ELSEVIER

Carbohydrate Research 269 (1995) 319–331

CARBOHYDRATE RESEARCH

NMR Studies of acetan and the related bacterial polysaccharide, CR1/4, produced by a mutant strain of *Acetobacter xylinum*

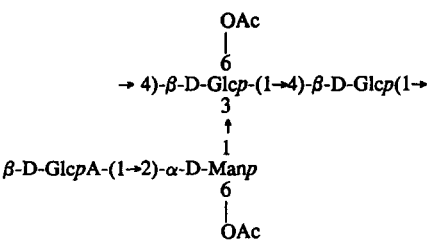
Ian J. Colquhoun *, Marianne Defernez, Victor J. Morris

Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UA United Kingdom

Received 18 August 1994; accepted 11 November 1994

Abstract

Acetan is a bacterial polysaccharide produced by *Acetobacter xylinum* NRRL B42. Chemical mutagenesis of *A.xylinum* allowed selection of a mutant strain which produced a new polysaccharide, CR1/4. 2D NMR methods have been used to assign the ^{1}H and ^{13}C spectra of the two polysaccharides and to determine that CR1/4 has the structure shown below. The total number of *O*-acetyl groups is slightly less than two per repeating unit,



The pentasaccharide side chain of acetan is truncated to a disaccharide unit in CR1/4, but the structures are otherwise identical. In particular, the degree of acetylation is about the same and the *O*-acetyl groups are located at the same positions in both polysaccharides.

Keywords: Bacterial polysaccharides; Acetan; *Acetobacter xylinum*; NMR Spectroscopy

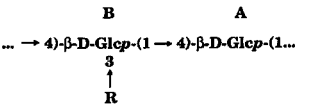
* Corresponding author.

SSDI 0008-6215(94)00367-X

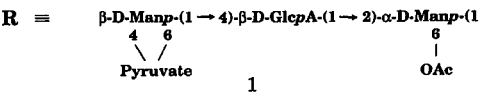
1. Introduction

Xanthan gum, the microbial polysaccharide produced by *Xanthomonas campestris*, has found widespread applications as a food ingredient thanks to its properties as a thickening and suspending agent. Native xanthan has the structure [1], **1**. The polysaccharide acetan [2], produced by *Acetobacter xylinum* NRRL B42, shows interesting structural and functional similarities to xanthan [3]. Following earlier biosynthetic [4] and structural studies [2,5], the structure of acetan was recently confirmed [6] as **2**. There are also one to two *O*-acetyl substituents per repeating unit in positions which have not previously been determined. Acetan resembles xanthan in having a cellulosic backbone substituted on alternate glucose residues with a side-chain. Although the lengths of the side-chains are different, the residues adjacent to the backbone (Man and GlcA) are the same in the two polysaccharides and they have the same types of linkage.

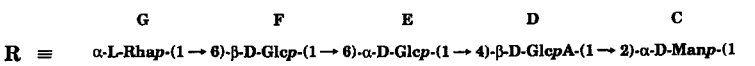
Backbone



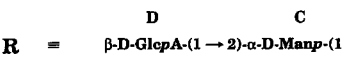
Side-chains



1



2



3

Acetan can be regarded as the most complex of a family of polysaccharides in which the side-chain may be modified by removal of one residue at a time. Studies of the biosynthesis of acetan [4] and successful experiments with xanthan [7] suggest that it may be possible to produce the complete family of modified polysaccharides by genetic engineering. Chemical mutagenesis of *A.xylinum* has allowed selection of mutants which produce one of these polysaccharides [8], CR1/4. Preliminary structural analysis showed that the polysaccharide contained t-GlcA, 1,2-Man, 1,3,4-Glc, and 1,4-Glc units. By analogy with acetan, the structure **3** was proposed for CR1/4. It is presumed that the biosynthetic pathway, which proceeds by step-wise addition of sugar units to form a lipid linked oligosaccharide intermediate [4], has been blocked after addition of the GlcA residue. A polysaccharide with the same basic structure, called xanthan polytetramer, was previously isolated from mutant strains of *X.campestris* [7]. Full structural charac-

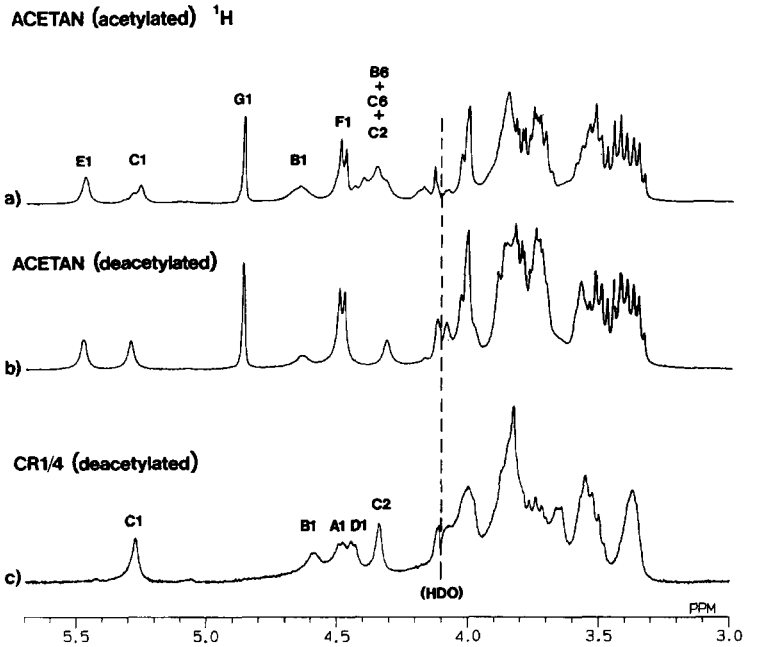


Fig. 1. 400-MHz ^{1}H NMR spectra (D_2O, 95°C) of (a) native (acetylated) acetan (the Me resonances of Rha and the *O*-Ac group are not shown); (b) deacetylated acetan; (c) deacetylated CR1/4.

