Carbohydrate Research, 57 (1977) 39–49

^{13}C-N.M.R. SPECTROSCOPY OF CELLULOSE ETHERS*

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(Received December 6th, 1976; accepted for publication, December 30th, 1976)

ABSTRACT

Characteristics of the ^{13}C-n.m.r. spectra of cellulose ethers (methyl, carboxymethyl, and hydroxyethyl) have been examined at 22.6 MHz. Partial depolymerization with acid or cellulase proved to be a requisite preliminary step. Strong deshielding of ^{13}C nuclei bearing alkoxyl groups was clearly evident in these spectra, which permitted an assessment of the degree of substitution at individual positions of the D-glucose residues. Better resolved spectra, and more-detailed structural analyses, were afforded by complete hydrolysates of the polymers. The findings are wholly consistent with data obtained for these derivatives by other methods, showing that the reactivities of the hydroxyl groups of cellulose are OH-2 > OH-6 ≫ OH-3. It is also shown that reducing-end residues liberated during enzymic hydrolysis of the cellulose derivatives are not substituted at the 2-position.

INTRODUCTION

Fourier-transform ^{13}C-n.m.r. spectroscopy is very useful for the characterization of carbohydrate polymers in solution, and has been fruitfully applied[1–4] to a variety of polysaccharides in recent years. Since many derivatives of cellulose are appreciably soluble, they should be amenable to study by this technique. The current article examines possibilities for the analysis of commercially important cellulose ethers (methyl, carboxymethyl, and hydroxyethyl).

RESULTS AND DISCUSSION

The introduction of an *O*-alkyl group promotes strong deshielding of the ^{13}C nucleus of the substituted carbinol group, usually by ~9 p.p.m.[5–6]. In the spectra of cellulose ethers, this characteristic should be reflected in the chemical shifts of carbons bearing alkoxyl substituents. Therefore, signals that exhibit such large, downfield shifts relative to those of cellulose itself, and their intensities, should define the location and content of the substituent groups in the polymer. Although this is a

*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.

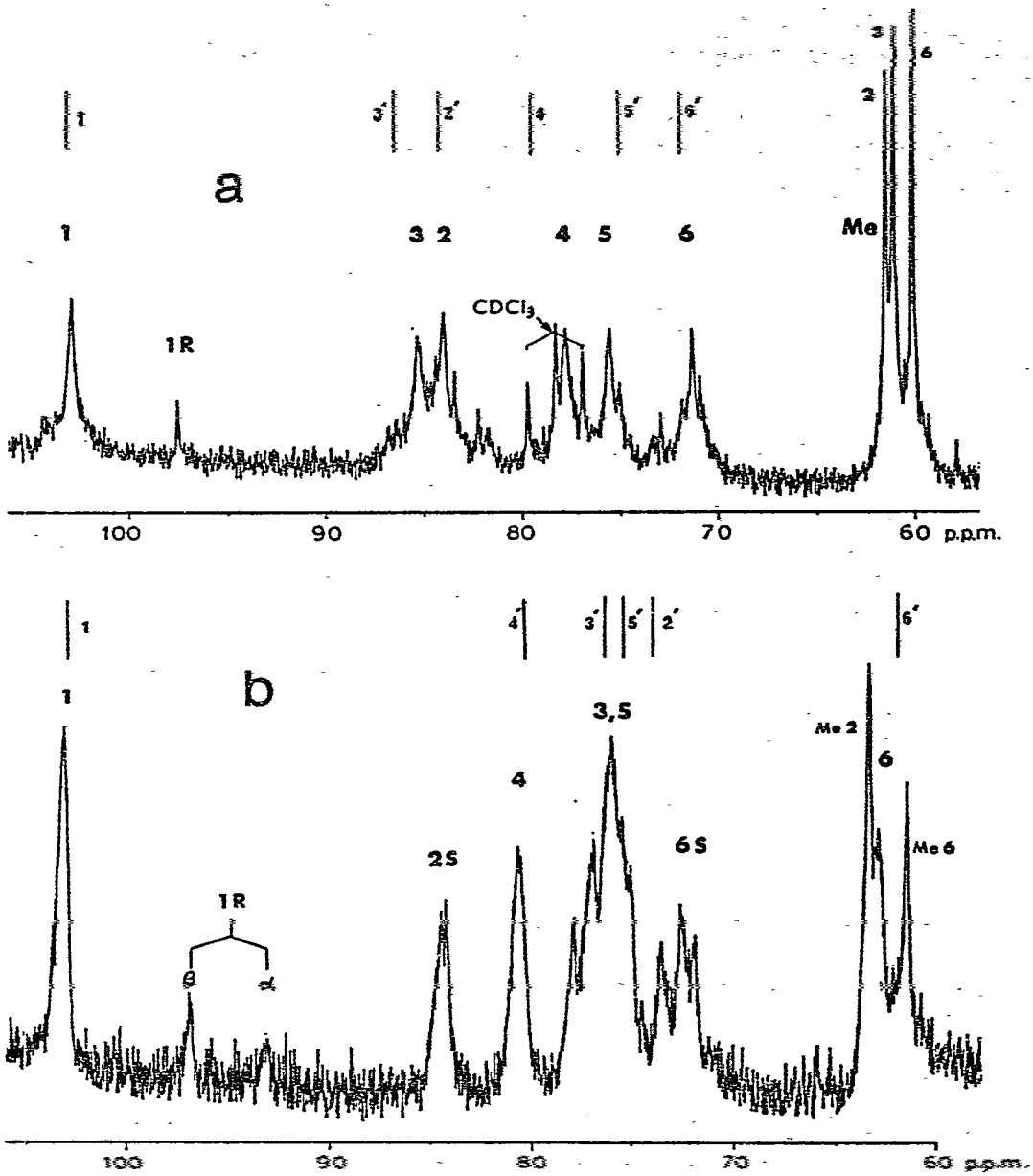
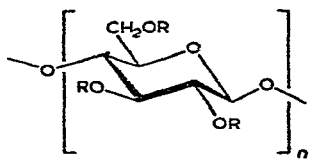


