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2D-N.M.R. STUDIES OF THE RELATED BACTERIAL POLYSACCHARIDES K54 AND XM6

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ABSTRACT

2D-N.m.r. (COSY) spectra of tetrasaccharides derived from the bacterial polysaccharides K54, produced by *Klebsiella aerogenes* serotype K54, and XM6 (from *Enterobacter* NCIB 11870) have enabled full assignment of the ^{1}H-n.m.r. spectra. The 2D spectra, together with n.O.e. experiments, indicate that the *O*-acetyl group of K54 is located at O-2 of the L-fucose residue. The ^{13}C-n.m.r. spectra of the polysaccharides have been assigned with the help of 2D-^{13}C/^{1}H shift-correlation methods.

INTRODUCTION

The extracellular polysaccharide XM6, which is produced by *Enterobacter* (NCIB 11870), is of potential commercial interest because it forms thermoreversible gels at extremely low concentrations[1] in aqueous salt solutions. The polysaccharide comprises a tetrasaccharide repeating-unit[2] which is identical with that[3] of the capsular polysaccharide produced by *Klebsiella aerogenes* serotype K54 except that, in the latter, every other repeating unit carries an *O*-acetyl group[3]. Comparison of the structures of these polysaccharides is of interest in studying the formation of gels[4], since the K54 polysaccharide forms gels only after *O*-deacetylation. Likewise, adsorption of the K54 polysaccharide onto the surface of erythrocytes occurs only after deacetylation[5,6].

X-Ray diffraction has been used[7] to study the effects of *O*-deacetylation on the molecular structure and intermolecular association of polysaccharide K54. Fibre X-ray diffraction patterns of the XM6 and K54 polysaccharides were interpreted in terms of a double helical structure (eight tetrasaccharide repeat-units per three turns of the helix) for each polymer, but the pattern for the XM6 polysacchar-

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ide was characteristic of a highly crystalline material, whereas that for the K54 polysaccharide indicated poor crystallinity. It was suggested[7] that the *O*-acetyl groups present in the K54 polysaccharide prevent intermolecular association of neighbouring double-helical segments and, thus, inhibit the crystallisation processes that are essential in the formation of gels.

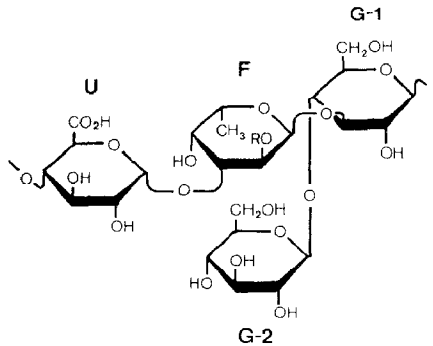
Attempts to model the 3D structure of the XM6 and K54 molecules[8] have emphasized the need to define the location of the *O*-acetyl groups in the latter. It was concluded[3] from a comparison of the ^{13}C-n.m.r. spectra of the acetylated and deacetylated tetrasaccharides, obtained by enzymic degradation of K54, that the *O*-acetyl substituent was located at O-2 of the L-fucose residue. Unfortunately, the assignment of these spectra was only partial and we have sought further confirmation of the position of the *O*-acetyl substituent. Mass spectrometry of oligosaccharides obtained from the K54 polymer confirmed that only the L-fucosyl residue was acetylated[2] at O-2 or O-4. The first computer-generated model of the 8_3 double helix strongly favoured O-4 as the acetylation site[8], placing the acetate group on the outside of the helix, whereas a substituent at O-2 would have had a sterically unfavourable position in the helix interior.

We now report 2D-n.m.r. (COSY) experiments which have enabled complete assignment of the ^{1}H-n.m.r. spectra of deacetylated (**1**) and acetylated (**2**) tetrasaccharides derived from the K54 polysaccharide, and establish that the *O*-acetyl group is located at O-2 of the L-fucosyl residue.

RESULTS AND DISCUSSION

The sugar residues of the poly- and oligo-saccharides are coded as shown in the annexed structure of the repeating units.

The XM6 and K54 polysaccharides have the same sequence of four sugar residues[2,3], but the latter has a single *O*-acetyl group on every second L-fucose residue. The tetrasaccharides **1** and **2**, obtained by enzymic degradation of the polysaccharides, comprise this repeating unit.