terisation of CR1/4 by NMR, including details of acetylation, is reported here, and the spectra are compared with those given by acetan. In a recent report [6], the ^{1}H NMR spectrum of a heptasaccharide produced by enzymic degradation of deacetylated acetan was assigned. In the present work we have assigned the ^{13}C and ^{1}H NMR spectra of the intact native and deacetylated polysaccharides.

2. Results and discussion

Individual sugar residues of the polysaccharides are coded as shown in structures **2** and **3**. Both CR1/4 and acetan were *O*-deacetylated by controlled alkaline hydrolysis (16 h at 1°C, pH 12.5) of the native samples. ^{1}H NMR spectra of deacetylated CR1/4, and native and deacetylated acetan are shown in Fig. 1. The ^{1}H NMR spectrum of native CR1/4 was not of good quality and is not shown here. Depending on the sample, native acetan was found to have a total of between 1.5 and 2 *O*-acetyl groups per repeating unit, as determined by integration of the methyl signals at δ 2.16 relative to the signal from the **G6** protons at δ 1.3 (d, 3 H). Two signals, of equal intensity, were resolved at δ 2.16, indicating at least two locations for the *O*-acetyl groups. Aqueous solutions of acetan and CR1/4 are highly viscous at room temperature, and even at 95°C the ^{1}H line-widths are rather broad. The sugar residues in acetan have different mobilities, depending on their position in the repeating unit, and this gives a range of line-widths.

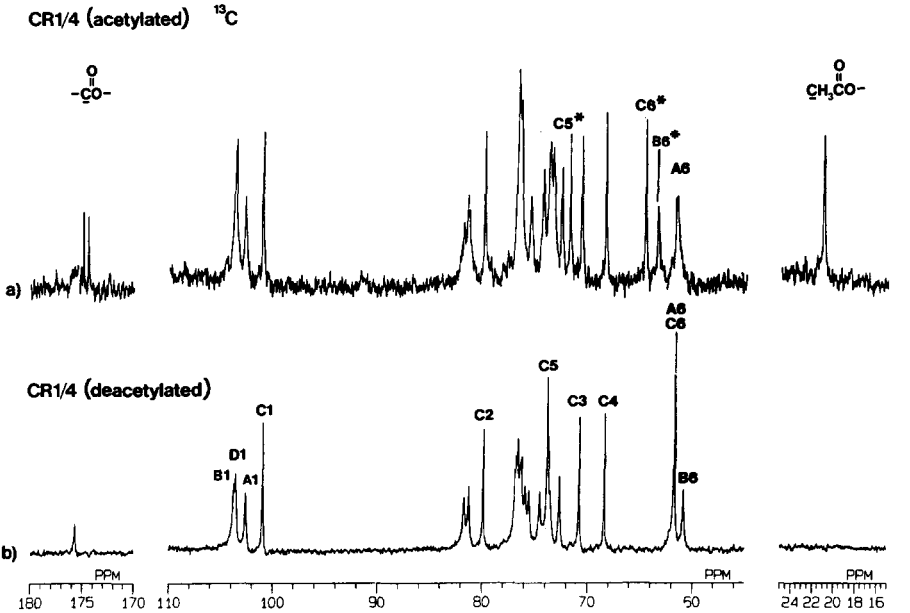


Fig. 2. 100-MHz ^{13}C NMR spectra (D_2O, 95°C) of (a) native (acetylated) CR1/4; (b) deacetylated CR1/4. Resonances which show appreciable chemical shift changes on deacetylation are marked *.

Narrow lines arise from protons belonging to the most mobile units (**G** and **F**) at the terminus of the acetan side-chain. Sharp features are absent from the spectrum of CR1/4, but the broad resonances associated with sugar units **A–D** are more clearly revealed than in the spectra of acetan.

Comparison of the ^{13}C spectra of native and deacetylated CR1/4 (Fig. 2) shows obvious differences in that the signals from the acetyl groups at δ 21.1 (methyl) and δ 174.4, 174.9 (carbonyl) have disappeared in Fig. 2b. There are also clear differences in the region (60–65 ppm) associated with C-6 resonances of the sugar rings. Signals at 64.6 and 63.4 ppm (marked * in Fig. 2a) disappear on deacetylation. There is an increase in intensity of the signal at 61.7 ppm and a new signal appears at 60.7 ppm (Fig. 2b). This implies that the *O*-acetyl groups are located at the carbon-6 position in two of the sugar units. The anomeric region of the deacetylated sample contains the expected four signals and the line-width variation apparent in the ^{1}H spectra is again evident. The ^{13}C spectra of acetan (Fig. 3) in the carbon-6 region show that the degree of acetylation of this polysaccharide is similar to that of CR1/4. From inspection of structure **2** it is clear that the *O*-acetyl groups must be located on rings **A**, **B**, or **C** since these are the only residues with free CH_2OH groups.