Fig. 1. FT ^{13}C-n.m.r. spectrum at 22.63 MHz, ^{1}H-decoupled of (*a*) *O*-methylcellulose (d.s. ~2.8, partially depolymerized) in $CDCl_3$ at 30°; R, signal due to reducing-end residue; Me, *O*-methyl. Inset lines give the chemical shifts of the corresponding carbons in methyl hepta-*O*-methyl-β-cellobioside[7]. (*b*) *O*-Methylcellulose (d.s. ~0.7, partially degraded by cellulase) in D_2O at 30°; R, signal due to reducing-end residue; S, C is bonded to methoxyl group; Me, *O*-methyl. Inset lines give the chemical shifts of the corresponding carbons in methyl β-cellobioside[13].

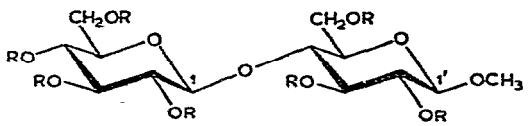
straightforward proposition, in principle, it entails some experimental limitations. Thus, cellulose derivatives of high molecular weight give dilute, highly viscous solutions which necessitate very long experimental runs and tend to produce broad signals. In the current study, these problems were materially alleviated by the use of saturated solutions of cellulose ethers that had been partially depolymerized either chemically or enzymically.

O-Methylcellulose. — A sample of *O*-methylcellulose (**1**) of d.s. ~2.8, given a prior treatment with hydrogen chloride in dichloroethylene, afforded the proton-decoupled ^{13}C-spectrum shown in Fig. 1a, with the use of deuteriochloroform as solvent. The six signals corresponding to the carbons of the glucose residues are clearly resolved and of comparable intensity, as are also the 2-, 3-, and 6-*O*-methyl resonances. A number of minor peaks present are consistent with the fact that the material was not fully methylated. Also, the weak signal at 97.5 p.p.m., which corresponds to that of C-1 of 2-*O*-methyl-β-D-glucose[4], shows that moderate depolymerization of the original material had taken place; since the intensity of this signal representing reducing-end units is ~5% of that of C-1 of glycosidically bound residues, the average d.p. is estimated to be ~20. Chemical shifts for the signals in Fig. 1a are close to those reported[7] for the 4-linked residue of methyl hepta-*O*-methyl-β-cellobioside (**2**) (inset lines of Fig. 1a), which are the basis of the assignments given; the largest difference is that C-4 of the polymer resonates ~2 p.p.m. upfield of C-4 in **2***. Similarly, the chemical shift of C-1 is close to that of C-1 of the non-reducing



