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DETERMINATION OF SUBSTITUENT DISTRIBUTION IN CELLULOSE ETHERS BY ^{13}C- AND ^{1}H-N.M.R. STUDIES OF THEIR ACETYLATED DERIVATIVES: *O*-(2-HYDROXYPROPYL)CELLULOSE

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ABSTRACT

Structural characteristics of *O*-(2-hydroxypropyl)cellulose samples, namely the molar substitution (mol. subst.), the total degree of substitution (d.s.), and the individual degree of substitution of hydroxyl groups on the glucose residues for a wide mol. subst. range of samples were determined after peracetylation by ^{1}H- and ^{13}C-n.m.r. analyses. The mol. subst. value was determined by comparing the acetyl methyl proton signal with that of the 2-hydroxypropyl methyl group. The acetyl carbonyl carbon signal of acetylated *O*-(2-hydroxypropyl)cellulose samples was found to split into a quadruplet in Me_2SO at 100°, reflecting the position of the substituent (2, 3, and 6) on the glucose residue and of the (oligo-)propylene oxide substituent end-group, and allowing determination of the substituent distribution as well as the total degree of substitution in a series of *O*-(2-hydroxypropyl)-cellulose samples.

INTRODUCTION

The distribution of substituents in cellulose derivatives is considered to strongly influence the properties of these derivatives both in bulk and in solution. The precise determination and control of substituent distribution are thus important in understanding structure–property relationships and for quality control in production.

Although n.m.r., particularly ^{13}C-n.m.r., has been widely applied with cellulose derivatives[1], the lack of a common n.m.r. solvent for such samples over a wide range of degrees of substitution (d.s.) has restricted its practical use, particularly with nondegraded samples.

On the other hand, analytical methods applied to hydrolyzates or

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alcoholyzates have been extensively used for the structural analysis of cellulose derivatives having hydrolytically stable substituents. Such techniques as g.l.c.[2,3] and n.m.r.[4–10] have been widely examined; the former appears to give the most detailed structural information.

We have recently proposed an alternative technique[1,11–13] in which acetylated cellulose derivatives are used for n.m.r. analysis. By this method, the derivatives retain their polymeric form to provide, at least in principle, information on higher-order polymer structures and on the distribution of substituted glucose residues along the polymer chain.

The foregoing studies on a series of *O*-methylcellulose and *O*-(2-hydroxyethyl)cellulose samples revealed a number of advantages to this technique as compared with previous methods involving untreated cellulose derivatives. These are: (*a*) the sample becomes soluble in common n.m.r. solvents over a wide range of d.s., which facilitates comparison with model polymers; (*b*) the acetyl carbonyl carbon signal is highly sensitive to its location on the glucose residue[14,15] allowing direct determination of the substituent distribution; (*c*) acetylation of the hydroxyl groups eliminates the spectral complication arising from hydrogen-bonding interactions[16]; and (*d*) the cellulose derivatives retain their polymeric form, thus obviating the sometimes cumbersome hydrolysis pretreatment.

We describe here the results of structural studies on a series of *O*-(2-hydroxypropyl)cellulose ("hydroxypropylcellulose") samples by means of ^{1}H- and ^{13}C-n.m.r. analyses of their acetylated derivatives[17]. As hydroxypropylcellulose is produced by reaction of "alkali cellulose" with propylene oxide, whereby anionic ring-opening polymerization occurs with scission of the methylene–oxygen bond[18], the product is etherified by a mixture of "oligo-propylene oxides" having various degrees of polymerization. Thus the molar substitution value (mol. subst.), which refers to the number of propylene oxide units per glucose residue, is commonly used for the designation of hydroxypropylcellulose samples.

Hydroxypropylcellulose is considered to be especially interesting for the present technique, since existing g.l.c. of n.m.r. methods using hydrolyzates or methanolyzates suffer serious chromatographic or spectral complications arising from the presence of diastereomeric mixture produced through the reaction of either the *R* or *S* enantiomer of propylene oxide. In addition, isomeric mixtures of bicyclic acetals are formed during the hydrolysis[10], and anomerization at C-1 of the glucose residue may cause another problem. Furthermore, acid degradation of the oligo-propylene oxide substituent unit to form cyclic dimers[19,20] may take place unless special care is taken in choosing the hydrolytic conditions.