Spectral assignment and structure of CR1 / 4.—Detailed assignment of the ^{1}H and ^{13}C spectra of deacetylated CR1/4 relied on a combination of 2D NMR techniques (COSY, HOHAHA, NOESY and C/H correlation). For native CR1/4 the ^{1}H chemical shifts could only be obtained indirectly from the C/H correlation spectrum. In the low-field region of the ^{1}H spectrum of deacetylated CR1/4, the COSY spectrum

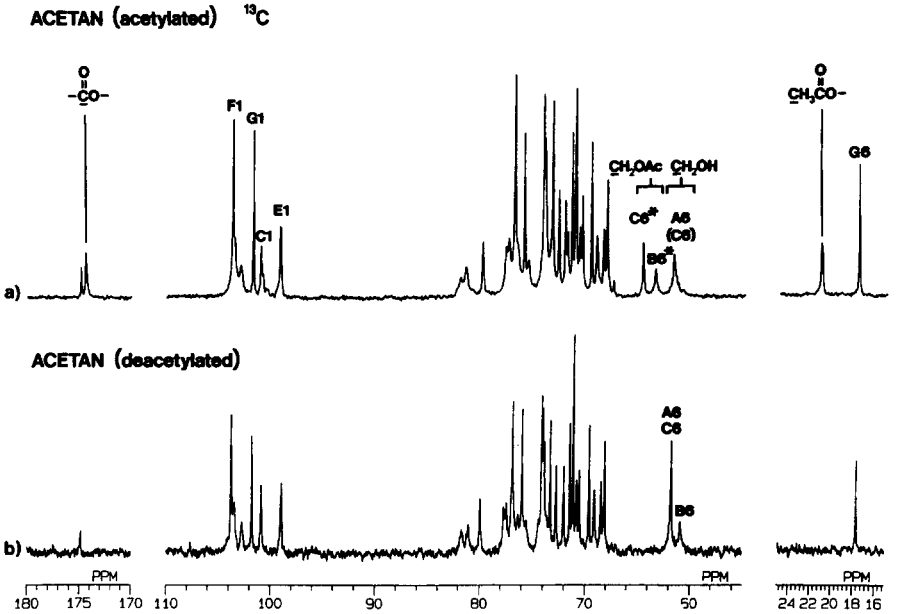


Fig. 3. 100-MHz ^{13}C NMR spectra (D_2O, 95°C) of (a) native (acetylated) acetan; (b) deacetylated acetan.

showed a cross-peak between two resonances at δ 5.28 and 4.35. These signals can therefore be assigned to protons **C**1 and **C**2, respectively, of the α-D-Man*p* residue [6]. The **C**2 resonance shows a marked down-field displacement when compared with the equivalent signal (at δ 4.20) in the linear acetan heptasaccharide [6]. Depending on the conformation the branching at residue **B** in the polysaccharide could introduce a short range interaction between the **C**2 proton and an oxygen atom on residue **A**. This would result in a down-field shift of the proton signal. No such interaction is possible in the heptasaccharide which was obtained by hydrolysis of the (1 → 4) linkage between **A** and **B**. The three remaining resonances between δ 4.45–4.60 are doublets with $J_{1,2} \sim 7$Hz and all can be assigned as anomeric protons of β-linked residues. Chemical shifts of protons up to H-5 in residue **C** and up to H-3 in the other residues could be determined from the COSY spectrum.

Further assignments depended on comparison of HOHAHA (Fig. 4) and NOESY (Fig. 5) spectra. Cross-peaks common to both spectra arise from pairs of protons belonging to the same sugar unit. The protons are part of the same coupling network (HOHAHA) and they may be spatially close (NOESY). Under the conditions employed to obtain the HOHAHA spectrum, correlations were observed between H-1 and all protons up to H-5 in the Glc and GlcA residues. However, in the NOESY spectra, intra-ring NOEs could only be observed between H-1 and the H-2, H-3, and H-5 protons. This distinction simplified the assignments of H-4 and H-5 for these residues. Cross-peaks appearing in the NOESY but not in the HOHAHA spectrum correlate signals from protons in different rings. The protons concerned are separated by short distances, and the interactions are principally transmitted across glycosidic linkages. In