1 R = H
2 R = Ac

^{1}H-N.m.r. spectra. — The 1D spectra of **1** and **2** are shown in Fig. 1. Assignment of complex spectra of carbohydrates by 2D techniques requires the prior assignment of at least one resonance in each sugar ring. The spectral region 4–6 p.p.m., which contains the resonances of the anomeric protons, is the usual starting point for such analyses. The region from 4.1–5.5 p.p.m. for **1** contains 7 d and a dq (at 4.76 p.p.m.). The m at 4.76 p.p.m. can arise only from H-5(F) and this assignment was confirmed by a homonuclear decoupling experiment in which H-6,6,6(F) at 1.18 p.p.m. was irradiated. In the work of Dutton and Merrifield[3], the m for H-5(F) appears to have been attributed to an *O*-formyl group, but it was confirmed subsequently that no *O*-formyl groups are present in the polysaccharide[9]. The d at 4.2 p.p.m. may be assigned to H-5(U) since its coupling constant (10 Hz) is outside the normal range for anomeric protons and this is the only proton in **1** [apart from the anomeric protons and H-6(F)] expected to give rise to a simple d. The slight line-broadening observed for this d may reflect an exchange process involving the salt and acid forms, since the chemical shift of the resonance H-5 in uronic acids is sensitive to protonation of the carboxyl group[10]. The spectra in Fig. 1 were obtained from solutions (pD 3.0) of **1** and **2** in the acid form. On adjusting the pD to 8.0 by the addition of sodium hydroxide, the chemical shift of the signal of H-5(U) in **1** was shifted from 4.21 to 4.05 p.p.m. The chemical shifts of the signals of H-1(U) and H-4(U) were also shifted upfield by 0.05 p.p.m. and that of H-4(G-2) was shifted downfield by 0.03 p.p.m. All other changes in chemical shifts were <0.01 p.p.m. Assignment of the resonances for H-1 of the fucose and glucuronic acid residues may then be obtained from the COSY spectra (see below) and, for the

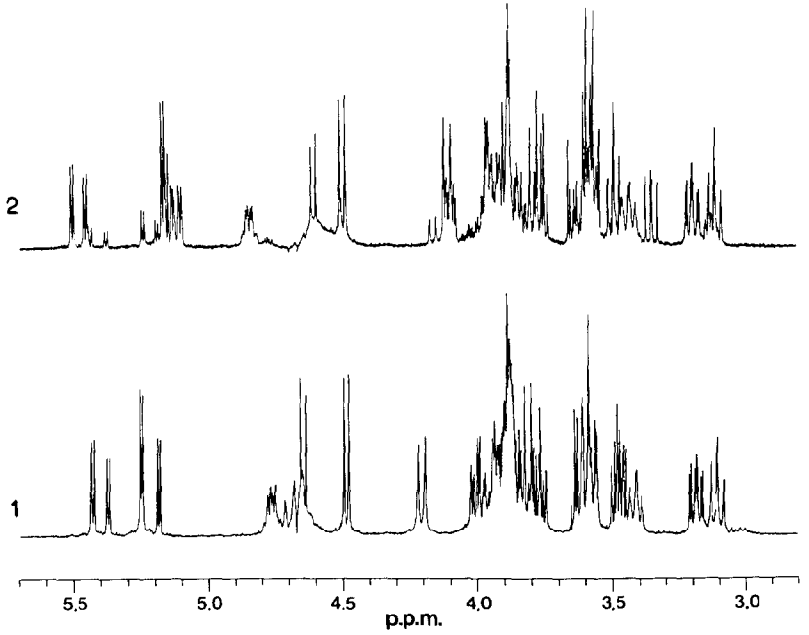


Fig. 1. 400-MHz ^{1}H-n.m.r. spectra of **1** and **2** (AcO resonances not shown).