It should also be pointed out that hydroxypropylcellulose forms a thermotropic cholesteric liquid-crystalline phase[21,22]. It is thus of urgent importance to develop a facile and convenient analytical method to provide more structural information on hydroxypropylcellulose in order to elucidate structure–liquid-crystalline property relationship for this unique polymer.

EXPERIMENTAL

Samples. — *O*-(2-Hydroxypropyl)cellulose ("hydroxypropylcellulose") samples having various values of molar substitution were supplied by Shin-Etsu Chemical Co., Ltd. The samples were acetylated as already described[1]. Polypropylene glycol (PPG 400, Yoneyama Yakuhin Co.) was acetylated similarly.

Measurements. — ^{1}H- and ^{13}C-n.m.r. analyses were performed at 270.8 and 67.8 MHz, respectively, by means of a JEOL JNM-GX270 spectrometer equipped with a 5-mm (i.d.) C-H dual probe at 100° in Me_2SO-d_6, or at 40° in D_2O. Chemical-shift values were referenced either to the solvent signal of Me_2SO (2.5 p.p.m. for ^{1}H and 43.5 p.p.m. for ^{13}C) or from sodium 4,4-dimethyl-4-silapentanoate-d_4 (0.0 p.p.m.) in D_2O. The general measurement conditions were similar to those of the previous study[1] except for the quantitative analysis, where ^{13}C-n.m.r. measurements were carried out with a pulse-repetition time of 100 sec with 1500–2000 transients. The sample solution of ~7–10% (w/v) was used with the bilevel complete decoupling mode by using a flip angle of 45°. Spectrum width was 20 000 Hz with 32K data points. The spin–lattice relaxation time, T_1, was measured by the inversion–recovery method using an acetylated hydroxypropylcellulose sample of mol. subst. 1.27.

I.r. spectra were taken on a Hitachi 260-10 spectrophotometer.

G.l.c. analyses on hydrolyzates of hydroxypropylcellulose samples were performed by a slight modification of the method described for *O*-methylcellulose[1]. Hydroxypropylcellulose (50 mg) was hydrolyzed with 2.0 mL of 3.0% H_2SO_4 for 3 h at 130°. No detectable degradation of the polypropylene glycol chain was observed under these conditions. After neutralization with $BaCO_3$, the sample was redissolved in 3.0 mL of MeOH and filtered through a 0.45-μm filter. Then 100 μL of $NaBH_4$solution, prepared with 1.5 g of $NaBH_4$ in 10 mL of 0.2M NaOH, was added and stirred for 1 h. Acetic acid, (10 μL) was then added and the solution was evaporated by heating, with subsequent washing with 3.0 mL of MeOH and evaporating (twice). Finally, 1.0 mL of Ac_2O and 1.0 mL of C_5H_5N were added and the mixture was heated for 3 h at 120°. The solution was directly analyzed by injecting 1 μL of it into a Shimadzu GC-7A gas chromatography equipped with a capillary column (0.2-mm i.d. × 10 m) of cross-linked 5% phenylmethylsilicone (Hewlett–Packard) with a column temperature of 190–320° and a heating rate of 4°.min^{-1}. G.l.c. peak assignments were made by analysis of the mass-spectrometric molecular-ion peaks recorded by a Hitachi M80B mass spectrometer in the c.i. mode. These analyses showed a distribution of glucose residues possessing substituents up to octa-propylene oxide units.