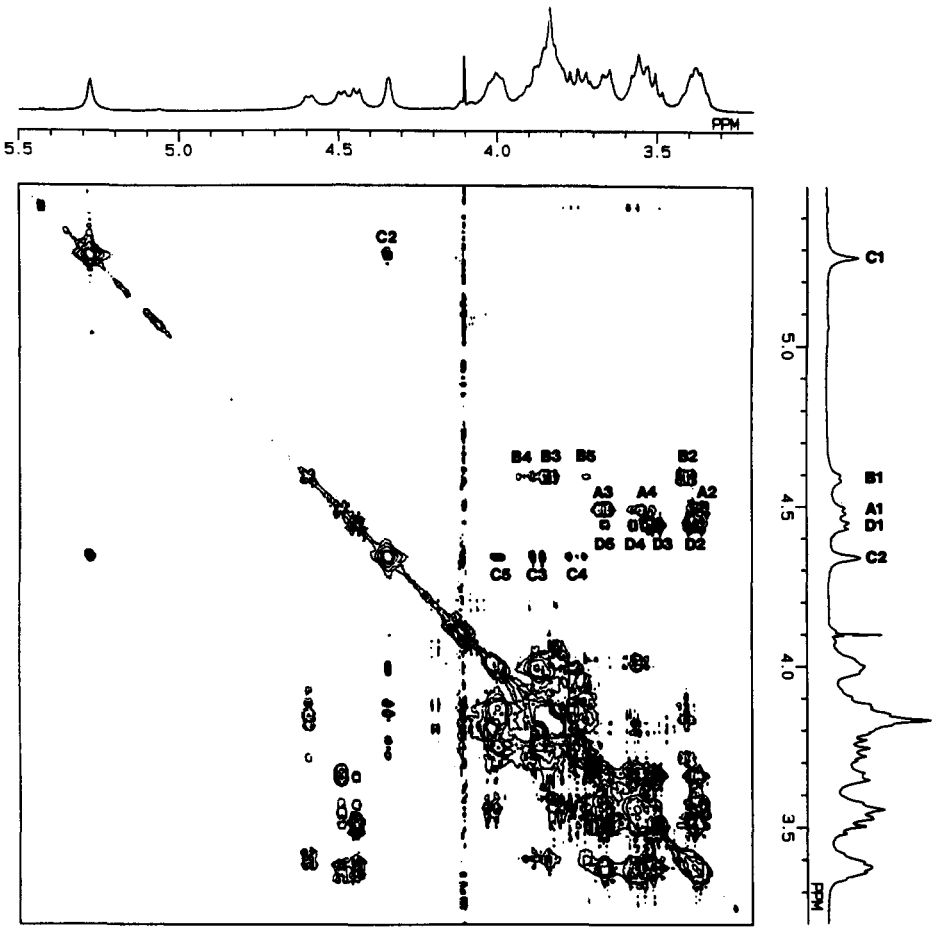


Fig. 4. 400-MHz HOHAHA spectrum of deacetylated CR1/4. Assignments of anomeric protons and C2 are indicated on the right hand side. Labels within the frame refer to chemical shifts on the horizontal axis.

polymers, however, cross-peaks can also arise as a result of relayed NOEs (spin diffusion), so the mixing time was kept relatively short (100 ms) to simplify the spectra as much as possible.

In the NOESY spectrum the **C1** proton gave a strong cross-peak, *a*, with one of the other anomeric protons at δ 4.45. In turn this anomeric proton had a cross-peak, *e*, with **C2** but no additional inter-ring interactions were evident. This identifies the anomeric proton as **D1** and the structural fragment as **4**.

β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→

4

An interaction between the anomeric protons of the two residues concerned is typical for a (1 → 2) linkage [9]. Sets of weak cross-peaks, *f* and *g*, were also observed

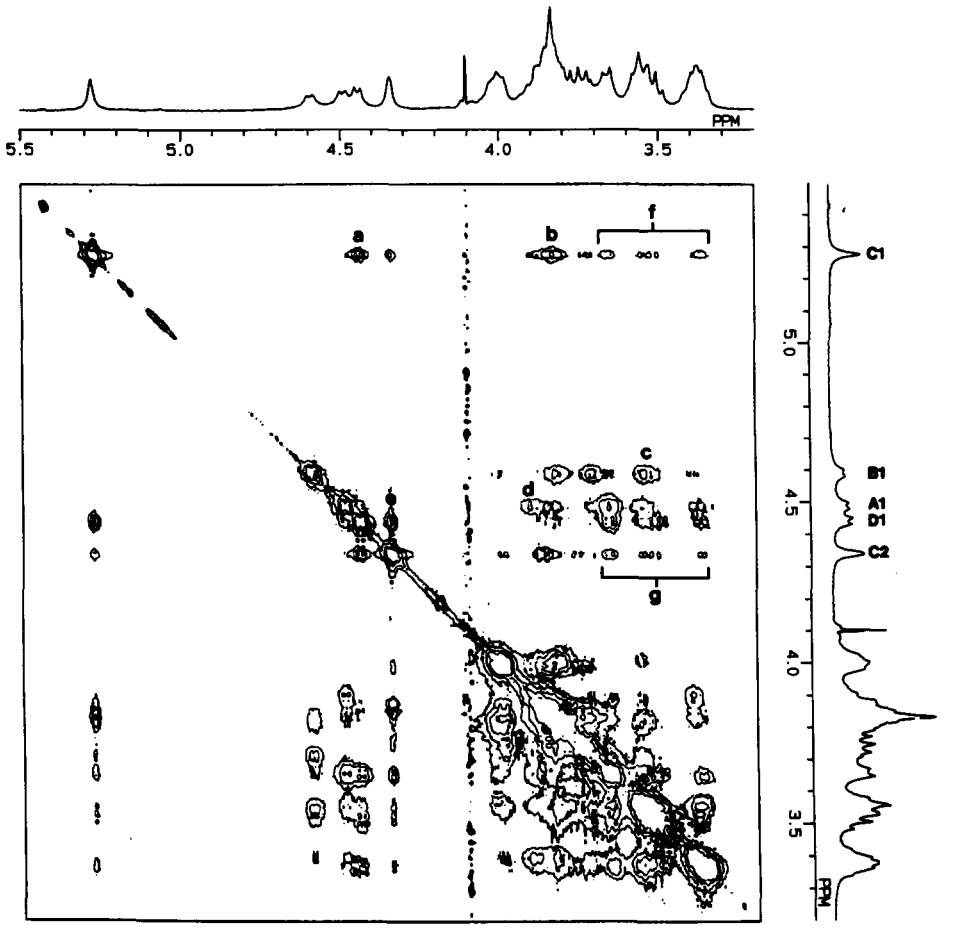


Fig. 5. 400-MHz NOESY spectrum (100 ms mixing time) of deacetylated CR1/4. Labelled cross-peaks arise from interunit NOEs. Assignments are discussed in the text.

between **C**1, **C**2 and **D**2, **D**3, **D**5. These NOEs were undoubtedly relayed from residue **C** via **D**1. The second strong cross-peak, involving proton **C**1, *b*, was to a signal at δ 3.84. This can be assigned to **B**3 from the HOHAHA spectrum, and thus the anomeric proton at δ 4.60 is assigned to **B**1. This leaves the last anomeric signal at δ 4.50 as **A**1 and the two remaining strong inter-ring cross peaks, *c* and *d* can be identified as **B**1-**A**4 and **A**1-**B**4, respectively, confirming that **3** is the correct structure of CR1/4. The ^{1}H chemical shifts are listed in Table 1. Further support for this structure comes from assignment of the ^{13}C spectrum using 2D C/H correlation NMR.