1 R = CH_3 5 R = CH_2CO_2H or/and H

3 R = CH_3 or/and H 6 R = $(CH_2CH_2O)_x$-$CH_2C'H_2OH$ or/and H



2 R = CH_3

4 R = H

*The macromolecular structure *per se* appears to make little contribution to the chemical-shift values. A similarly close affinity in chemical shifts has been noted recently in comparing[8] glycosaminoglycans and model compounds of low molecular weight.

residue of the permethylated disaccharide[7], whereas the *O*-methyl resonances are close to those for 2-, 3- and 6-mono-*O*-methyl-D-glucose[4].

For the examination of a water-soluble, commercial *O*-methylcellulose (3) of low methoxyl content (d.s. $\simeq 0.7$), a prior treatment with a cellulase preparation was carried out. Since this type of enzyme preferentially degrades regions of cellulose ethers that bear fewest substituents[9–12], the undegraded residue examined spectroscopically was more fully methylated than the original sample. As seen in Fig. 1b, the spectrum of this enzyme-resistant material shows that the position of highest substitution is at O-2. That is, the signals at 84 and 62 p.p.m. correspond closely in chemical shift to those of C-2 and O–CH_3, respectively, of 2-*O*-methyl-*β*-D-glucopyranose[4]. This information is reinforced by the fact that there is no strong signal near 74 p.p.m., the resonance position of C-2 in methyl *β*-D-glucopyranoside[6] or C-2′ in methyl *β*-cellobioside[13] (*4*). A lower content of 6-*O*-methyl groups in the sample is demonstrated by the relatively weaker resonances at 72 and 60 p.p.m., which are the chemical shifts of C-6 and OCH_3, respectively, of 6-*O*-methyl-*β*-D-glucose[4]. No direct evidence is given in Fig. 1b that this *O*-methylcellulose contains 3-*O*-methyl groups. However, a commercial preparation of *O*-methylcellulose having a d.s. of 1.5–2, examined after partial hydrolysis with acid, produced a relatively weak signal at 87 p.p.m., which is consistent with a low proportion of substitution at C-3 (*i.e.*, C-3 of 3-*O*-methyl-*β*-D-glucose resonates at[4] 86.7 p.p.m.).

Hence, these findings are in qualitative accord with long-established information[14] about the substitution pattern of *O*-methylcelluloses, *i.e.*, the ease of methylation is O-2 > O-6 ⩾ O-3.

Other signals in Fig. 1b deserve comment. Those of the C-1*β* and C-1*α* reducing-ends show that the enzyme-resistant material consists of relatively short chains; by reference to the intensity of the C-1 signal, the average d.p. is estimated to be ~ 15. The cluster of strong signals around 75–77 p.p.m. is attributed mainly to C-5 and C-3, by analogy with the chemical shifts of C-5′ and C-3′ of methyl *β*-cellobioside[13] (**4**). These nuclei are adjacent to both methylated and non-methylated 2- and 6-positions, and a range of chemical shifts is therefore to be expected, because neighbouring ether groups cause appreciable upfield displacements[4–6]*. Other, more minor signals probably represent the end-groups; *e.g.*, the signal at 71 p.p.m. corresponds to that of the C-4 of the non-reducing residue of **4** (71.2 p.p.m.).

The spectrum shown in Fig. 1b was obtained at 30°. Although the spectra of polymers may be improved by raising the sample temperature, this possibility was not examined with solutions of *O*-methylcellulose, because of their tendency to gel on heating. As noted below for *O*-(carboxymethyl)cellulose, however, increasing the temperature to 60° offered little advantage.

O-(Carboxymethyl)cellulose. — Possible shielding effects of an *O*-carboxymethyl group on the ^{13}C nuclei of cellulose were examined by reference to 2-, 3-, and

*For example, C-3 of 2-*O*-methyl-*β*-D-glucose resonates 0.5 p.p.m. upfield of C-3 of *β*-D-glucose, and for C-5 of the 6-*O*-methyl *β*-anomer the upfield displacement[4] is 1.5 p.p.m.

