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Preliminary communication

Determination of the primary structure of polysaccharides and their derivatives by solid-state carbon-13 n.m.r. spectroscopy

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High-resolution ^{13}C-n.m.r. spectroscopy has become firmly established[1] as a powerful tool for the structural elucidation of natural polysaccharides and, to a lesser extent, their derivatives. Native and modified polysaccharides are not always amenable to analysis by this technique, however, because of such intrinsic properties as insolubility in common solvents (as with cellulose and chitin), and gel formation, or extremely high solution-viscosities (as with xanthan gum). It has been obvious for some time that ^{13}C-n.m.r. of solid materials, measured with magic-angle spinning (m.a.s.) and cross polarization (c.p.)[2], should provide a welcome supplement to the conventional solution-state experiment, and two recent studies characterizing the morphology of native cellulose[3,4] have provided support for this supposition. In the present work, we draw attention to the use of ^{13}C m.a.s.–c.p. n.m.r. for determination of the primary structure of polysaccharides and their chemical derivatives.

The utility of the m.a.s.–c.p. ^{13}C-n.m.r. method is nicely illustrated by the spectra in Fig. 1 for xanthan gum **1** (unpurified Keltrol) and its octadecylamide **2** (d.s. ~1.0, prepared by treatment of **1** with octadecylamine in an adaption of a procedure reported for alginate[5]). The spectrum of **1** (Fig. 1-A) shows the carbonyl signals at 173 p.p.m., the partially resolved anomeric signals at 102 p.p.m., the overlapping signals from C-2 to C-6 of the various monosaccharide residues at 73 p.p.m., and the pyruvate methyl resonance at 21 p.p.m. The spectrum of the amide **2** (Fig. 1-B) reveals an additional resonance at 33 p.p.m., which may be assigned to the methylene carbon atoms of the hydrophobic side-chain. Although the resolution of the individual carbon resonances is far from perfect, it is clear that this technique provides considerably more detail than the corresponding ^{13}C solution experiments (Fig. 1-C) in an equivalent period (3 h) of experimental time for similar quantities of material.

The m.a.s.–c.p. method is of particular importance for insoluble substances, as exemplified here by the ^{13}C-n.m.r. spectrum (Fig. 2) of [*N*-(3-fluorophenyl)methyl]-chitosan (**3**, d.s. 0.9, obtained by reductive alkylation of chitosan with *m*-fluorobenz-

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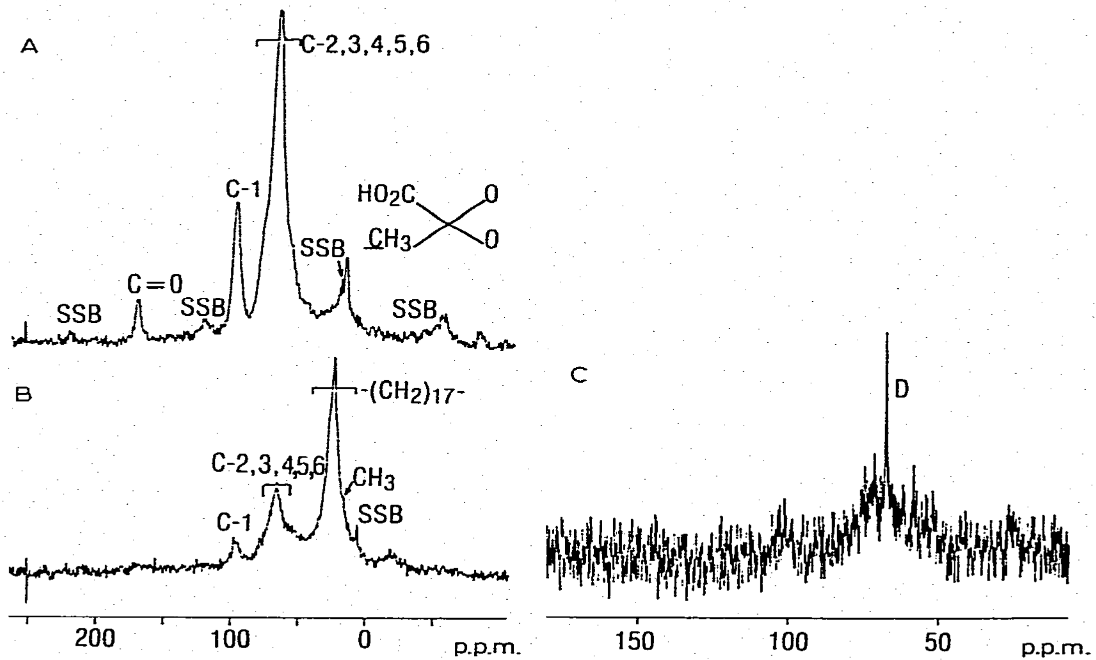


Fig. 1. Magic-angle spinning, cross-polarization ^{13}C-n.m.r. spectra of (A) xanthan gum (unpurified Keltrol) 1, 10,000 scans, 2-s repetition, 0.5-ms contact time, 2-kHz spinning, 10G ^{1}H decoupling; (B) xanthan octadecylamide (2), 100-mg sample, same conditions as A. Spectra were obtained with a Bruker CXP-200 spectrometer using a Kel-F spinner. Assignments are based on solution spectra. (C) 100.6-MHz ^{13}C-n.m.r. spectrum of 1 (purified[8]) in D_2O (~80 mg/2 mL) at 310 K; sweep-width 23,000 Hz, 25,000 scans, pulse-width 14 μs, delay 0.1 s, and internal 1,4-dioxane (D; 67.40 p.p.m.) used as reference. Spectrum was obtained with a Bruker WH-400 spectrometer. Spinning side-bands are indicated as SSB.