TABLE I

^{1}H-N.M.R. DATA FOR TETRASACCHARIDES **1** AND **2**

Atom	*Chemical shifts*[a] *(p.p.m.)*				
	α-GlcA (U)	*α-Fuc (F)*	*α-Glc ~OH (αG-1)*	*β-Glc ~OH (βG-1)*	*β-Glc (G2)*
1					
H-1	5.25	5.43, 5.37[b]	5.19	4.64	4.49
H-2	3.62	4.01[c]	3.77	3.47	3.18[c]
H-3	3.82	3.91[c]	3.94	3.77	3.48[c]
H-4	3.58	3.89	3.87	3.88	3.11[c]
H-5	4.21	4.76	3.95	3.58	3.41[c]
H-6		1.17[c]	n.a.[d]	3.82	3.58
H-6′			n.a.	3.96	3.89
2					
H-1	5.16	5.50, 5.45[b]	5.15	4.60	4.49
H-2	3.58	5.11[c]	3.64	3.35	3.19[c]
H-3	3.77	4.10[c]	3.92	3.76	3.49[c]
H-4	3.56	3.96	3.85	3.87	3.11[c]
H-5	4.10	4.84	3.95	3.56	3.43[c]
H-6		1.20[c]	n.a.	3.82	3.58
H-6′			n.a.	3.96	3.90
OAc		2.14[c]			
Coupling constants[e] *(Hz)*					
1					
$J_{1,2}$	3.9	4.0	3.7	8.0	7.8
$J_{2,3}$	9.8	10.1	9.5	9.2	9.4
$J_{3,4}$	9.2	2.0	n.a.	9.3	9.4
$J_{4,5}$	10.1	3.8	n.a.	9.3	9.6
$J_{5,6}$		6.7	n.a.	4.7	8.1
$J_{5,6'}$			n.a.	2.1	1.9
$J_{6,6'}$			n.a.	12.3	12.2

[a]±0.01 p.p.m. [b]Values for molecules containing αG-1 and βG-1. [c]Two sets of signals observed with $\Delta\delta \leq 0.01$ p.p.m. (see Footnote *b*). [d]Not assigned. [e]±0.1 Hz.

glucose residues, from the integrated intensities of the remaining d. These assignments, which are in agreement with those of Dutton and Merrifield[3], are given in Table I. The spectrum of **2** shows some similarities to that of **1**, but is slightly complicated by the presence of ~20% of **1** as impurity. Considerations similar to those given above permit the assignment of the resonances of the anomeric protons, together with those of H-5(F) and H-5(U). The signal for H-5(U) (d, 4.1 p.p.m.) of **2** overlaps with an m subsequently shown to be due to H-3(F).

The assignment of signals in the region 4.1–5.5 p.p.m. forms the basis for the assignment of the entire spectrum by means of the COSY technique[11,12]. COSY spectra of **1** and **2** are shown in Figs. 2 and 3. The step-wise assignment procedure,

beginning at H-5(F), is illustrated for the protons of the fucose rings. The chemical shifts of the resonances of the remaining protons in the other sugar rings were determined in similar fashion. Modified COSY experiments (RELAY[13] and f_1-decoupled COSY[12,14]) were used in order to resolve some ambiguities in the crowded central region of the spectrum where several cross-peaks overlapped. A further aid to assignment is provided by the cross-peaks which are attributable to long-range coupling. These signals are readily distinguishable, because of their low intensity. In Fig. 2, for example, weak cross-peaks may be discerned linking H-1(F) with H-5(F), and H-1(U) with H-3,5(U). These small couplings do not give rise to resolvable line-splitting in the 1D spectra.

The ^{1}H-n.m.r. data for **1** and **2** are given in Table I. The resonance for H-2(F) is shifted downfield by 1.1 p.p.m. on going from **1** to the acetylated molecule **2**; all other changes in chemical shifts were <0.1 p.p.m. This is convincing evidence that it is position 2 in the fucose moiety which is acetylated in **2** and also, therefore, in the K54 polysaccharide. The coupling constants given in Table I indicate that the