RESULTS AND DISCUSSION

Proton-[23,24] and ^{13}C[8–10,25]-n.m.r. spectroscopic analyses on polymeric hydroxypropylcellulose or on its hydrolyzate (or alcoholyzate) give only limited

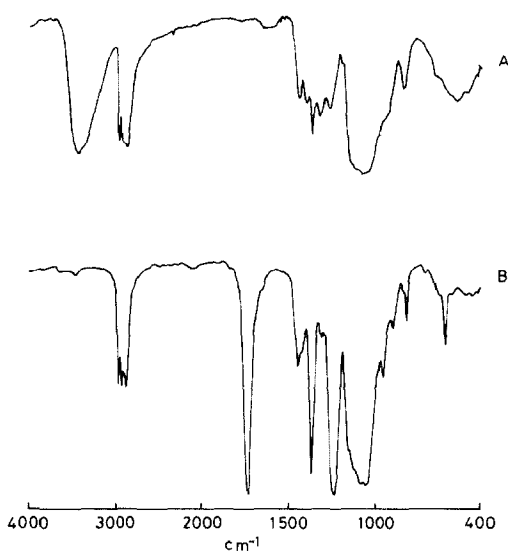

Fig. 1. I.r. spectra of hydroxypropylcellulose (A) and acetylated hydroxypropylcellulose (B) (sample 6 in Table I).

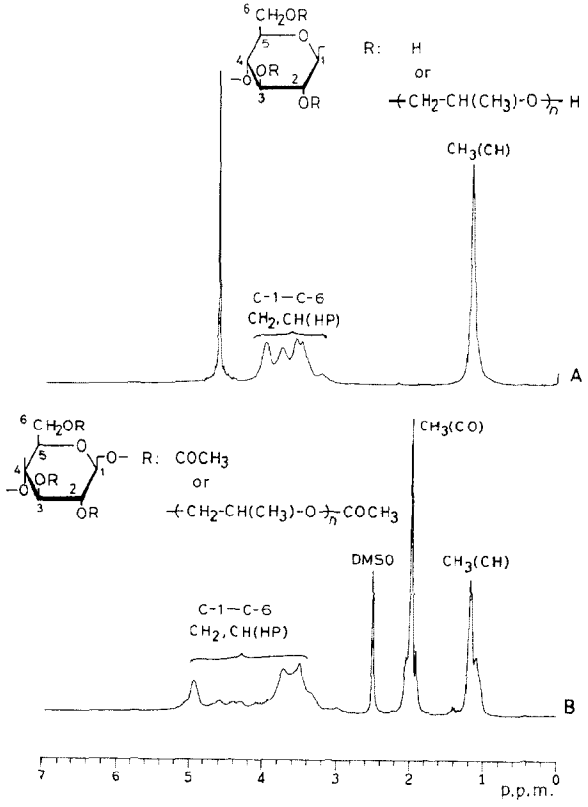

Fig. 2. ^{1}H-N.m.r. spectra of hydroxypropylcellulose (A) of mol. subst. 3.68 in D_2O at 40° (sample 7 in Table I) and acetylated hydroxypropylcellulose (B) of mol. subst. 2.45 in Me_2SO-d_6 at 100° (sample 6 in Table I).

structural information because of the lack of a common n.m.r. solvent for a wide range of mol. subst. samples and to the serious spectral complications arising from signal overlap. The present technique employing acetylated hydroxypropylcellulose improves the situation substantially.

I.r. spectra of the original hydroxypropylcellulose and its acetylated (Ac_2O–C_5H_5N) derivative are shown in Fig. 1. Quantitative acetylation is evident from the disappearance of the hydroxyl absorption at 3300 cm^{-1} along with appearance of ester carbonyl absorption at 1735 cm^{-1}.

Acetylated hydroxypropylcellulose samples of a wide mol. subst. range are found to be soluble in Me_2SO-d_6, thus facilitating high-temperature n.m.r. measurements.

Proton- and ^{13}C-n.m.r. measurements were performed in Me_2SO-d_6 at 100° and the spectra were compared with those of untreated hydroxypropylcellulose in D_2O at 40°. Fig. 2 shows ^1H-n.m.r. spectra of the untreated and the acetylated hydroxypropylcellulose derivative. The mol. subst. value was estimated more accurately by use of the latter spectrum by reference to the well-resolved and separated acetyl methyl and hydroxypropyl methyl proton signals.

