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Note

Identification by ^{13}C-n.m.r. spectroscopy of oligosaccharides derived from alginic acid*

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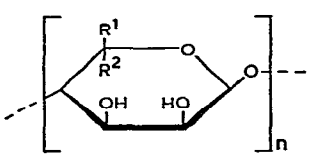
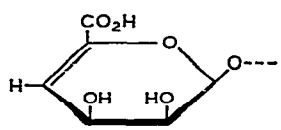
It has been observed that alginic acid samples and blocks derived from alginic acid show well defined ^{13}C-n.m.r. spectra[1]. The three types of blocks, β-D-mannuronan [poly(ManA), **1**], α-L-guluronan [(poly)GulA), **2**], and blocks in which these two uronic acids occur in some sort of alternation [poly(ManA-GulA)][2,3], may be readily distinguished, particularly by the position of the resonances due to the anomeric carbon atoms. We wished to see if these observations could be extended to help characterise oligosaccharides derived from alginic acid either by acid hydrolysis or by enzymic degradation with an α-L-guluronanate lyase[4]. This enzyme appears to be specific for the α-L-gulosyluronic acid linkages in the poly(GulA) blocks and has no action on poly(ManA) blocks. However, like other alginate lyases reported in the literature[5], it also attacks poly(ManA-GulA) blocks, and it became of importance to identify the oligosaccharides produced by this action. The conventional technique of Taylor and Conrad[6] for identifying oligouronic acids by lactonisation, reduction with borohydride, and acid hydrolysis worked well for us but it is costly, time-consuming and destructive of samples. N.m.r. spectroscopy seemed a possible non-destructive technique and we now report on this aspect.

The ^{13}C-n.m.r. absorptions of carbon atoms in sugars have been extensively studied and used in structural assignments of mono- and oligosaccharides[7–9]. The



1 $R^1 = CO_2H$, $R^2 = H$
2 $R^1 = H$, $R^2 = CO_2H$



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*Dedicated to Professor Dexter French on the occasion of his 60th birthday.

techniques has been extended, particularly for the anomeric carbon atom, to the differentiation of the same monosaccharide at the reducing end and as an internal residue in an oligosaccharide[10,11]; more recently it has been used to examine the products of enzymic hydrolysis of agarose[12].

The proton-decoupled ^{13}C-n.m.r. spectra of sodium D-mannuronate (ManA) and sodium L-guluronate (GulA) in D_2O show well defined resonances assigned as in Table I. The disaccharides, 4-*O*-(β-D-mannosyluronic acid)-D-mannuronic acid (ManA-ManA) and 4-*O*-(α-L-gulosyluronic acid)-L-guluronic acid (GulA–GulA) show resonances for the anomeric carbons as given in Table II. When glycosidically linked, the C-1 resonance of a ManA residue occurs at δ_c 101.5, whereas the reducing end-group resonates at δ_c 94.8; the corresponding figures for the GulA residues are 102.1 (glycosidic) and 94.4 (reducing end), respectively. The pattern is repeated in the homologous trisaccharides, with the resonances for the glycosidically linked carbon atoms more intense than those for the reducing end-groups. In the polymeric species, poly(ManA) and poly(GulA), the resonances due to the reducing end-groups are either very weak or absent, but internally linked, anomeric carbon atoms resonate at δ_c 101.3 for poly(ManA) and δ_c 102.0 for poly(GulA). Of equal interest in distinguishing GulA residues from ManA residues is the observation that resonances at δ_c 65.8, 69.0, and 81.2 are associated with other carbon atoms in GulA residues, the resonances for C-2 to C-5 in ManA residues all appearing in the range δ_c 69.9 to 78.8. The carboxyl carbon resonances, however, are not diagnostic, as they always appear in the range δ_c 176.5 to 177.5 and are rarely separated by our spectrometer.