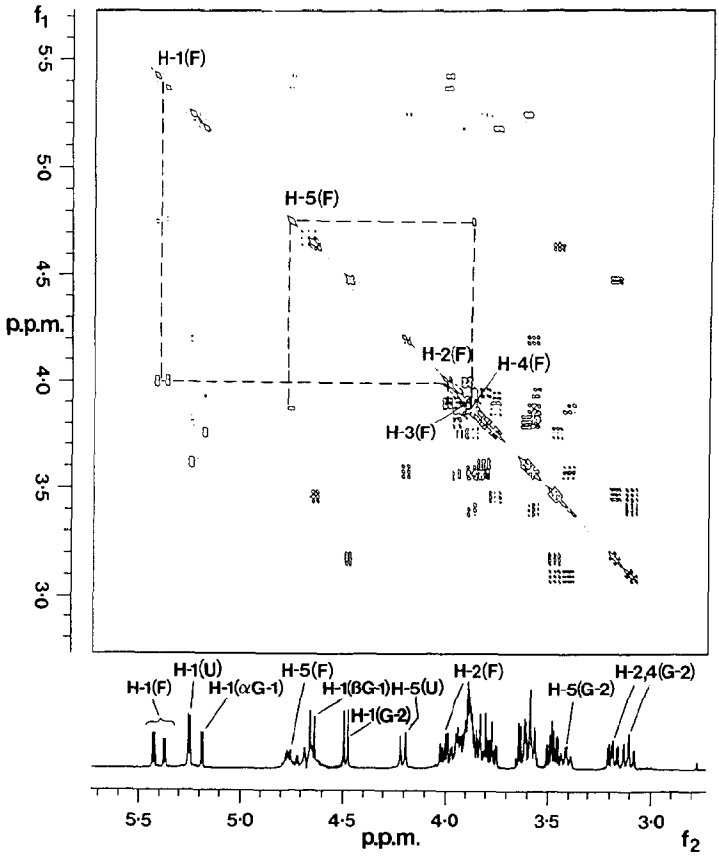


Fig. 2. COSY spectrum of **1**. Dotted lines illustrate connectivities between *J*-coupled fucose (F) resonances.

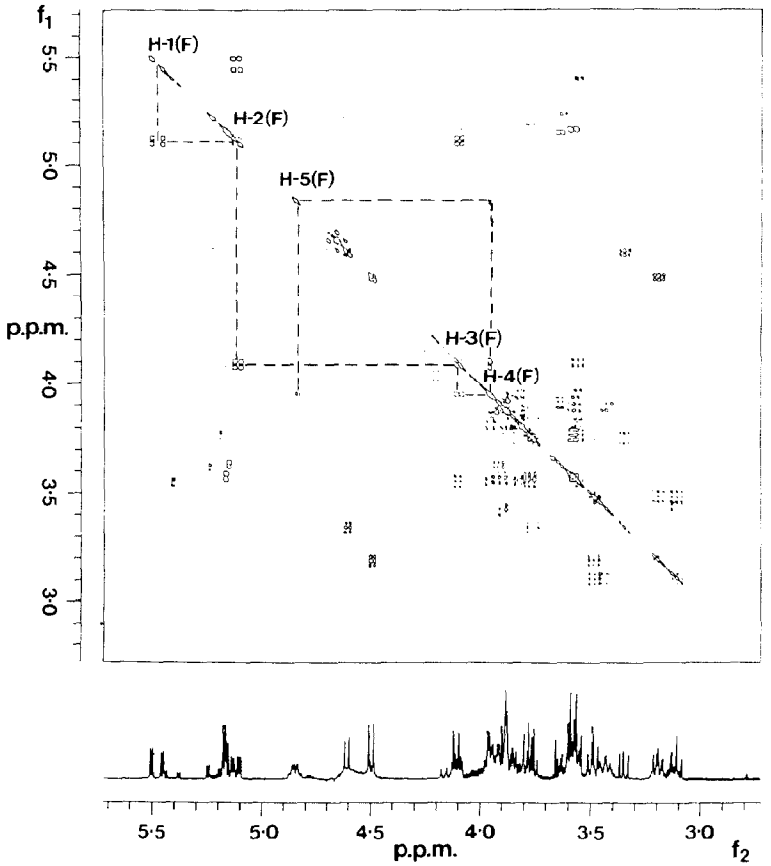


Fig. 3. COSY spectrum of **2**. Dotted lines illustrate connectivities between *J*-coupled fucose (F) resonances.

sugar residues are in the expected chair conformations, *i.e.*, $^1C_4$ for L-fucose ($J_{2,3}$ 10.1 Hz) and $^4C_1$ for the other residues.

In the 1D spectra (Fig. 1), many of the m of the F and G-2 residues have twice the expected number of lines, *e.g.*, that for H-2(G-2) of **1** at 3.18 p.p.m. consists of eight rather than four lines. The doubling occurs for protons H-1,2,3,6(F) and H-2,3,4,5(G-2) in both **1** and **2**. In addition, **2** gave two peaks for OAc. Residues F and G-2 are linked to the reducing D-glucose residue (G-1), and therefore the resonances of their protons have slightly different chemical shifts depending upon whether G-1 is α or β. The largest of these differences (0.06 p.p.m.) is associated with the resonance of H-1(F), which is to be expected. The high-frequency signal for H-1(F) (d 5.43 p.p.m., 55% intensity) originates from the molecule where G-1 is β. The m for H-2(F) in **1** (Figs. 1 and 2) exhibits a second-order intensity pattern because of strong coupling between H-2,3(F). In **2**, the difference in chemical shift of the resonances for H-2,3(F) has increased sufficiently to give a first-order intensity pattern for the H-2(F) m in which the smallest splitting

can be attributed to the $\alpha\beta$-effect. No such effect was observed for H-1(G-2), but the remaining proton resonances of G-2 were split.

