytic in a neighborhood of the origin with $G(0,0) \neq 0$ and $H(0,0) \neq 0$. Then, for any $P(a,b)$ analytic (or even meromorphic) at (0,0)

$$\mathcal{C}(y^m z^n)\{P(a,b)\} = \mathcal{C}(a^m b^n)\{P(a,b)[G(a,b)]^m[H(a,b)]^n \det \mathbf{D}\}$$

where

$$D = \begin{bmatrix} 1 - a\frac{\partial}{\partial a}(\ln G) & -a\frac{\partial}{\partial a}(\ln H) \\ -b\frac{\partial}{\partial b}(\ln G) & 1 - b\frac{\partial}{\partial b}(\ln H) \end{bmatrix}$$

and "ln" denotes log natural to the base e.

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Notes

Molecular Motion of Branched-Chain Polysaccharides Studied by ^{13}C NMR Spin–Lattice Relaxation Rates

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Structural determination of the sequence of polysaccharides by chemical and enzymatic means is a time-consuming and difficult study. However, NMR spectroscopy, particularly ^{13}C NMR, has proved to be an indispensable tool in the structural analysis of polysaccharides.[1] Today NMR spectroscopy is extensively used for structure elucidation as it demonstrates the existence of a regularly repeated unit in the sequence and as it is an essential tool for obtaining direct evidence of the number of sugar residues in the repeating unit. Furthermore, NMR spectroscopy makes it easy to ascertain the anomeric configuration of glycosidic linkages in polysaccharide structures through the one-bond ^{13}C–^{1}H coupling constants.[1,2] ^{13}C NMR spectroscopy is thus well suited for structural analysis of polysaccharides because it gives well-defined spectra even in the case of complex and large repeating units. However, an analysis of the ^{13}C chemical shifts and coupling constants does not unambiguously determine the sequence of the monosaccharides that constitute the polysaccharide structure. Additional information on the sequence within the repeating unit can be obtained on the native polysaccharide by use of spin–lattice relaxation studies (R_1 values).

The background for the application of ^{13}C NMR spin–lattice relaxation rates in the study of macromolecular dynamics has recently been discussed in a review by Jardetzky[3] and in a theoretical paper by Bull.[4] Studies on the relaxation behavior of mono- and oligosaccharides have been carried out in order to prove that the relaxation mechanism for this type of compounds is dominated by the intramolecular dipole–dipole relaxation mechanism.[5,6]

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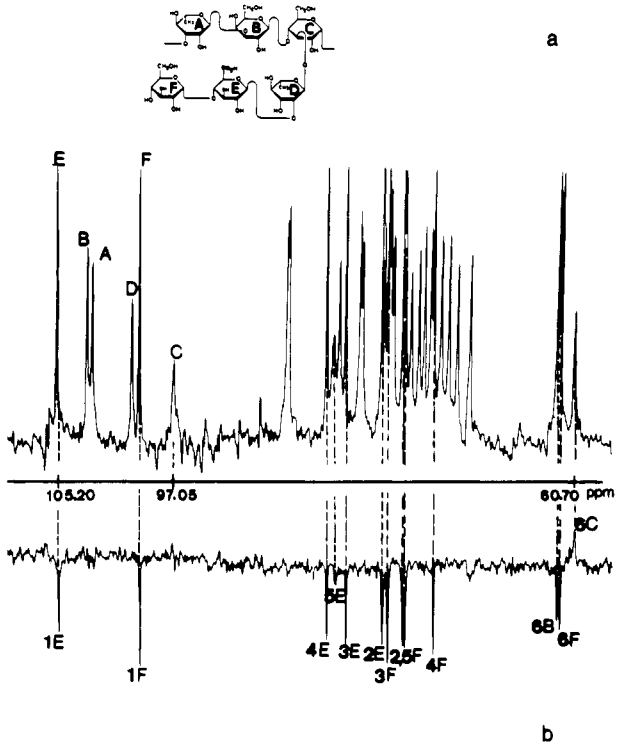

Figure 1. ^{13}C NMR spectra of the K-18 *Klebsiella* polysaccharide: (a) fully relaxed, (b) partially relaxed (t = 0.1 s).