6-*O*-carboxymethyl derivatives of D-glucose (Table I). As can be seen, this type of ether substituent exerts about the same strong deshielding of the α-carbon[15] and slight shielding of adjacent (β) carbons as does an *O*-methyl group. Consequently, there are close similarities between the spectra of *O*-(carboxymethyl)celluloses and *O*-methylcelluloses. A pretreatment with cellulase was important in this series also, in order to obtain solutions of an adequately high concentration.

TABLE I

^{13}C-CHEMICAL SHIFTS OF MONO-*O*-CARBOXYMETHYL- AND -*O*-(2-HYDROXYETHYL)-D-GLUCOSES

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_2	CH_2'
α-D-Glucose[a]	93.3	73.1	74.4	71.2	72.9	62.4		
β-D-Glucose[a]	97.1	75.6	77.3	71.2	77.3	62.4		
2-*O*-Carboxymethyl-α-D-glucose	91.1	81.3	73.0	70.8	72.4	61.3[b]	70.8[c]	
2-*O*-Carboxymethyl-β-D-glucose	96.7	84.5	76.1	70.8	77.0	61.9[b]		
3-*O*-Carboxymethyl-α-D-glucose	92.9	72.3	83.9	70.2[b]	72.0	61.5[b]	70.1[c]	
3-*O*-Carboxymethyl-β-D-glucose	96.7	74.7	86.4	70.4[b]	76.6	61.6[b]		
6-*O*-Carboxymethyl-α-D-glucose	92.9	72.3	73.5	70.1	71.1	70.5	68.9[c]	
6-*O*-Carboxymethyl-β-D-glucose	96.8	74.9	76.5	70.1	75.5	70.5		
3-*O*-(2-Hydroxyethyl)-α-D-glucose	93.4	72.6	83.3	70.6[b]	72.8	62.2[b]	75.3	62.8
3-*O*-(2-Hydroxyethyl)-β-D-glucose	97.2	75.1	85.9	70.4[b]	77.3	62.3[b]	75.3	62.8
6-*O*-(2-Hydroxyethyl)-α-D-glucose	93.3	72.6	73.8	70.4	71.5	70.5	75.5	62.9
6-*O*-(2-Hydroxyethyl)-β-D-glucose	97.2	75.2	76.7	70.4	75.9	70.5	75.5	62.9

[a]Data from Ref. 6. [b]Chemical shifts for α and β anomers may be reversed. [c]Chemical shifts for the appended carboxyl carbons, at ~185 p.p.m.

Fig. 2a shows the spectrum of a preparation from an *O*-(carboxymethyl)-cellulose (5) of d.s. = 0.7; the spectrum was obtained at 30°; but was substantially the same at 60°. The relative prominence of the downfield signal assigned to substituted C-2 demonstrates a high d.s. at the 2-position, whereas the strong signal at 62 p.p.m. (due to unsubstituted C-6) provides evidence that little derivatization had occurred at the primary hydroxyl group of the cellulose. There appears to be no signal attributable (Table I) to a 3-*O*-carboxymethyl substituent. A strong group of signals centered at ~75 p.p.m. is ascribed to C-5, C-3, and unsubstituted C-2. Reference to Table I suggests that peaks in the region 69–72 p.p.m. include those of methylenes of the 2- (major) and 6- (minor) substituents, C-6 bearing a substituent, and, as mentioned above for *O*-methylcellulose, C-4 of non-reducing-end residues. Several small signals (106, 103, and 81 p.p.m.) are probably spinning side-bands. As noted in Table I, all of the carboxyl carbons of the monosubstituted glucoses have essentially the same shift, and hence offer little information. Furthermore, these signals are relatively weak, because of the long relaxation times of carboxyl carbons, and were even weaker in the polymer spectra.

A sample of lower d.s. (0.5), being appreciably more degradable by the enzyme, gave a slightly better resolved spectrum than that of Fig. 2a, although its general features were closely similar. At an *O*-carboxymethyl level of d.s. = 1.2, the relative

