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Analysis of positions of substitution of *O*-carboxymethyl groups in partially *O*-carboxymethylated cellulose by the reductive-cleavage method*

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ABSTRACT

A method is described for the analysis of positions of substitution of *O*-carboxylated groups in commercial samples of *O*-carboxymethylcellulose. Sequential permethylation of the polymer and reductive cleavage gives eight products, which are analyzed as their *O*-acetyl derivatives by gas–liquid chromatography.

INTRODUCTION

Several procedures have been developed for analysis of the positions of substitution by carboxymethyl groups in samples of commercial *O*-carboxymethylcellulose (CMC). All of these methods have employed acid-catalyzed hydrolysis of the polymer, and the mixtures of monomers so generated have either been analyzed directly by ^{13}C-n.m.r. spectroscopy[1–3] and ^{1}H-n.m.r. spectroscopy[4], or, after silylation, by gas–liquid chromatography–mass spectrometry[5,6]. Due to the fact that CMC is only partially alkylated, hydrolysis gives rise to eight monomers, namely, D-glucose, the three mono-*O*-carboxymethyl derivatives (2-, 3-, and 6-), the three di-*O*-carboxymethyl derivatives (2,3-, 2,6-, and 3,6-), and 2,3,6-tri-*O*-carboxymethyl-D-glucose. Analysis of this mixture is complicated by the presence of both pyranose and furanose[6] anomers of these monomers, as well as lactonization products[6]. Thus, the gas–liquid chromatograms from silylated CMC hydrolysates contained 70–80 peaks, some of which have yet to be identified[6].

The reductive-cleavage technique[7] offers an alternative to these methods in that mixtures of tautomers are not formed. Indeed, analysis of samples of commercial *O*-methylcellulose and *O*-ethylcellulose by this technique indicated the presence of only eight compounds, as expected[8]. Furthermore, the reductive-cleavage technique is directly applicable to the analysis of carboxylic acid ester-containing polysaccharides, owing to the stability[9–12] of such esters under reaction conditions. The analysis of fully

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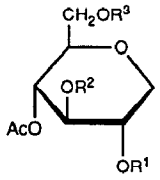

1	$R^1 = R^2 = R^3 = Me$	**5**	$R^1 = R^2 = CH_2CO_2Me$, $R^3 = Me$
2	$R^1 = R^2 = Me$, $R^3 = CH_2CO_2Me$	**6**	$R^1 = R^3 = CH_2CO_2Me$, $R^2 = Me$
3	$R^1 = R^3 = Me$, $R^2 = CH_2CO_2Me$	**7**	$R^1 = Me$, $R^2 = R^3 = CH_2CO_2Me$
4	$R^1 = CH_2CO_2Me$, $R^2 = R^3 = Me$	**8**	$R^1 = R^2 = R^3 = CH_2CO_2Me$

methylated CMC by the reductive-cleavage technique should thus also avoid the complications arising as a consequence of the presence of lactonization products. Reductive cleavage of fully methylated CMC, and acetylation of the product, is therefore expected to give rise to eight 4-*O*-acetyl-1,5-anhydro-D-glucitol derivatives, namely compounds **1–8**. As a test of this procedure, samples of commercial CMC having different degrees of substitution (d.s.) have been analyzed.

RESULTS AND DISCUSSION

Shown in Fig. 1 is the gas–liquid chromatogram obtained when fully methylated, medium viscosity CMC was subjected to reductive cleavage in the presence of triethylsilane as the reducing agent and a mixture of Me_3SiOSO_2Me and $BF_3{\cdot}Et_2O$ as the catalyst. These conditions had previously been shown[13] to effect the reductive cleavage of fully methylated cellulose to give the expected product **1** and none of the undesired product, 5-*O*-acetyl-1,4-anhydro-2,3,6-tri-*O*-methyl-D-glucitol, which arises in $Me_3SiOSO_2CF_3$-catalyzed reductive cleavages if traces of water are present[14]. The numbered peaks were identified by comparison to independently synthesized standards[15] by chemical-ionization (NH_3) mass spectrometry (c.i.m.s.), electron-ionization mass spectrometry (e.i.m.s.), and g.l.c. retention time.