The relaxation rates for oligosaccharides have furthermore been shown to give information about the molecular motion of these molecules[7,8] and proved useful for the identification of carbon atoms that belong to the same unit of an oligosaccharide.[9-12] If the segmental motion determined from R_1 values is greater for a side chain than for the main chain in a polysaccharide, a distinction could be possible between those sugar units that are in the side chain or in the main chain or that occupy a branch-point position. In this work, we wish to report on the application of ^{13}C relaxation rates in the study of the molecular motion and also in the assignment of ^{13}C NMR signals of polysaccharides related to the capsular polysaccharides associated with *Klebsiella* bacteria. These compounds provide a wide choice among the approximately 80 different serotypes of easily available regular repeating models[13] with a great variety of branched-type structures.

Serotypes K-18[14] and K-41[15] were selected for this study because their trisaccharide side chains are the longest found in this series of polysaccharides. In addition both are devoid of other substituents, pyruvate, formate, or acetate, which are common in many other serotypes. They are therefore simple models for the study of the ^{13}C NMR relaxation rate of the different sugar residues depending on their position within the side chain relative to the branch point and to the other residues of the main chain.

^{13}C NMR spectra of K-18 and K-41 antigen polysaccharides were obtained on a Bruker WM 250 instrument operating at 62.84 MHz using 5% (w/v) solutions in 99.96% D_2O at 360 K. The inversion recovery method was used for T_1 determinations [two pulse sequences $(T-180-t-90°)_n$] with eight t values. The peak heights of the different carbons were measured as a function of delay time (t), and these data were then used in a computer program that determines the relaxation rates (±10%).

The fully relaxed spectrum of K-18 is presented in Figure 1 together with one of the partially relaxed spectra. The fully relaxed spectrum for the hexasaccharide repeating unit is well resolved and shows 35 separate signals, including the C-6 of the glucuronic acid and of the rhamnose residues, not shown in Figure 1. As seen in the anomeric region, large differences in the relative intensities of the signals are observed. Two anomeric signals have a high intensity, three signals have a medium intensity, and the last signal has a very low intensity. The R_1^{obsd} values for each of the resonances are reported in Table I. The discrepancies in the ^{13}C R_1 values for the ring carbon atoms are the same as those found for the anomeric signals. Since the anomeric carbon resonances were previously unambiguously assigned,[16] identification of the remaining signals in the partially relaxed spectrum was possible (t value of 0.1 s). A number of signals are eliminated in this partially relaxed spectrum because the magnetization vectors of these carbon atoms have reached the null point. The remaining inverted signals correspond to two sugar residues. The knowledge[16] of the assignments for the anomeric resonances allowed the identification of the two inverted signals as being those of sugar residues E and F at 105.2 and 99.85 ppm, respectively. Without taking into account the distinct C-6 resonances of the quaternary carbon of the uronic acid and the primary hydroxyl carbons of the hexoses, the former relaxing much slower and the latter much faster, 10 remaining inverted signals can be identified. The α and β configuration of residues E and F was previously established by the ^1H NMR[14] of the aldobiouronic acid E–D.