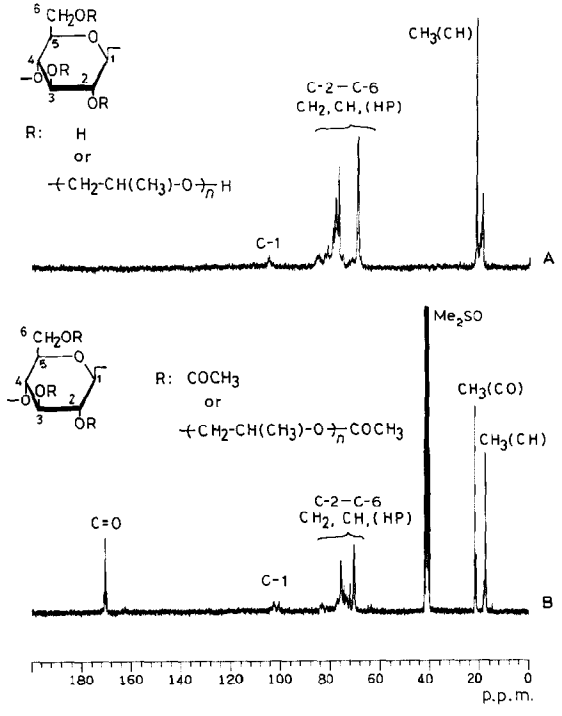

Fig. 3. ^{13}C-N.m.r. spectra of hydroxypropylcellulose (A) of mol. subst. 3.68 in D_2O at 40° (sample 7 in Table I) and acetylated hydroxypropylcellulose (B) of mol. subst. 2.45 in Me_2SO-d_6 at 100° (sample 6 in Table I).

The ^{13}C-n.m.r. spectra of the starting hydroxypropylcellulose and of its peracetate are shown in Fig. 3. The informative peaks of the former are the split hydroxypropyl methyl signals, which have been reported[8] to correspond respectively to the inner and the end-units of the oligo-propylene oxide substituent. The latter shows nicely-resolved carbon peaks for the carbonyl groups, C-1 acetal, acetyl methyl and hydroxypropyl methyl, to provide significantly more structural information.

The spectra of a series of acetylated hydroxypropylcellulose samples, expanded in the carbonyl region, are collected in Fig. 4. The carbonyl carbon signal was observed to be split into an apparently resolved quadruplet at 172.2, 172.6, 172.9, and 173.4 p.p.m., corresponding not only to the position of the acetyl substituents on the glucose residue [positions 2 (172.2), 3 (172.6), and 6 (173.4), respectively[1]], but also to the end group of the (oligo-)propylene oxide substituent

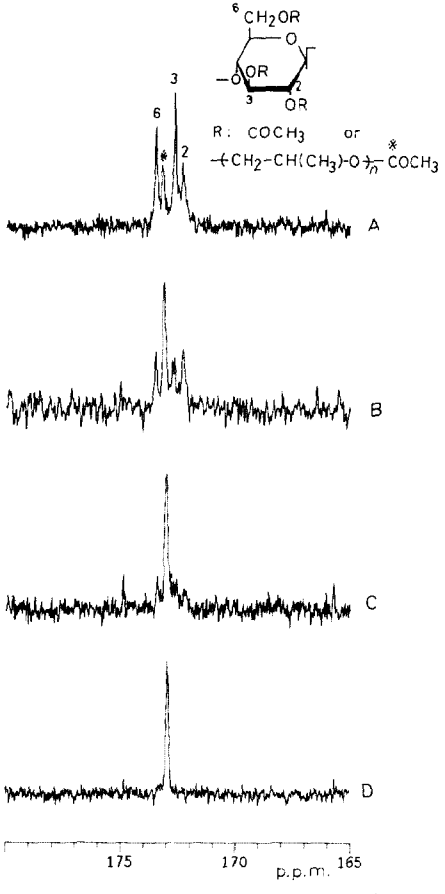

Fig. 4. Carbonyl region of the ^{13}C-n.m.r. spectra of acetylated hydroxypropylcelluloses in Me_2SO-d_6 at 100° [mol. subst. of sample: (A) 0.36; (B) 1.27; (C) 2.45; and (D) 3.68].