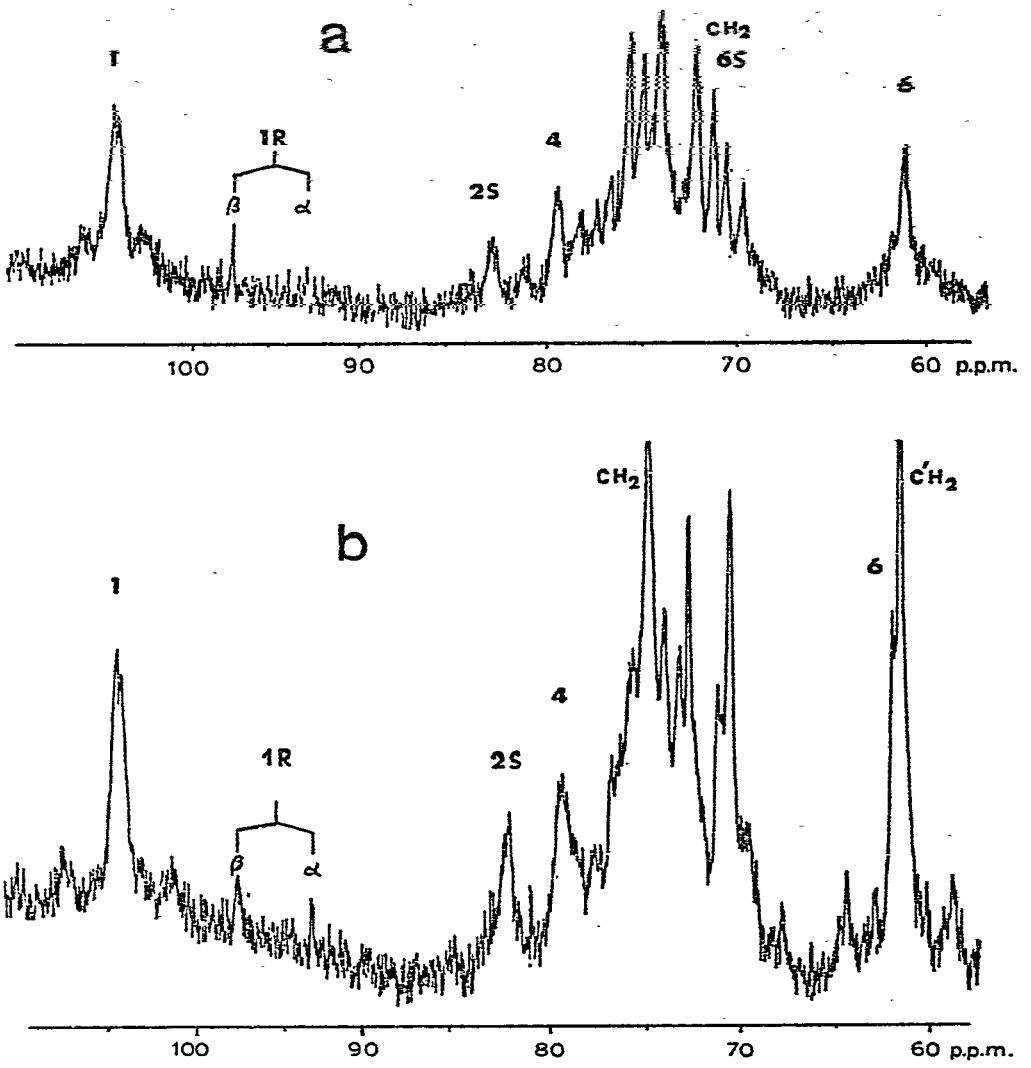


Fig. 2. FT ^{13}C-n.m.r. spectrum (at 22.63 MHz, ^{1}H-decoupled) of (*a*) *O*-(carboxymethyl)cellulose (d.s. 0.7, partially degraded by cellulase) in D_2O at 30° (a similar spectrum was obtained at 60°); R, signal of reducing-end residue; S, C is bonded to alkoxyl group. (*b*) *O*-(2-Hydroxyethyl)cellulose (d.s. 0.8, partially degraded by cellulase) in D_2O at 30°; R, signal due to reducing-end residue; S, C is bonded to alkoxyl group.

intensities of the peaks attributable to substituted C-2 and C-6 increased by comparison with those of Fig. 2a, but the resolution was poorer because the molecular weight of the sample recovered after enzymic digestion was higher than that from *O*-(carboxymethyl)cellulose of d.s. = 0.7.

In general, then, these data reflect the fact, already noted in dealing with *O*-methylcellulose, that ether formation by the alkali-cellulose used in the preparation

of *O*-carboxymethyl derivatives[16,17] shows a strong preference for substitution of OH-2, less for OH-6, and least for OH-3.