The ^{1}H signals of the anomeric protons are well resolved so that an unambiguous identification of the corresponding ^{13}C resonances is possible (Table 2). The narrowest of the four resonances is found to be **C**1 and further assignment of the D-Man*p* unit shows that all carbons in this residue give narrow lines (Fig. 2b) with linewidths

Table 1
^{1}H chemical shifts for CR1/4 and acetan [a]

Compound	Residue	Chemical shift (δ)						
		H-1	H-2	H-3	H-4	H-5	H-6	H-6′
CR1/4	(1,4)-β-D-Glc*p* **A**	4.50	3.37	3.67	3.53	3.58	3.83	4.01
	(1,3,4)-β-D-Glc*p* **B**	4.60	3.41	3.84	3.91	3.72 3.92 *	3.83 4.33 * [b]	4.01
	(1,2)-α-D-Man*p* **C**	5.28	4.35	3.88	3.76	4.00	3.83 4.36 *	
	t-β-D-Glc*p*A **D**	4.45	3.39	3.51	3.57	3.68		
Acetan	(1,2)-α-D-Man*p* **C**	5.27	4.31	3.84	3.74	3.98 4.16 *	3.82 4.33 *	4.42 *
	(1,6)-α-D-Glc*p* **E**	5.46	3.53	3.73	3.51	3.88	3.88	4.10
	(1,6)-β-D-Glc*p* **F**	4.49	3.35	3.53	3.41	3.57	3.73	4.02
	t-α-L-Rha*p* **G**	4.86	4.00	3.80	3.45	3.74	1.30	

[a] Chemical shifts for the deacetylated polysaccharides are given in the top row for each residue. Chemical shifts which change appreciably in the native samples are given underneath, marked *.
[b] Value taken from the acetan NOESY spectrum.

Table 2
^{13}C chemical shifts for CR1/4 and acetan [a]

Compound	Residue	Chemical Shift (δ)					
		C-1	C-2	C-3	C-4	C-5	C-6
CR1/4	(1,4)-β-D-Glc*p* **A**	102.32	74.34 [b]	75.31	81.03	76.06	61.57
	(1,3,4)-β-D-Glc*p* **B**	103.48	73.31 [b]	81.46	76.20	75.69 73.0 *	60.68 63.4 *
	(1,2)-α-D-Man*p* **C**	100.70	79.65	70.59	68.18	73.66 71.73 *	61.57 64.56 *
	t-β-D-Glc*p*A **D**	103.29	73.58 [b]	76.41	72.47	76.63	176
Acetan	(1,2)-α-D-Man*p* **C**	100.74	79.83	70.71	68.39	73.81 71.8 *	61.72 64.6 *
	(1,6)-α-D-Glc*p* **E**	98.85	72.65	73.92	70.56	71.92	69.03
	(1,6)-β-D-Glc*p* **F**	103.66	74.05	76.75	71.03	75.86	68.06
	t-α-L-Rha*p* **G**	101.66	71.06	71.36	73.22	69.52	17.8

[a] Chemical shifts for the deacetylated polysaccharides are given in the top row for each residue. Chemical shifts which change appreciably in the native samples are given underneath, marked *.
[b] Assignments may be interchanged.

comparable to **C**1. Up-field of the anomeric region are two signals identified by C/H correlation as the glycosidic linkage positions, **B**3 (δ 81.46) and **A**4 (δ 81.03). The signal from the remaining linkage position, **B**4, is found at δ 76.2, in the centre of a cluster of peaks which is otherwise made up of signals from the 3- and 5-carbons of rings **A**, **B**, and **D**. Di-substitution of ring **B** at neighbouring positions affects the ^{13}C and ^{1}H chemical shifts of positions **B**3 and **B**4. In both cases the ^{13}C shifts are lower and the ^{1}H shifts higher than would be expected for singly substituted 3- or 4-linked β-Glc rings, on the basis of reported data for model disaccharides [10,11]. The 2-carbons of rings **A**, **B**, and **D** (δ 73.3–74.3) are difficult to assign because of the similarity of their ^{1}H chemical shifts and the limited resolution in the ^{1}H dimension. The last signal in this region is from one of the carbons (**D**4, δ 72.47) in the t-GlcA residue, and because it is well-resolved (Fig. 2b), its line-width can be compared with the line-widths of the Man*p* signals. Surprisingly, the terminal residue has broader lines (is less mobile) than the Man*p* residue which is linked to the backbone. Possibly an interaction between the GlcA residue and the backbone, or an intra- or inter-molecular interaction between side-chains imposes a constraint on the mobility of this unit.