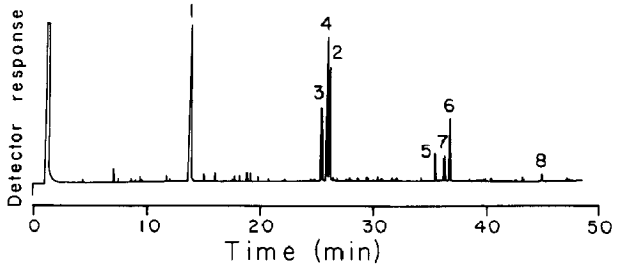

Fig. 1. Gas–liquid chromatogram of the anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated, medium-viscosity CMC. The peaks are numbered with the compound numbers. Unnumbered peaks were not of carbohydrate origin.

TABLE I

Mole fractions of products (compounds **1–8**) derived by reductive cleavage of per-*O*-methylated carboxymethylcellulose

Compound	*Parameter*	*Sample*[a] *(Mole fractions)*		
		A	*B*	*C*
1	s_0	0.428	0.491	0.512
2	s_6	0.155	0.140	0.135
3	s_3	0.094	0.078	0.069
4	s_2	0.203	0.184	0.191
5	s_{23}	0.028	0.023	0.021
6	s_{26}	0.055	0.057	0.054
7	s_{36}	0.022	0.021	0.017
8	s_{236}	0.014	0.005	trace
	d.s.[b]	0.704	0.619	0.579

[a] Sample A: high-viscosity CMC, sodium salt; B: medium-viscosity CMC, sodium salt; C: low-viscosity CMC, sodium salt. [b] d.s. $= x_2 + x_3 + x_6$; $x_2 = s_2 + s_{23} + s_{26} + s_{236}$; $x_3 = s_3 + s_{23} + s_{36} + s_{236}$; $x_6 = s_6 + s_{26} + s_{36} + s_{236}$.

Integration of all peaks and correction for molar response[16,17] gave the mole fraction(s) of each product **1–8** in each sample of CMC analyzed (see Table I). Using these values, the fractional degree of substitution, x_i, at each of the three positions on the D-glucopyranosyl residues of cellulose and the average degree of substitution (d.s. $= x_2 + x_3 + x_6$) were calculated[2]. Further manipulation of these values as described by Reuben and Conner[2] yielded the relative first-order rate constants, $k_i/(k_2 + k_3 + k_6)$, for carboxymethylation at each of the three positions of the D-glucopyranosyl residues. For samples A, B, and C, respectively, $k_2/(k_2 + k_3 + k_6) = 0.420, 0.440$, and 0.462, $k_3/(k_2 + k_3 + k_6) = 0.203, 0.191$, and 0.169, and $k_6/(k_2 + k_3 + k_6) = 0.332, 0.352$, and 0.345. The average values for these relative first-order rate constants were therefore $k_2/(k_2 + k_3 + k_6) = 0.441 \pm 0.021$, $k_3/(k_2 + k_3 + k_6) = 0.188 \pm 0.019$, and $k_6/(k_2 + k_3 + k_6) = 0.343 \pm 0.011$.

Qualitatively, the results obtained by this method indicate that the reactivities of the hydroxyl groups of cellulose toward carboxymethylation are the same as the reactivities toward methylation[8], *i.e.*, OH-2 > OH-6 > OH-3. Quantitatively, the relative first-order rate constants for carboxymethylation obtained by the reductive-cleavage method are in good agreement with the values obtained by ^{13}C-n.m.r. spectroscopy[2], *i.e.*, $k_2/(k_2 + k_3 + k_6) = 0.470 \pm 0.030$, $k_3/(k_2 + k_3 + k_6) = 0.220 \pm 0.008$, and $k_6/(k_2 + k_3 + k_6) = 0.348 \pm 0.041$. However, the averaged relative rate constants are in better agreement with those of Buytenhuys and Bonn[5], obtained by g.l.c.–m.s. analysis of the mixture of silylated monosaccharides (see Table II).