O-(2-Hydroxyethyl)cellulose. — The *O*-(2-hydroxyethyl) substituent exerts about the same effects on the α- and β-carbons as found for the other ether substituents (Table I). A sample of *O*-(2-hydroxyethyl)cellulose (6) (d.s. = 0.8), after partial degradation by cellulase, gave the spectrum shown in Fig. 2b. In several respects, this spectrum resembles that of the *O*-(carboxymethyl)cellulose sample presented in Fig. 2a, which is in accord with other evidence[12] to the effect that these two preparations have similar substitution patterns.

The strength of the signals produced by CH_2 and CH_2' of the substituents (at 76 and 62 p.p.m., respectively) is taken as an indication of the extent to which the ethylene oxide reacts with newly generated carbinol groups in side-chains to form linear dimethyleneoxy oligomers [$-O-(CH_2-CH_2-O)_n$] during derivatization of the cellulose[18]. Although signal overlap interferes with a quantitative measure of this process, it appears that these chains may average 2–3 units. Two other strong peaks are found at 71 and 73 p.p.m. Their identity is unclear, but it is not improbable that they represent adsorbed condensation products of ethylene oxide that have not been detected in *O*-(2-hydroxyethyl)celluloses by other analytical methods.

Spectra of total hydrolysates of cellulose ethers. — Another way in which ^{13}C-n.m.r. spectroscopy may be used for the characterization of cellulose ethers is to examine the products of complete acid hydrolysis. Understandably, much better spectra are obtained in this way than with the intact or partially degraded polymer, because the problems of low concentration and high viscosity are greatly minimized. An example is provided in Fig. 3 by the spectrum of the hydrolysate of the *O*-(carboxymethyl)cellulose used above to obtain spectrum 2a. No signal was detected in the 105-p.p.m. region—the resonance position of a glycosidically bound C-1—which shows that the hydrolysis was virtually complete. Signals in the 80–86 p.p.m. region provide a clear description of the pattern of substitution at the 2- and 3-positions. Although here, again, a preponderance of the 2-*O*-carboxymethyl group is found, substitution of the 3-position is appreciable (from relative intensities, the ratio of 2- to 3- is ~4:1), whereas 3-substitution was not detectable in Fig. 2a*. A moderately strong signal at 70 p.p.m. is probably due to the C-6 atoms that are bearing an ether substituent, and this view is supported by the fact that the signal attributable to CH_2-6 is of a similar intensity. These signals indicate once again that the extent of carboxymethylation at C-6 is intermediate between that at C-2 and C-3. Some of the other assignments refer to prominent signals characteristic of unsubstituted α,β-D-glucose (Table I).

*The material described in Fig. 2a was enzymically modified, and may represent some selective fractionation of differently substituted portions of the original sample. Di-*O*-substituted glucose was barely detectable in this sample by paper chromatography. With cellulose of higher d.s., however, the co-occurrence of 2,3-di-*O*-carboxymethyl-D-glucose with the 2- and 3-monosubstituted derivatives is expected to give rise to extra peaks in this region ascribable to C-2 and C-3, because of mutual shielding by the neighboring *O*-carboxymethyl groups in the disubstituted compound.