Selected n.O.e. spectroscopy[15] experiments on **1** and **2**, illustrated in Fig. 4, were undertaken in order to confirm the spectral assignments and the proposed linkage pattern. Detailed investigations of molecular conformation, which would have required quantitative measurement of n.O.e. values by either 1D or 2D methods, were not pursued because of the extremely small values of the n.O.e. Heteronuclear n.O.e. measurements showed that, at 30°, the average $^{13}C-\{^1H\}$ n.O.e. for the ring carbons of **1** was 2.1, corresponding to a correlation time for isotropic reorientation (τ_c) of $\sim 4 \times 10^{-10}$ s. At a proton operating frequency of 400 MHz, this value of τ_c is very close to ω_L^{-1} (where ω_L is the Larmor frequency in rad/s); under these conditions, small $^1H-\{^1H\}$ n.O.e. values are to be expected.

Selective irradiation of the high-frequency d for H-1(F) of **1** at 5.43 p.p.m. (Fig. 4a) gave comparable n.O.e.s for two protons identified from the COSY spectrum as H-2(F), the proton closest to H-1(F) within the fucose ring, and H-3(βG-1), the proton directly across the glycosidic linkage from H-1(F). Likewise, for **2** (Fig. 4b), there were n.O.e.s for H-2(F) and H-3(βG-1). The large change in

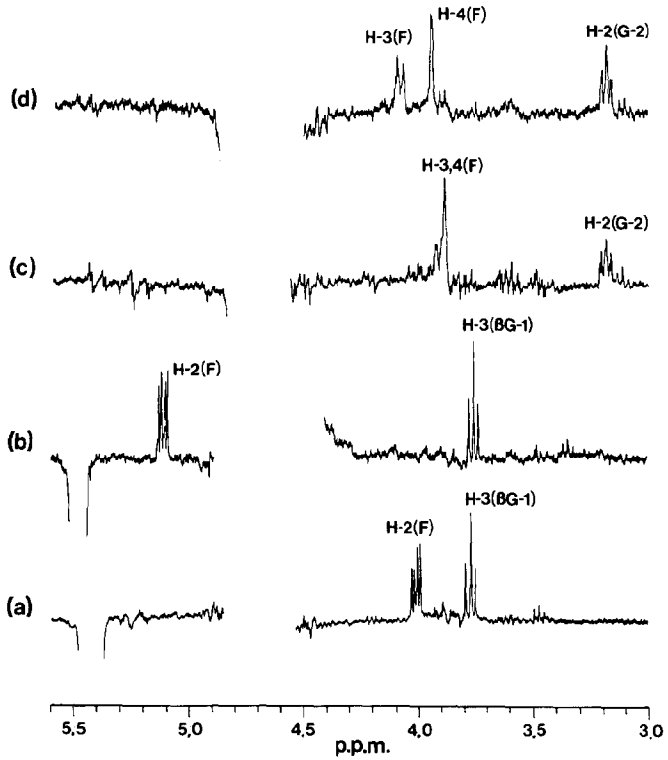


Fig. 4. ^1H-N.O.e. difference spectra of **1** and **2**. The strong peak (~4.7 p.p.m.) given by residual water protons has been omitted from the spectra: saturation of (*a*) H-1(F) resonance (5.43 p.p.m.) in **1**, (*b*) H-1(F) resonance (5.50 p.p.m.) in **2**, (*c*) H-5(F) resonance in **1**, and (*d*) H-5(F) resonance in **2**.

the chemical shift of the resonance of H-2(F) on acetylation is made obvious by comparison of Figs. 4a and 4b. Further n.O.e. experiments involving irradiation of the well-resolved resonances in the high-frequency part of the spectrum confirmed the proposed sequence of sugar units. N.O.e. connectivities were observed not only for protons within the same residue, *e.g.*, H-1(G-2) with H-3,5(G-2), but also across all the glycosidic linkages: H-1(G-2)–H-4($\alpha\beta$G-1), H-1(F)(5.43 p.p.m.)–H-3(βG-1), and H-1(F)(5.37 p.p.m.)–H-3(αG-1), H-1(U)–H-3(F). Observation of an n.O.e. between H-5(F) and H-2(G-2) on saturation of H-5(F) (Figs. 4c and 4d) is of particular interest for future modelling studies. The magnitude of this inter-residue n.O.e. is comparable to that for the intra-residue n.O.e. between H-4,5(F) and H-3,5(F), indicating that H-5(F) and H-2(G-2) must be within 2.5–3 Å of each other in the preferred conformations of **1** and **2**. Irradiation of the remaining well-resolved resonances in the high-frequency part of the spectrum failed to reveal any further significant inter-residue n.O.e.s.