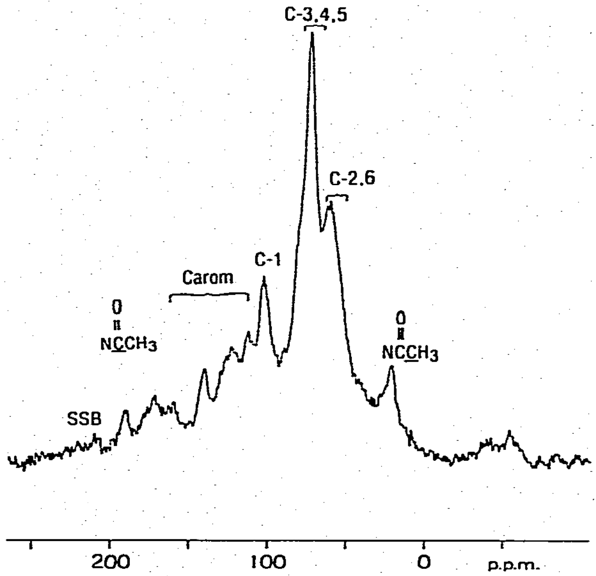
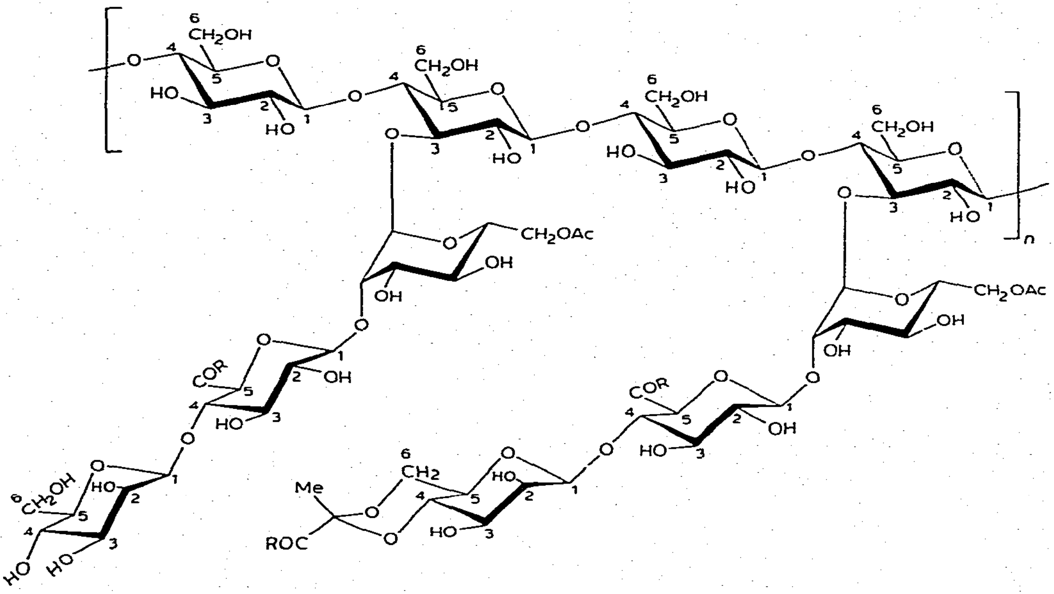
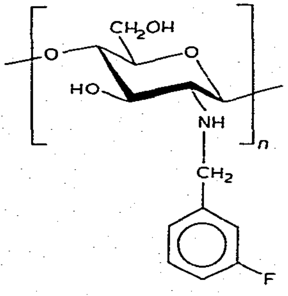


Fig. 2. Magic-angle spinning, cross-polarization ^{13}C-n.m.r. spectrum of [*N*-(3-fluorophenyl)methyl-chitosan (3), 20,000 scans, same conditions as in Fig. 1 A,B.



1 R = OH

2 R = $NH(CH_2)_{17}CH_3$



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aldehyde and sodium cyanoborohydride[6]). The carbonyl signal* of 3 is observed at 173 p.p.m., the aromatic signals appear between 110 and 167 p.p.m., the anomeric signals at 104 p.p.m., the unresolved resonances of C-3, C-4, C-5 at 73 p.p.m., C-6 and C-2 at 62 p.p.m., and the *N*-acetyl* methyl resonance at 23 p.p.m.

There are numerous other circumstances where solid-state ^{13}C-n.m.r. studies have unique potential for the carbohydrate chemist, particularly for the analysis of *selectively* modified polysaccharides[7] (partly exemplified here by the novel derivatives 2 and 3), which are attracting growing interest in a variety of areas.

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*Of the residual (~5–10%) NAc groups in the chitosan sample.

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