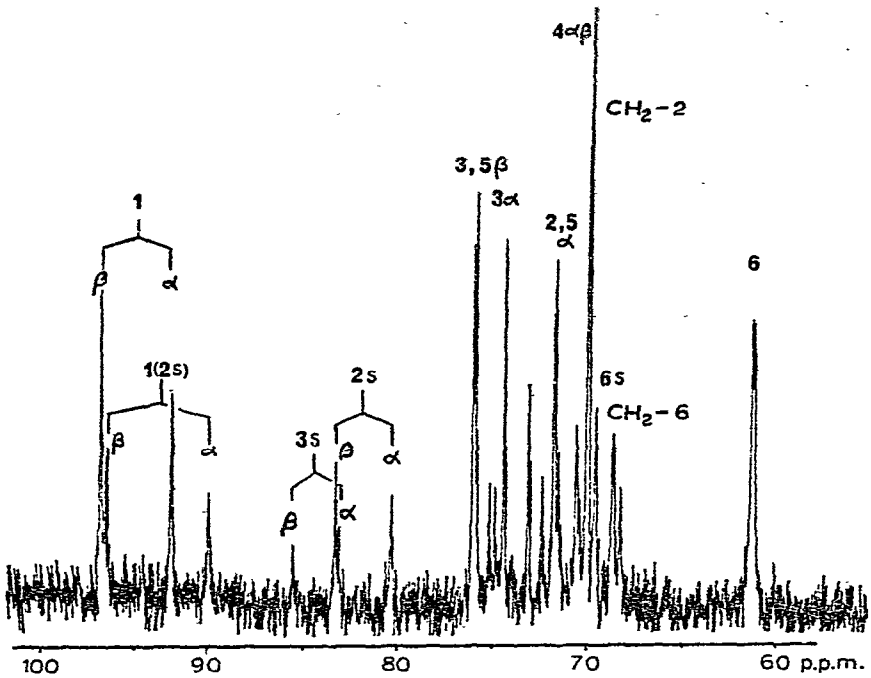


Fig. 3. FT ^{13}C-n.m.r. spectrum (at 22.63 MHz, ^{1}H-decoupled) of complete hydrolysate of *O*-(carboxymethyl)cellulose (d.s. 0.7) in D_2O. S, C is bonded to alkoxyl group; (2S), due to 2-substituted glucose.

Such hydrolysates may be analysed with good accuracy, of course, by chromatographic procedures[12,19]. However, ^{13}C-n.m.r. spectroscopy eliminates the need to isolate the products and (for g.l.c.) to prepare[12] derivatives. Hence, although measurements of spectral intensity are probably no more accurate than area measurements of chromatograms, they involve fewer sources of error introduced in the handling of hydrolysates.

Identification of reducing-end residues in enzymically degraded cellulose ethers. — As seen in Table I, the presence of an ether substituent at the 2-position causes an upfield shift of the C-1 resonances relative to those of glucose. This effect is greater with the α anomer, so that $\Delta\delta$ for the C-1α and C-1β signals is larger with the derivatives than with glucose itself: *i.e.*, $\Delta\delta$ is 5.6 p.p.m. for 2-*O*-carboxymethyl-α- and -β-D-glucose, 6.4 p.p.m. for the 2-*O*-methyl sugar[4], and 3.8 p.p.m. for the unsubstituted compound (see Table I). Therefore, it is noteworthy that the C-1 signals of reducing-end residues, visible in Figs. 1b, 2a, and 2b, correspond closely to those of α- and β-D-glucose (or α- and β-cellobiose), which is evidence that these reducing residues are not substituted at the 2-position. This finding is in accord with earlier proposals[9–12] to the effect that the scission of chains in cellulose ethers by cellulase takes place preferentially at residues that do not bear an *O*-alkyl substituent.

SUMMARY

The ^{13}C-n.m.r. spectrometer that has been used in the current study, which operates at 22.6 MHz and with 10-mm sample tubes, is unsuitable for the direct examination of cellulose ethers of high molecular weight. However, it affords moderately well-resolved spectra with samples that have been partially depolymerized, and these spectra have characteristics closely akin to those of model compounds of low molecular weight. A limited hydrolysis with acid in a non-polar solvent is effective for dealing with highly substituted cellulose ethers, whereas for those having a moderate-to-low d.s., partial degradation with an enzyme affords solutions that are satisfactory for spectroscopy.

Improvements in instrumentation and technique will enhance the attractiveness of ^{13}C-n.m.r. applications in this area. For example, instruments of higher operating frequencies and/or with sample tubes of larger diameter, now coming into wider use, by offering greater sensitivity may permit a direct examination of unmodified cellulose ethers. Although the measurement of signal intensities in their spectra could be seriously complicated by such sensitive variables as relaxation times and the Overhauser enhancement, as has been well-recognized with other polymers[20,21], continuing advances in technique can be expected to alleviate this difficulty. In some instances, the use of elevated temperatures may be beneficial.

Clearly, the direct study of intact polymers is the ideal objective for the future. At present, however, well-resolved spectra can be obtained most readily by resorting to the other extreme, *i.e.*, by using a complete hydrolysate of the polymer. Although this hydrolysate can also be analyzed by chromatographic procedures, ^{13}C-n.m.r. spectroscopy is a more direct method and offers the additional advantage that it simultaneously provides fuller information as to the identity of the products formed.