^{13}C-N.m.r. spectra. — Although extensive compilations of ^{13}C chemical shifts

TABLE II

^{13}C-CHEMICAL SHIFTS[a] (P.P.M.) FOR TETRASACCHARIDES **1** AND **2**, AND SELECTED DATA FOR THE DEACETYLATED OCTASACCHARIDE **3**

Atom	*α-GlcA (U)*	*α-Fuc (F)*	*α-Glc ~OH (αG-1)*	*β-Glc ~OH (βG-1)*	*β-Glc (G2)*
1					
C-1	101.60	99.66, 99.77[b]	92.82	96.64	102.06, 102.03[b]
C-2	72.32	68.06, 68.09[b]	73.49	76.35	74.59
C-3	73.39	78.65, 78.71[b]	76.53	78.78	76.44
C-4	72.48	72.77	73.55	73.55	71.53
C-5	73.17	67.04, 67.07[b]	71.78	76.19	77.12
C-6	175.0	16.12	60.51	60.58	62.91
2					
C-1	101.72	97.29	92.94	96.93	102.19
C-2	72.05	70.55, 70.59[b]	73.67	76.47	74.57, 74.59[b]
C-3	73.34	75.31, 75.34[b]	76.42	78.63	76.36
C-4	72.70	72.79, 72.82[b]	73.54	73.54	71.44
C-5	n.a.[c]	67.30, 67.36[b]	71.82	76.21	77.26
C-6	175.6	16.01, 16.12[b]	60.42	60.50	63.04
Me (OAc)		21.15			
CO (OAc)		174.0			
3[d]					
C-1	101.34			102.78	
C-2	72.16			75.37	
C-3	72.11			78.33	
C-4	80.86			73.51	
C-5	n.a.			76.19	
C-6	175.2			60.51	

[a]±0.01 p.p.m. [b]Two signals observed. [c]Not assigned. [d]Values given for U and G-1 residues of **3**. Other values were similar to those for **1**.

of mono- and di-saccharides are available[16–18], the introduction of 2D-$^{13}C/^{1}H$ shift-correlation experiments[19] has provided a direct method by which the spectra of oligo- and poly-saccharides may be assigned. When the ^{1}H-n.m.r. spectrum has already been assigned, a 2D heteronuclear shift-correlation experiment can provide an assignment of the ^{13}C-n.m.r. spectrum.

The $^{13}C/^{1}H$ shift-correlation experiments on the oligosaccharides were carried out using the version of the technique[20] in which a BIRD (bilinear rotation decoupling[21]) pulse sequence is inserted at the centre of the evolution period. This method has the advantage that all couplings between protons on different carbon atoms are eliminated from the f_1 dimension, benefitting both sensitivity and resolution. The ^{13}C chemical shift assignments (Table II) for **1** and **2** were determined in this way, using the ^{1}H assignments of Table I. Literature values[16] for the relevant monosaccharides are for the most part in reasonable agreement (±0.5 p.p.m.) with the values for **1** and **2**, except for those carbon atoms which are involved in glycosidic linkages where the anticipated downfield shifts (2–9 p.p.m.) are observed. Acetylation at position 2 of the fucose residue produces a downfield shift of 2.5 p.p.m. in the resonance of C-2(F). Upfield shifts of about the same magnitude are observed for C-1,3(F), but the other resonances are affected little. The linewidth of the resonance of C-5(U) in **1** (at pD 3.0) was ~25 Hz at 30°, again reflecting the exchange process referred to above. The chemical shift of this resonance is sensitive to pH, as shown by studies of the monosaccharide[22,23], changing from 76.9 (pH 7.8) to 71.4 p.p.m. (pH 1.8) in α-D-glucopyranuronic acid. All other lines in the ^{13}C-n.m.r. spectrum had widths of <2 Hz. For **1**, on increasing the pD from 3.0