(172.9). The spin–lattice relaxation time (T_1) values for the carbonyl carbon signals at the 2, 3, and 6 position of the glucose residue were 1.98, 2.24, and 3.10 sec, respectively, while that at the end of hydroxypropyl group was significantly longer (9.89 sec), indicating high flexibility of the substituent end-groups. Quantitative estimation of the degrees of substitution was performed by measurement with the long pulse-repetition time of 100 sec. Integration of each peak allows direct determination of not only the total degree of substitution but also the distribution of acetyl groups in acetylated hydroxypropylcellulose samples. The substituent distribution of (oligo-)propylene oxide groups was subsequently determined by subtracting the acetyl group distribution in the acetylated hydroxypropylcellulose sample.

The C-1 acetal carbon analysis, which is known[1] to be indicative of the type

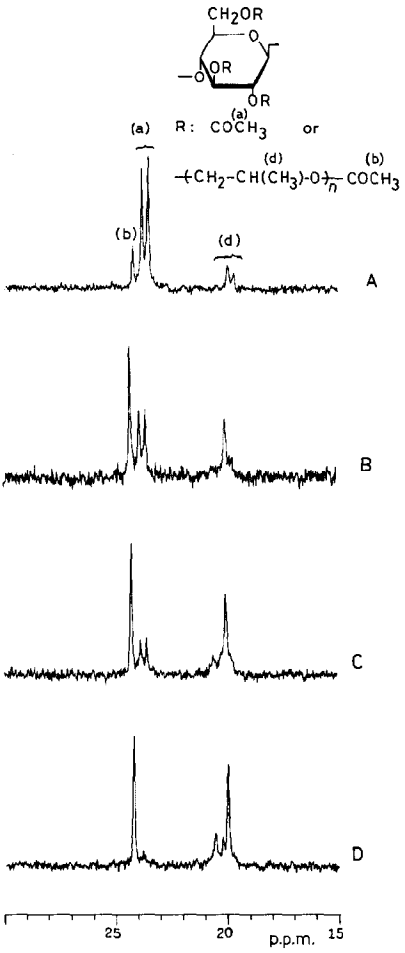

Fig. 5. Acetyl-methyl and hydroxypropyl-methyl region of the ^{13}C-n.m.r. spectra of acetylated hydroxypropylcelluloses in Me_2SO-d_6 at 100° [mol. subst. of sample: (A) 0.36; (B) 1.27; (C) 2.45; and (D) 3.68].

of substituent on O-2 [at 104.8 p.p.m. for 2-*O*-(hydroxypropyl) and at 102.8 p.p.m. For 2-*O*-acetyl] permits determination of the degree of substitution at O-2 separately.

The expanded acetyl-methyl and hydroxypropyl-methyl regions of the spectra of a series of acetylated hydroxypropylcellulose samples are collected in Fig. 5. The acetyl-methyl signal was found to be split into three peaks at 23.5, 23.8, and 24.2 p.p.m., corresponding to the location of the acetyl function on either the glucose residue (23.5 and 23.8) or at the end group of an (oligo-)propylene oxide substituent (24.2). The T_1 values were 1.30 and 1.13 sec for the former two carbons and 3.40 sec for the latter. Integration of each signal again provides the total degree of substitution.

The hydroxypropyl-methyl signal at ~20.0 p.p.m. showed fine splitting, presumably reflecting the substituent position on the glucose residue and/or the distribution of enantiomers on the hydroxypropyl unit. With increasing mol. subst., signal at 20.5 p.p.m. becomes clearly visible; it was assigned to the inner methyl group of the oligo-propylene oxide substituent by comparison with the spectrum of acetylated polypropylene glycol. Thus the average degree of polymerization of the oligo-propylene oxide substituent may be determined by the integration of these two signals.