Table I
^{13}C NMR Data for *Klebsiella* K-18 Capsular Polysaccharide

chem shift[a]	R_1, s^{-1} 360 K	assign	chem shift[a]	R_1, s^{-1} 360 K	assign
105.20	3.2	C-1E	73.60	5.6	
103.10	4.2	C-1B	72.95	3.0	} C-2F, C-5F
102.70	5.3	C-1A	72.85	2.7	
99.95	5.0	C-1D	72.25	5.9	
99.35	3.4	C-1F	71.80	5.0	
97.05	6.7	C-1C	71.40	4.0	
81.20	5.3		70.95	4.8	
81.10	5.3	C-3B	70.75	2.9	C-4F
78.40	3.0	C-4E	70.20	3.8	
77.90	4.5	C-5E	69.65	5.0	
77.20	(NC)[b]		69.00	5.9	
77.05	3.2	C-3E	68.10	3.8	
76.00	(NC)[b]		61.90	4.2	C-6B
75.85	(NC)[b]		61.70	2.9	C-6F
74.45	4.5	C-2E	60.70	(NC)[b]	C-6C
74.25	5.3		17.7	(NC)[b]	C-6A
74.05	3.6	C-3F	17.4	(NC)[b]	C-6D
73.85	5.0		174.00	(NC)[b]	C-6E

[a] Chemical shifts are given in ppm, relative to internal acetone, δ 31.07 downfield from sodium 4,4-dimethyl-4-silapentanesulfonate (DSS). [b] NC: not calculated.

Because E and F residues are homologous sugars with D-gluco configuration, but β-linked (1,2 trans) and α-linked (1,2 cis), respectively, the resonances of the corresponding carbon atoms have distinct chemical shifts and allowed the assignment indicated in Figure 1. It should be noted that the signal from C-5E has a particularly low intensity. This is probably caused by line broadening due to the presence of the adjacent carboxyl group. This has been interpreted by Casu and co-workers on monomeric model compounds as an additional relaxation contribution induced by a paramagnetic impurity.[17] The assignment of the three signals, two negative and one positive, corresponding to the primary hydroxyl carbon atoms is not straightforward because of the internal motion around the C-5–C-6 bond. However, of the three hexose residues of the repeating unit, residue C can be expected to exhibit less segmental motion because of its location at a branch point, which makes it relax faster than B and F.

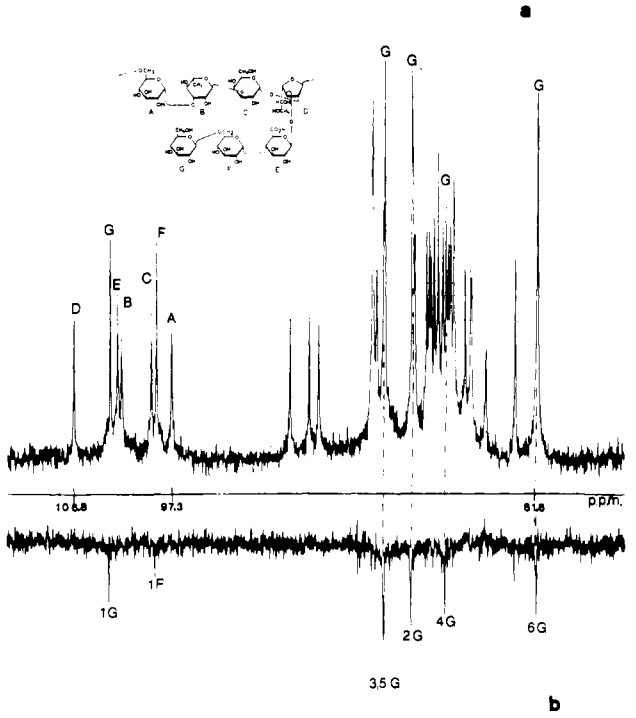

Figure 2. ^{13}C NMR spectra of the K-41 *Klebsiella* polysaccharide: (a) fully relaxed, (b) partially relaxed (t = 0.08 s).

The same is true for the anomeric signals, where unit C has the fastest R_1 values. From Table I, only two sets of values can be distinguished: above 4 s^{-1} and below 3.34 s^{-1}. It is observed that the fast R_1 values expected for all the carbon atoms of unit C are difficult to identify because of the small chemical shift differences between the signals. It can be seen that only the two terminal sugar residues E and F from the trisaccharide side chain give rise to slow ^{13}C R_1 values. It appears in this case that residue D, directly attached to the branch point, behaves in the same manner as those of the main chain, in agreement with the decreasing segmental motion as one moves away from the end unit.[10]

In the same way the C-6 signals of residues B and F, which have very similar chemical shifts, were tentatively assigned on the basis of their relaxation rates. Unit B, which is in the main chain, was expected to have less mobility than unit F, as is shown in Table I.