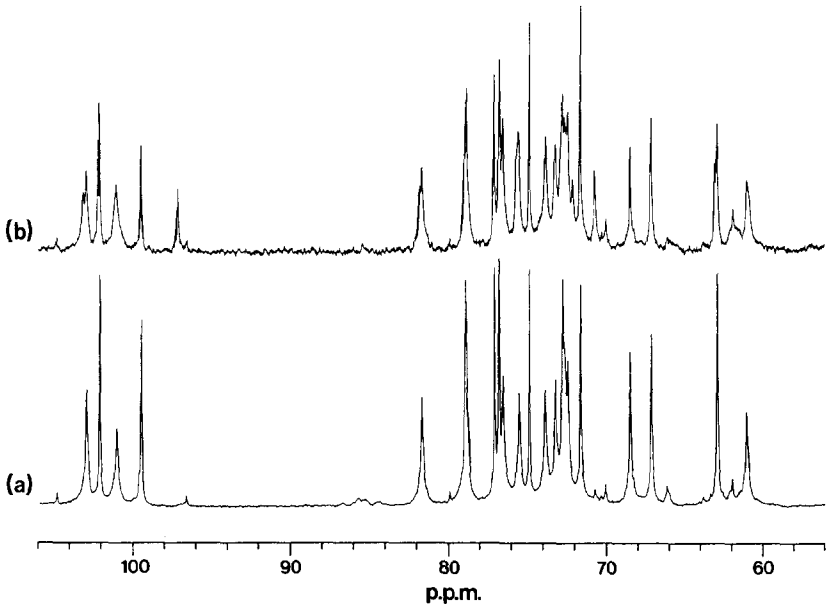


Fig. 5. 100-MHz ^{13}C-n.m.r. spectra (95°) of polysaccharides: (*a*) XM6, (*b*) K54 (acetyl resonances not shown).

to 8.0, the resonance for C-5(U) was shifted from 72.7 to 74.2 p.p.m. and sharpened. The resonances of C-4,6(U) were also shifted (72.5 to 73.0, and 175.0 to 177.1 p.p.m., respectively) at pD 8.0, but changes in the other ^{13}C shifts were small (<0.2 p.p.m.).

^{13}C-N.m.r. spectra of the acetylated and deacetylated polysaccharides, obtained at 95°, are shown in Fig. 5. As indicated above, complete assignments of the spectra for the tetrasaccharides assisted interpretation of the spectra of the polymers. However, the tetrasaccharides contain terminal α-glucuronic acid (non-reducing end) and glucose (reducing end) residues, whereas the polymer contains these residues as 4-linked α- and β-forms, respectively, in the backbone.

Parameters for uronic acid and glucose residues incorporated in the chain were obtained from COSY and $^{13}C/^{1}H$ correlation spectra of the deacetylated octasaccharide **3**. The large downfield shifts of the resonances for C-4(U) and C-1(G-1) associated with the linkages are indicated in Table II. Smaller, upfield shifts (~1 p.p.m.) were observed for the resonances of the neighbouring carbon atoms C-2(G-1) or C-3(U). With this information, it was possible to assign the spectra of the polysaccharides in Fig. 5. The chemical shift data for the deacetylated polysaccharide (XM6) are given in Table III together with values in parentheses for carbon atoms in the acetylated tetrasaccharide unit of polysaccharide K54, where they differ significantly from the values for XM6. The effects of acetylation (*i.e.*, large shift changes in the resonances for C-1,2,3(F) and minor changes for the other resonances) are analogous to the differences found between **1** and **2**. It was possible to obtain 2D spectra of the polysaccharides, but the loss of sensitivity and resolution, which is a consequence of increased line-widths, would have made it difficult to assign the spectra without associated studies of the oligosaccharides.

The ^{13}C-n.m.r. spectrum of the polysaccharide K54 provides an additional method of assessing the extent of acetylation in the native polysaccharide, although the same information would be available more rapidly from the ^{1}H-n.m.r. spectra.

TABLE III

^{13}C CHEMICAL SHIFTS[a] (P.P.M.) FOR THE DEACETYLATED (XM6) AND ACETYLATED (K54) POLYSACCHARIDES[b]

Atom	*α-GlcA (U)*	*α-Fuc (F)*	*β-Glc (G-1)*	*β-Glc (G-2)*
C-1	101.14	99.47 (97.17)	103.0 (103.18)	102.12 (102.24)
C-2	72.50	68.53 (70.72)	75.58	74.89
C-3	72.68	78.92 (75.69)	78.92	76.77
C-4	81.72 (81.82)	72.81	73.89	71.65
C-5	73.28	67.14	76.54	77.09
C-6	176.09	16.11	61.07	63.0 (63.12)
Me (OAc)		(21.19)		
CO (OAc)		(174.13)		

[a]±0.02 p.p.m. [b]The first value given is for XM6. A second value is given in parentheses for the acetylated tetrasaccharide unit of K54 where it differs significantly from that for XM6.