Assignment of acetan spectra; position of acetyl groups in acetan and CR1 / 4.—The spectra of deacetylated acetan were assigned first. Partial occupancy of the acetylation sites slightly complicates spectra of the native samples. For example the low-field shoulder of the **C**1 resonance in the native sample (Fig. 1a) is at the same chemical shift as **C**1 in the deacetylated acetan. Identification of proton resonances in the anomeric region of the spectrum was straightforward from comparisons with CR1/4 and with previous studies of the repeating unit [6]. **F**1 was the only anomeric signal for which the coupling $J_{1,2}$ (7.8 Hz) gave a resolved doublet, and this strong signal obscured the **A**1 and **D**1 resonances. Complete sets of ^{1}H chemical shifts could be obtained for rings **F** and **G** from a COSY experiment, and for ring **E**, which had somewhat broader lines, from RELAY and C/H correlation spectra. The latter also provided ^{13}C and ^{1}H assignments for **C**, which were very similar to those in CR1/4. ^{1}H and ^{13}C chemical shifts are listed in Tables 1 and 2 for residues **C**, **E**, **F** and **G**. The configuration of the terminal Rha*p* residue was confirmed as α by measurement of $^{1}J_{C,H}$ for **G**1 (171 Hz). This is in agreement with a recent report where the same conclusion was reached from consideration of ^{1}H chemical shifts [6]. A full assignment was not possible for rings **A**, **B** and **D** which have broader lines and weaker signals than the other residues. However, the ^{1}H chemical shifts of the linkage positions **B**1, **B**3, and **A**4 could be obtained and all were within 0.03 ppm of corresponding values in CR1/4. This suggests that there is little change in average backbone conformation on addition of the extra side-chain units. Cross-peaks in the C/H correlation spectrum at δ 77.6/3.82 and 77.4/3.82 were assigned to **D**4 and **D**3 respectively. ^{13}C and ^{1}H shifts of **D**4 are both displaced down-field in comparison with CR1/4 where **D** (β-GlcA) is a terminal residue. The NOESY spectrum of deacetylated acetan showed that the ^{1}H chemical shift of **D**1 (from the **D**1/**C**2 cross-peak) was identical to that of **F**1. The ^{1}H chemical shifts of **D**1 and **C**2 were displaced downfield (0.04 ppm) and upfield (also 0.04 ppm), respectively, from the corresponding shifts in CR1/4. This may indicate that the extension of the side-chain beyond ring **D** leads to a conformational change in the linkage between **D** and **C**.

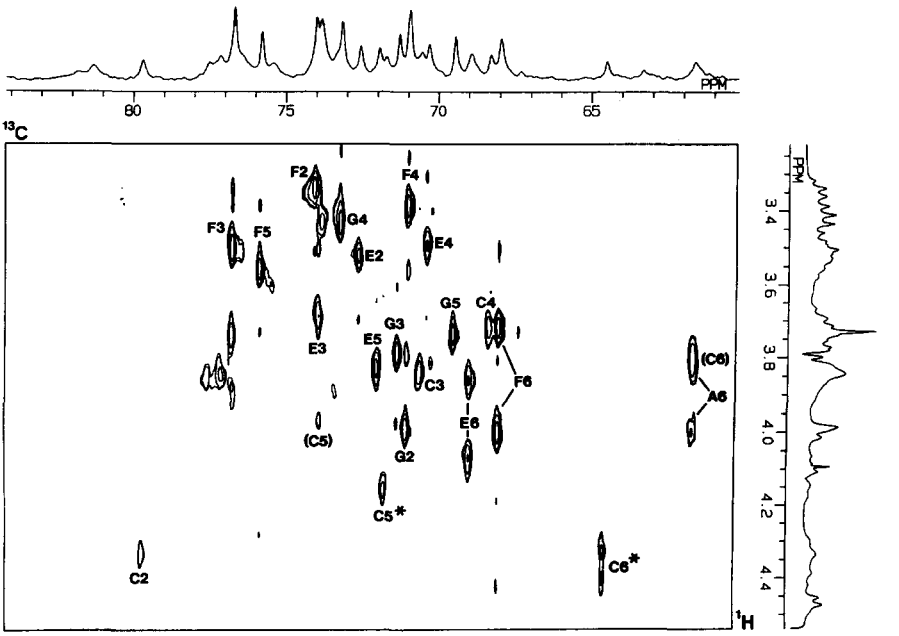


Fig. 6. $^{13}C/^{1}H$ correlation spectrum of native acetan. Cross-peak labels of C5 and C6 in the acetylated Man unit are marked *; for the deacetylated unit, they are shown in parenthesis.

The C/H correlation spectrum of native acetan is shown in Fig. 6, together with assignments for rings **C**, **E**, **F**, and **G**. For these rings, the only differences between the correlation spectra of native (acetylated) and deacetylated samples were the positions of the **C5** and **C6** cross-peaks. Because of the incomplete acetylation, cross-peaks are evident in Fig. 6 for both substituted and non-substituted α-Man*p* residues. The large down-field displacement of the ^{1}H and ^{13}C resonances of **C6** with acetylation shows that one of the *O*-acetyl groups is located at position 6 of the α-Man*p* residue. Identical observations were made for native and deacetylated CR1/4 (Tables 1 and 2).