Another model, serotype K-41, with a trisaccharide side-chain moiety was also studied. The fully relaxed spectrum and one of the partially relaxed spectra for K-41 are presented in Figure 2. The heptasaccharide repeating unit gives a fully relaxed spectrum that shows 34 separate signals, including the C-6 resonances of one glucuronic acid and one rhamnose residue not shown in Figure 2. Partially relaxed spectra corresponding to different t values were recorded, but the most significant was obtained with a delay time of 0.08 s (Figure 2). In this case, only six signals are observed. In previous results[16] two of these signals were unambiguously assigned to C-1G and C-6G. The other four negative signals can then be easily ascribed to the C-3, C-5, C-2, and C-4 of residue G, in agreement with the known chemical shifts of a β-1,6-linked glucose, gentiobiose.[18] A delay time of 0.05 s produced inversion of all the carbon signals corresponding to the gentiobiosyl terminal side-chain unit (G and F). This allowed the assignment of the carbons from glucosyl residue F. However, as seen from Table II, one must be cautious in considering the values for the signals of the ring carbon atoms since some signals are so close in chemical shift that the observed

Table II
^{13}C NMR Data for *Klebsiella* K-41 Capsular Polysaccharide

chem shift[a]	R_1, s^{-1} 360 K	assign	chem shift[a]	R_1, s^{-1} 360 K	assign
106.8	5.0	C-1D	72.40	3.4	C-2F
103.3	2.0	C-1G	72.15	3.7	C-4F
102.6	3.9	C-1E	71.95	4.3	
102.25	4.2	C-1B	71.65	3.6	
99.35	4.0	C-1C	71.25	5.0	
98.85	3.2	C-1F	70.80	4.5	
97.3	4.6	C-1A	70.50	2.0	C-4G
85.75	4.3	C-2D	70.30	4.5	
83.90	5.0	C-3D	70.10	4.3	
82.95	5.0	C-4D	69.75	4.5	
77.70	4.6		68.65	5.3	C-6F
77.35	4.0		68.10	4.5	
76.65	1.9	C-3G	68.00	5.0	
76.50	1.9	C-5G	66.60	9.1	
73.80	2.6	C-2G	63.80	3.0	
73.55	3.6	C-3F	61.60	2.9	C-6G

[a] Chemical shifts are given in ppm, relative to internal acetone, δ 31.07 downfield from sodium 4,4-dimethyl-4-silapentanesulfonate (DSS).

R_1 value for one given carbon may be averaged by the relaxation of the adjacent overlapping signal. This problem could be solved by the use of a spectrometer operating at higher field.

On the basis of these two branched polysaccharides, it can be concluded that the ^{13}C R_1 values for some of the anomeric carbon atoms can be interpreted in terms of their chemical structure. In both examples the fastest R_1 values correspond to branch-point residues. This is further confirmed in the case of the K-41 antigen, since three other signals belonging to the branched sugar units and appearing in a well-separated region also have the fastest R_1 values. This indicates restricted mobility for the sugar residue to which the side chain is attached. Another characteristic indication is provided by the much smaller R_1 value of the end group of the side chain. This is particularly clear for residue G of K-41, which not only occupies a terminal position but also is glycosidically linked to the C-6 of the neighboring residue. This type of linkage through the primary hydroxyl carbon introduces an additional degree of freedom due to the ω torsion angle. For such branched-chain heteropolysaccharides, the two residues at the nonreducing end of the side chain are more mobile whereas the residue directly attached to the branch point behaves like the sugar units of the main chain. Thus, there is a limitation in the application of R_1 values for complete sequencing of the side chains in branched polysaccharides.

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