It is apparent from Fig. 5b that 40% of the tetrasaccharide repeat-units were acetylated, in contrast to the 50% reported in earlier work[3]. The lower figure may reflect some *O*-deacetylation under the mild alkaline conditions[3] and the sample was slightly heterogeneous with regard to acetate content.

The spectra in Fig. 5 show that the ^{13}C resonances may be classified into three groups on the basis of their line-width. The data in Fig. 5 and in Table III show that all the carbon atoms of a given sugar residue have the same line-width. The resonances of the side-chain glucose (G-2) had the narrowest lines (T_2 0.1 s, estimated from line-width = $1/\pi T_2$), intermediate values were found for the fucose residue (T_2 0.07 s), and the in-chain glucose (G-1) and glucuronic acid residues had the broadest lines (T_2 ~0.04 s). It was reported[3] that satisfactory ^{13}C-n.m.r. spectra of the native K54 polysaccharide could not be obtained, but we have found that corresponding lines in the spectra of the polysaccharides K54 and XM6 have similar line-widths at 95°.

It has been suggested[24] that ^{13}C T_1 values may be used to assess the mobility of carbon atoms in polysaccharides and that differences in T_1 might prove useful in making signal assignments by use of partly relaxed F.t. spectra. In the present instance, T_1 values (at 95°) of the methine carbons of the polysaccharide XM6 showed an overall variation of only 30% (average value, 0.36 s) and there was no correlation between the T_1 values and particular residues. The same inference was true of measurements of the ^{13}C–{^1H} n.O.e.s. (average value, 2.0). Therefore, on the limited basis of the present work, it appears that the line-widths of the ^{13}C resonances of heteropolysaccharides are more useful than longitudinal relaxation times for purposes of assignment. Relatively narrow lines can be observed not only for the carbon atoms in terminal residues of short side-chains but also for certain residues which form part of the backbone. Factors affecting the line-width would be expected to include the type of linkage and the degree of branching. Since T_2 values are expected to be particularly sensitive to slow motions, detailed study of ^{13}C relaxation parameters may provide information on ordering and stiffness of polysaccharide chains in solution.

The precise location of commonly occurring non-carbohydrate substituents in complex bacterial polysaccharide structures may be difficult to determine. The present work has shown that a combination of enzymic degradation to oligosaccharides, 2D-n.m.r., and n.O.e. methods can provide a solution to this problem.

EXPERIMENTAL

Bacteriophage-induced enzymic depolymerisation of the polysaccharides K54 and XM6 and the purification and isolation of tetra- and octa-saccharides was carried out as described[2]. The ^{13}C- and ^1H-n.m.r. spectra were recorded with a JEOL GX-400 spectrometer at operating frequencies of 100.4 and 399.65 MHz, respectively. The oligosaccharides were examined in the acid form as solutions in D_2O (20 mg/0.6 mL; pD 3.0) in 5-mm(o.d.) tubes at 30°. The polysaccharides were

examined in the sodium salt form as 2% (w/w) solution in D_2O (XM6, pD 8.0; K54, pD 6.0) in 10-mm(o.d.) tubes at 95°; pD measurements were made at room temperature and are pH meter readings, uncorrected for the isotope effect. Acetone was the internal reference and the chemical shifts of the acetone methyl group were taken to be 2.217 (^{1}H) and 31.07 p.p.m. (^{13}C) with respect to the signals for Me_4Si. COSY spectra were acquired, using a 45° observation pulse, into a 2048 (t_2) × 512 (t_1) data matrix. The spectral width was 1200 Hz in both dimensions, giving digital resolution of 1.17 and 2.34 Hz in f_2 and f_1, respectively. Gated irradiation was used to suppress the signal from residual protons of the solvent in both 1D and 2D ^{1}H-n.m.r. spectra.

2D-^{13}C/^{1}H Shift-correlation spectra were acquired into a 2048 (t_2) × 512 (t_1) data matrix for the oligosaccharides **1–3**. The spectral widths were 5000 Hz in the f_2 (^{13}C) dimension and 1200 Hz in the f_1 (^{1}H) dimension, giving digital resolution of 4.88 and 2.34 Hz, respectively. The pulse sequence used included[20], a BIRD pulse sandwich[21] at the centre of the evolution period. ^{13}C/^{1}H Shift-correlation spectra (2048 × 256 data matrix, same spectral widths as above) were obtained for the polysaccharides using the conventional pulse sequence[25], with BIRD sequence omitted. All 2D spectra were processed using standard JEOL software and were examined in the absolute value mode.

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