EXPERIMENTAL

Proton-decoupled ^{13}C-n.m.r. spectra were recorded with a Bruker WH-90 spectrometer operating at 22.63 MHz. Spectral accumulation and Fourier transformation were accomplished with a B-NC12 computer having an 8K data memory. Generally, a pulse width of 18–24 μs (*i.e.*, 70–90°), a repetition time of 2.8 sec, and a total acquisition time of 16 h, were used and, unless otherwise specified, hexadeuteriobenzene contained in a coaxial capillary was employed to provide a lock signal. The sample tube was 10 mm in diameter, the sample concentration was usually 100–200 mg/ml, and temperature 30–60°.

Synthesis of reference compounds. — Samples of 2-, 3- and 6-mono-*O*-carboxymethyl-D-glucose were prepared essentially as described by Shyluk and Timell[19]. Crystalline intermediates obtained were 3-*O*-carboxymethyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose methyl ester, m.p. 103° (lit.[19] m.p. 103–104.5°), and 6-*O*-carboxymethyl-1,2:3,5-di-*O*-methylene-α-D-glucofuranose methyl ester, m.p. 46° (lit.[19] m.p. 46.5–47.5°). These intermediates were also utilized, through reduction

with lithium aluminium hydride, for the preparation of the reference compounds 3- and 6-*O*-(2-hydroxyethyl)-D-glucose. None of these five model derivatives was crystalline, but each was judged to be of high purity by chromatography, and by both ^{1}H- and ^{13}C-n.m.r. spectroscopy.

Preparation of solutions of cellulose derivatives. — (*a*) *Partial hydrolysis of O-methylcellulose with acid* (*i*). A solution of *O*-methylcellulose (d.s. ~2.8) in dichloroethylene (10 ml) and conc. hydrochloric acid (1.5 ml) was boiled under reflux for 1.5 h, and then concentrated *in vacuo* to dryness. The residue was dissolved in deuteriochloroform, which also provided the (internal) lock signal. (*ii*) A sample of *O*-methylcellulose (d.s. 1.5–2) was triturated with M hydrochloric acid (10 ml), the slurry was heated at 95° for 5 h, and the resulting solution was freeze-dried. The residue was dissolved in water, the lock signal being furnished by a capillary containing hexadeuteriobenzene.

(*b*) *Enzymic degradation of* O-(*carboxymethyl*)*cellulose*[12]. *O*-(Carboxymethyl)-cellulose (1 g; Hercules Powder Co., Wilmington, Del.) was triturated in 0.1M sodium acetate buffer (pH 5.5, 10 ml) containing cellulase (30 mg; *Streptomylces* sp. QMB814*). The resulting paste was stored at 40° for 12 h, causing a marked decrease in viscosity. The solution was heated at 100° for 15 min, dialysed against tap water for 48 h and distilled water for 8 h, and finally freeze-dried. Water was used as solvent for the residue, the lock signal being furnished by a capillary of hexadeuteriobenzene.

A similar procedure was used for *O*-(2-hydroxyethyl)- and *O*-methyl-cellulose samples of d.s. < 1.

(*c*) *Complete hydrolysis of* O-(*carboxymethyl*)*cellulose with acid.* A suspension of *O*-(carboxymethyl)cellulose [3 g of the same sample as in (*b*)] in 72% sulfuric acid (20 ml) was kept at 40° for 1 h, water (1.5 l) was introduced, and the solution was autoclaved (pressure, 2 atm.) for 1 h. Excess of barium carbonate was added to the cooled solution, the suspension was filtered through Celite, and the effluent was treated with Amberlite IR-120(H^+) resin and then freeze-dried. Deuterium oxide was used as solvent for the residue, and also to provide the lock signal. Paper-chromatographic examination of the solution[19] (ethyl acetate–formic acid–water, 12:1:12) showed the presence of two major, and one minor, monosubstituted sugars, and traces of more highly substituted species.

ACKNOWLEDGMENTS

The authors thank the "Ministère de l'Education du Québec" for a scholarship (to A.P.) under the France–Québec exchange program, and the Pulp and Paper Research Institute of Canada and the National Research Council of Canada for generous support. The kind interest and advice of Dr. S. S. Bhattacherjee is very much appreciated.

*Kindly furnished by E. T. Reese.

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