The structural parameters obtained for a series of hydroxypropylcellulose samples possessing various mol. subst. values, determined by ^{1}H-n.m.r. and g.l.c. techniques, are collected in Table I. The total d.s. values were obtained either from acetyl carbonyl or acetyl methyl carbon analysis and were in satisfactory agreement with each other. The degrees of substitution at individual positions on the glucose residue, estimated from acetyl carbonyl analysis, are also given in this Table. The degrees of substitution at O-2 were determined also from analysis of the C-1 region and were comparable with those from analysis of the acetyl carbon region; nevertheless, serious line-broadening of the C-1 acetal carbon signals is observed,

TABLE I

DISTRIBUTION OF (OLIGO-)PROPYLENE OXIDE GROUPS IN HYDROXYPROPYLCELLULOSE

Sample	*Mol. subst.*		*Total d.s.*		*Individual position*			*D.p. of substituent*	
	^{1}H	*G.l.c.*	*CO*[a]	*CH_3*[b]	*2*[a,c]	*3*[a]	*6*[a]	*Obs.*	*Calc.*[d]
1	0.36	0.24	0.49	0.41	0.40 (0.18)	0.01	0.08	1.00	0.88
2	0.90	0.75	1.00	0.95	0.41 (0.31)	0.29	0.26	1.00	0.90
3	1.27	1.17	1.26	1.25	0.57 (0.43)	0.28	0.42	1.14	1.00
4	2.37	2.12	1.95	1.94	0.77 (0.59)	0.60	0.60	1.20	1.22
5	2.37	2.20	2.12	1.93	0.73 (0.65)	0.66	0.73	1.30	1.12
6	2.45	2.60	1.85	1.82	0.64 (0.66)	0.55	0.64	1.23	1.32
7	3.68	3.76	2.86	2.65	1.00 (1.00)	0.86	1.00	1.36	1.29

[a]From carbonyl region analysis. [b]From acetyl methyl region analysis. [c](C-1) region analysis in parentheses. [d]Calculated by mol. subst. (^{1}H)/total d.s. (CO).

particularly at low degrees of substitution, where the largest discrepancy is observed.

The hydroxyl groups at O-2 appear to react most readily with propylene oxide in the initial stage of the reaction, while those at O-6 were gradually consumed until the degree of substitution at O-6 reached the same level of that at O-2 in a later stage. The 3-hydroxyl groups were found to be sluggish toward reaction with propylene oxide; nevertheless, they were mostly consumed when the mol. subst. value reached 3.68. The established reactivity of hydroxyl groups on glucose residues, namely[1] O-2>O-6>O-3, appears to be maintained here also. Nevertheless, care should be taken in the quantitative estimation of the reactivity of hydroxyl groups on glucose residues and at the substituent end-group because of the heterogeneous nature of the reaction between alkali cellulose and propylene oxide; reaction in the amorphous region may take place much more readily compared than in the crystalline phase of cellulose, resulting in a different substituent distribution from that estimated by statistical calculations[2].

The average degrees of polymerization of (oligo-)propylene oxide substituents, as determined by analysis of the hydroxypropyl methyl signals, are listed and compared to those estimated from the ratio of the mol. subst. and the total d.s., as shown in Table I. Both values are found to coincide and to remain near unity in the initial stage of the reaction. They were then observed to increase along with the mol. subst. value. It should be noted that mol. subst. values estimated by g.l.c. are lower than those determined by the ^{1}H-n.m.r. method, particularly for samples having low degrees of subsitution; they are even lower than total d.s. values estimated by the ^{13}C-n.m.r. method and are beyond the range of an experimental error. This anomaly may well be explained by undesired degradation and by loss of the sample during such consecutive pretreatments as hydrolysis, reduction, and acetylation as required for g.l.c. analysis.

In conclusion, the present n.m.r. technique using acetylated derivatives of cellulose ethers provides a convenient and reliable method to elucidate the microstructure of hydroxypropylcellulose. Further applications of the technique not only to other cellulose derivatives[13] but also to various carbohydrate polymers are in progress.

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