In spite of the good agreement between these methods, the method reported herein offers some distinct advantages over those wherein hydrolyzed samples are analyzed. The primary advantage of the reductive-cleavage technique is its simplicity,

TABLE II

Comparison of relative-rate constants for carboxymethylation of cellulose

Reference	$k_2 : k_3 : k_6$
This work	2.35 : 1.00 : 1.82
Reuben and Conner[2]	2.14 : 1.00 : 1.58
Buytenhuys and Bonn[5]	2.50 : 1.00: 1.8
Ho and Klosiewicz[4]	2.0 : 1.0 : 1.5
Croon and Purves[18]	2 : 1 : 2.5
Timell and Spurlin[19]	1 : 1 : 2

i.e., the identities and mole fractions of the monomers are obtained simply by integration of eight peaks in the g.l.c. profile. In contrast, n.m.r. methods require chemical-shift analysis and curve fitting of the resonances for mixtures comprised of $\geqq 16$ compounds (the α- and β-pyranose anomers of eight monosaccharide residues), and other g.l.c. methods require the identification and integration of > 70 compounds. Thus the reductive-cleavage technique nicely avoids the complexities of analysis introduced by the hydrolysis step. The sole disadvantage of the reductive-cleavage technique is that permethylation of the sample is required. However, methylation of CMC samples was readily accomplished by standard procedure, and no evidence for incomplete methylation was obtained.

Finally, it should be noted that the accuracy of d.s. values obtained using this method are not known for certain, since the d.s. of the commercial samples of CMC used in this study were not available for comparison. The d.s. values reported (Table I) might possibly be too low owing to degradation of the fully methylated glycosyl residues during reductive-cleavage, but several observations argue against this possibility. First, in all previous studies[9–12], there has been no evidence for the degradation of carboxylic acid methyl esters under reductive-cleavage conditions. Second, no side products of carbohydrate origin were detected (see Fig. 1). Third, the relative proportions of each of the substituted and unsubstituted glycosyl residues (see Table I) are those to be expected based upon the results of other methods[2,4,5]. It is therefore probable that the d.s. values determined by the reductive-cleavage method accurately reflect the degree of derivatization of the polymers.

EXPERIMENTAL

General. — Methylation was carried out as described by Blakeney and Stone[20] using lithium methylsulfinyl carbanion[8] as the base. Fully methylated polysaccharides were extracted into dichloromethane, then purified by chromatography on a column (2.5 × 30 cm) of Sephadex LH-20 in 2:1 (v/v) dichloromethane–methanol. Fractions testing positive in the phenol–sulfuric acid assay[21] were combined and used for further analysis. Reductive cleavage was performed as described by Jun and Gray[13]. The reaction was allowed to proceed for 2.5 h, and the products were isolated and acetylated

in the usual way[13]. Gas–liquid chromatography was performed in a Hewlett–Packard Model 5890A gas–liquid chromatograph equipped with a Hewlett–Packard Model 3392A integrator, a flame-ionization detector, and a J. and W. Scientific DB-5 fused-silica capillary column (0.25 mm × 30 m; film thickness 0.25 μm). The temperature of the column was held for 2 min at 110°, and then programmed to 300° at 3°/min. Low-viscosity carboxymethylcellulose, sodium salt (lot no. 55F-0608), medium-viscosity carboxymethylcellulose, sodium salt (lot no. 116F-0231), and high-viscosity carboxymethylcellulose, sodium salt (lot no. 76F-0318) were obtained from Sigma Chemical Co.

Molar response values (flame-ionization detection) of anhydroalditol derivatives **1–8**. — The integral values of all g.l.c. peaks were corrected for molar response by the effective carbon-response (e.c.r.) method[16,22–24], which had been shown[17] to be applicable to anhydroalditols. Integrated areas were divided by the appropriate e.c.r. value in order to correct for molar response. The e.c.r. values were normalized to **1** set at unity and are: **2** (1.10); **3** (1.10); **4** (1.10); **5** (1.20); **6** (1.20); **7** (1.20), and **8** (1.30).

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