Fig. 1 shows the additional intensity appearing between δ 4.3 and 4.4 in the ^{1}H spectrum of the native sample. This intensity is partly attributable to the down-field displacement of the **C6** protons on acetylation. Unfortunately the signal to noise ratio in the C/H spectrum was not good enough to observe a C/H correlation peak for the ^{13}C resonance at δ 63.4 (associated with the second presumed acetylation site). However, the NOESY spectrum of native acetan, recorded with a long (400 ms) mixing time, allows identification of further signals at δ 4.3–4.4. Cross-peaks *b* (Fig. 7) relate **C5** (δ 4.16) to **C6** (δ 4.33, 4.42) and occur in the positions expected from the C/H spectrum. The cross-peak *c* is assigned to **D1/C2** and corresponds to the same interaction in CR1/4 (Fig. 5, cross-peak *e*). The presence of another signal at $\delta \sim 4.33$ is shown by cross-peak *a* which links a resonance at this position to the anomeric proton **B1** at δ 4.62. Cross-peak *a* is assigned to **B1/B6**, and is probably a relayed cross-peak, with transfer via **B5** occurring as a result of the extended mixing time. **B5** itself has a

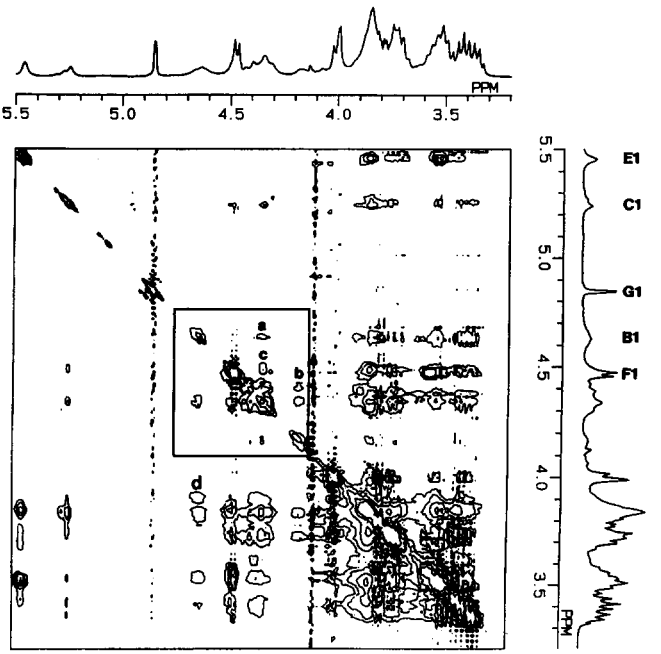


Fig. 7. 400-MHz NOESY spectrum (400 ms mixing time) of native acetan. Cross-peaks within the inner frame show origin of signals at ~ 4.33 ppm. Assignment of NOEs is discussed in the text.

chemical shift of 3.90 ppm (cross-peak *d*) and is shifted down-field from 3.69 ppm in the deacetylated sample. NOEs from **B1** to other protons (**B2**, **B3**, **A4**) show that the chemical shifts of these other protons are almost unchanged on deacetylation. Heteronuclear correlation spectra of both acetan and CR1/4 contained a cross-peak (**B5**) at δ 73.0/3.90 for the native, but not the deacetylated samples. Taken together, these observations suggest that in both acetan and CR1/4 the second *O*-acetyl group is attached at carbon-6 of **B**, the backbone β-Glc*p* residue to which the side-chain is linked. Acetylation results in downfield displacements of the ^{1}H signals of **B5**, **B6** and the ^{13}C signal of **B6**, but an up-field displacement of the ^{13}C resonance of **B5**. The behaviour of the **C5** and **C6** signals on acetylation of Man-6 is exactly the same.

Acetan and CR1/4 resemble xanthan and xanthan polytetramer in that all the polysaccharides are acetylated at Man-6. Xanthan and the polytetramer are not acetylated on the backbone but, apart from this, the structures of CR1/4 and the polytetramer are the same. The data presented for CR1/4 should help to provide a complete assignment of the NMR spectra of xanthan for which only partial assignments have been reported previously [12–14]. The presence of a backbone acetyl group in acetan and CR1/4 may affect the solution and gelling properties of the polysaccharides as well as their biochemical characteristics, such as susceptibility to enzymic cleavage. This remains to be tested.

The biosynthesis of bacterial polysaccharides is generally well understood but there is some doubt concerning the stage at which non-carbohydrate substituents are added.

Schemes for xanthan biosynthesis [15] show transfer of the acetyl group from Acetyl CoA occurring before polymerisation, but after assembly of the repeating unit has been completed. Recent studies of succinoglycan biosynthesis [16] have shown, however, that the acetylation step may take place during assembly of the repeating unit, between addition of successive sugar residues. Our finding that the two acetyl group locations are the same in acetan and CR1/4, and that the degree of acetylation is very similar in the two polysaccharides, support the latter proposal. The acetylases which catalyse transfer of the acetyl groups would then only have to recognise the same substrates (at di- and tri-saccharide intermediate stages) for both wild-type and mutant polysaccharides. It might be argued that the polymerase must recognise full length and truncated repeating units for the polymerisation process, but it should be noted that yields of truncated polysaccharides are decreased in comparison with wild-type acetan [8] and xanthan [15].

The NMR spectra of acetan were dominated by a set of signals from the four most mobile sugar units, and their presence made it difficult to observe and assign the broader, weaker signals from the three remaining units. The chemical shifts in these less mobile units were determined more readily for the simpler polysaccharide, CR1/4. The results for CR1/4 demonstrate that appropriate 2D NMR techniques can be used to analyse the spectra of intact polysaccharides even when the spin–spin splittings are rather poorly resolved.

3. Experimental

The preparation and purification of acetan and CR1/4 have been described previously [8]. The samples were deacetylated by treatment with alkali. A 0.25% w/v solution of the polysaccharide in distilled water was prepared by heating to 90°C with stirring (under N_2) for 0.5h. The solution was cooled to 1°C, titrated to pH 12.5 by addition of 0.1 M NaOH, and left at 1°C for 16 h. The pH was then adjusted to 8.5 by addition of 0.1 M HCl, the solution was dialysed against distilled water, and the polysaccharide was freeze-dried.