The spectra of oligosaccharides derived by action of alginate lyase on alginate fractions are complicated by the presence, at their non-reducing ends, of the unsaturated acid, 4-deoxy-L-*erythro*-hex-5-ulosuronic acid, glycosidically linked as in 3. This unsaturated group ("Δ") may arise from either a ManA or a GulA residue. The enzymically derived disaccharide, Δ-GulA[4], shows a resonance at δ_c 94.4, characteristic of a reducing-end GulA residue, and another resonance at δ_c 108.8, attributable to the unsaturated group. The homologous trisaccharide, Δ-GulA-GulA,

TABLE I

^{13}C CHEMICAL SHIFTS[a]

Compound	*δ-Value*[b]		
	C-1	*C-2 to C-5*	*C-6*
Sodium D-mannuronate	95.1(α), 94.8(β)	69.8(β), 70.2(α), 71.3, 71.6(α), 72.3(β), 74.7(α), 77.5(β)	178.1(α) 177.0(β)
Sodium L-guluronate	94.8	68.0, 70.3, 71.3, 72.0	176.6

[a] δ_c Values relative to external tetramethylsilane. [b] Anomeric assignments are based solely on the relative sizes of the resonance signals, the smaller being attributed to the β anomer.

TABLE II

^{13}C CHEMICAL SHIFTS OF ANOMERIC CARBON ATOMS IN OLIGOSACCHARIDES FROM ALGINIC ACID[a]

Oligosaccharide	*δ-Value*				
	Reducing end-group		*Internal residue*		*Unsaturated group*
	ManA	*GulA*	*ManA*	*GulA*	*Δ*
ManA-ManA	94.8		101.5		
ManA-ManA-ManA	94.8		101.3		
Poly(ManA)	94.9[b]		101.3		
GulA-GulA		94.4		102.1	
GulA-GulA-GulA		94.3		102.0	
Poly(GulA)		—		102.0	
Poly(ManA-GulA)	95.1[b]		101.1	102.2	
Δ-GulA		94.4			108.8
Δ-GulA-GulA		94.3		102.4	108.8
Δ-ManA-GulA		94.3	101.2		108.8

[a] δ_c relative to external tetramethylsilane. [b] Weak signals.

shows resonances as expected at δ_c 108.8, 102.4, and 94.3 from the unsaturated group, the internal GulA-residue, and the reducing-end GulA group, respectively.

An example of the use of this technique came during an examination of the products derived by the action of α-L-guluronanate lyase on poly(ManA-GulA) blocks. One oligosaccharide fraction was isolated that could not readily be identified. The fraction, which contained the unsaturated acid 3, appeared homogeneous on t.l.c. and from its order of elution on gel chromatography, and its R_F value on t.l.c. appeared to be that of a trisaccharide. Its ^{13}C-n.m.r. spectrum showed major resonances at δ_c 108.8, 101.2, and 94.3, together with a minor resonance at δ_c 102.4. From this, it was concluded that the fraction was a mixture of two trisaccharides, the major component being *Δ*-ManA-GulA, and the minor one *Δ*-GulA-GulA. In each trisaccharide, the reducing end-group is L-guluronic acid and the non-reducing end-group the unsaturated acid 3, as expected from the known specificity and action of this enzyme[4]. That two components were indeed present in the fraction was subsequently verified by a paper-chromatographic run for 162 h. The fraction was resolved into two components, one of which migrated 7.5 cm down the paper (identical with *Δ*-GulA-GulA, run as a control) and the other, presumably *Δ*-ManA-GulA, had migrated 6.1 cm. Visual inspection of the chromatogram suggested that the latter trisaccharide was present in four-fold excess over the former, in agreement with the qualitative results obtained by n.m.r. spectroscopy.

EXPERIMENTAL

Alginate blocks were prepared as described previously[4]. Oligosaccharides were obtained by partial hydrolysis of the blocks, and separation on columns of Biogel P-4

essentially as described by Larsen *et al.*[13]. T.l.c. was performed on Silica Gel G, developed with butan-1-ol–acetic acid–water (2:1:1); plates were sprayed with *p*-anisidine hydrochloride. Paper chromatography on Whatman no. 1 paper was carried out with the solvent system pyridine–ethyl acetate–acetic acid–water (5:5:1:3) and the foregoing spray-reagent.

For n.m.r. spectroscopy, oligosaccharides (40–100 mg) were dissolved in D_2O (2.5 ml) in 10-mm tubes. Spectra were recorded at 15 MHz on a JEOL FX-60 spectrometer with external tetramethylsilane as standard and the following instrumental parameters: spectral width, 4 kHz; pulse width, 60°; data points, 4096; repetition time, 1 sec; no. of transients 20000–120000; and temperature, ambient.

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