NMR experiments.—Acetan and CR1/4 were examined as 2 and 1% solutions, respectively, in D_2O. The polysaccharides were in the sodium form and the pH * was 5.0 ($\pm$0.5) for all solutions. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Jeol GX-400 spectrometer at 95°C. Samples for ^1H NMR, COSY, NOESY, etc., were contained in 5-mm o.d. tubes and for ^{13}C NMR and C/H correlation spectra in 10-mm o.d. tubes. HOHAHA and NOESY spectra of CR1/4 (deacetylated) were also recorded at 600 MHz on a Varian VXR600 spectrometer. Chemical shifts are reported relative to Me_4Si using dioxan as a secondary reference. δ values of dioxan were taken as 3.74 (^1H) and 67.4 (^{13}C). Phase sensitive [17] DQF-COSY [18], HOHAHA [19], and NOESY [20] spectra were acquired into a 2048 (t_2) $\times$ 256 (t_1) $\times$ 2 data matrix, with spectral widths of 1000 Hz in both dimensions. A mixing time of 60 ms (MLEV-17 sequence) was used in the HOHAHA experiment and mixing times of 100 or 400 ms in the NOESY experiments. The phase sensitive C/H correlation spectra [21] were acquired into a 4096 (t_2) $\times$ 32 (t_1) $\times$ 2 data matrix with spectral widths of 5555 (^{13}C) x 1000 Hz (^1H). The f_1 dimension was zero-filled to 128 points on Fourier

transformation giving final digital resolution of 2.7 and 7.8 Hz in f_2 and f_1, respectively. Between 1000 and 2000 scans were acquired for each t_1 increment.

Acknowledgements

M.D. was supported by a grant from the E.C. ERASMUS student exchange programme. We thank Dr. J.A. Parkinson (Edinburgh University Ultra High Field NMR Centre) for the 600 MHz spectra.

References

[1] P.-E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.
[2] R.O. Couso, L. Ielpi, and M.A. Dankert, *J. Gen. Microbiol.*, 133 (1987) 2123–2135.
[3] V.J. Morris, G.J. Brownsey, P. Cairns, G.R. Chilvers, and M.J. Miles, *Int. J. Biol. Macromol.*, 11 (1989) 326–328.
[4] R.O. Couso, L. Ielpi, R.C. Garcia, and M.A. Dankert, *Eur. J. Biochem.*, 123 (1982) 617–627.
[5] K. Tayama, H. Minakami, E. Entani, S. Fujiyama, and H. Masai, *Agric. Biol. Chem.*, 49 (1985) 959–966.
[6] P.-E. Jansson, J. Lindberg, K.M. Swarna Wimalasiri, and M.A. Dankert, *Carbohydr. Res.*, 245 (1993) 303–310.
[7] M.R. Betlach, M.A. Capage, D.H. Doherty, R.A. Hassler, N.M. Henderson, R.W. Vanderslice, J.D. Marelli, and M.B. Ward, in M. Yalpani (Ed.), *Industrial Polysaccharides: Genetic Engineering, Structure / Property Relations and Applications*, Elsevier, Amsterdam, 1987, pp 35–50.
[8] C.A. MacCormick, J.E. Harris, A.P. Gunning, and V.J. Morris, *J. Appl. Bacteriol.*, 74 (1993) 196–199.
[9] I.J. Colquhoun, G.A. de Ruiter, H.A. Schols, and A.G.J. Voragen, *Carbohydr. Res.*, 206 (1990) 131–144.
[10] I. Backman, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, (1988) 889–898.
[11] P.-E. Jansson, L. Kenne, K. Persson, and G. Widmalm, *J. Chem. Soc., Perkin Trans.*, 1 (1990) 591–598.
[12] M. Rinaudo, M. Milas, F. Lambert, and M. Vincendon, *Macromolecules*, 16 (1983) 816–819.
[13] D. Horton, O. Mols, Z. Walaszek, and W.C. Wernau, *Carbohydr. Res.*, 141 (1985) 340–346.
[14] N.W.H. Cheetham and E.N.M. Mashimba, *Carbohydr. Polym.*, 17 (1992) 127–136.
[15] R.W. Vanderslice, D.H. Doherty, M.A. Capage, M.R. Betlach, R.A. Hassler, N.M. Henderson, J. Ryan-Graniero, and M. Tecklenburg, in V. Crescenzi, I.C.M. Dea, S. Paoletti, S.S. Stivala, and I.W. Sutherland (Eds.), *Biomedical and Biotechnological Advances in Industrial Polysaccharides*, Gordon and Breach, New York, 1988, pp 145–156.
[16] T.L. Reuber and G.C. Walker, *Cell*, 74 (1993) 269–280.
[17] D.J. States, R.A. Haberkorn, and D.J. Ruben, *J. Magn. Reson.*, 48 (1982) 286–292.
[18] M. Rance, O.W. Sørensen, G. Bodenhausen, G. Wagner, R.R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.* 117 (1983) 479–485.
[19] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[20] Anil Kumar, G. Wagner, R.R. Ernst, and K. Wüthrich, *J. Am. Chem. Soc.*, 103 (1981) 3654–3658.
[21] M. Ohuchi, M. Hosono, K. Furihata, and H. Seto, *J. Magn. Reson.*, 72 (1987) 279–297.