Carbohydrate Research, 177 (1988) 111–116
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ANALYSIS OF POSITIONS OF SUBSTITUTION OF *O*-METHYL OR *O*-ETHYL GROUPS IN PARTIALLY METHYLATED OR ETHYLATED CELLULOSE BY THE REDUCTIVE-CLEAVAGE METHOD*

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(Received September 2nd, 1987; accepted for publication, November 10th, 1987)

ABSTRACT

A method is described for the analysis of positions of substitution of *O*-methyl and *O*-ethyl groups in commercial samples of *O*-methylcellulose and *O*-ethylcellulose, respectively. The method requires perethylation of *O*-methylcellulose and permethylation of *O*-ethylcellulose. Subsequent reductive cleavage of both polymers gives the same 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 methyl or ethyl groups in samples of commercial *O*-methylcellulose or *O*-ethylcellulose. All of these have employed acid-catalyzed hydrolysis or methanolysis and the mixtures of monomers so generated have been separated either directly[1] or after benzoylation[2] or analyzed without separation by ^{13}C-n.m.r. spectroscopy[3–5]. Because these cellulose ether derivatives are only partially alkylated, hydrolysis of each will give rise to eight monomers, namely, D-glucose, the three mono-*O*-methyl- or -ethyl-D-glucose derivatives (2-, 3-, and 6-), the three di-*O*-methyl- or -ethyl-D-glucose derivatives (2,3-, 2,6-, and 3,6-), and 2,3,6-tri-*O*-methyl- or -ethyl-D-glucose. Analysis of these mixtures directly by ^{13}C-n.m.r. spectroscopy[3–5] or by benzoylation and high-performance liquid chromatography[2], is further complicated by the presence of the pyranose anomers of each of the monomers. Thus, for either *O*-methylcellulose or *O*-ethylcellulose, a mixture comprised of up to 16 components is ultimately analyzed.

The reductive-cleavage technique[6,7] offers an alternative to these methods, in that mixtures of anomers are not formed. Furthermore, such analyses can be further so simplified that the same eight monomers are derived from either *O*-

*This investigation was supported by Grant GM34710 awarded by the Department of Health and Human Services.
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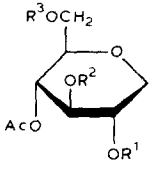
0008-6215/88/$ 03.50

methylcellulose or *O*-ethylcellulose. Described herein is an approach involving per-*O*-ethylation of *O*-methylcellulose and per-*O*-methylation of *O*-ethylcellulose. Reductive cleavage of either polymer, and acetylation of the product, is therefore expected to give rise to eight 4-*O*-acetyl-1,5-anhydro-D-glucitol derivatives, namely, **1–8**. As a test of this procedure, samples of commercial *O*-methyl- and *O*-ethyl-cellulose having different degrees of substitution (d.s.) have been analyzed.



	R^1	R^2	R^3
1	Me	Me	Me
2	Me	Me	Et
3	Me	Et	Me
4	Et	Me	Me
5	Et	Et	Me
6	Et	Me	Et
7	Me	Et	Et
8	Et	Et	Et

RESULTS AND DISCUSSION

Shown in Fig. 1 are the gas–liquid chromatograms obtained when fully methylated *O*-ethylcellulose (upper) and fully ethylated *O*-methylcellulose (lower) were subjected to reductive cleavage in the presence of a mixture of Me_3SiOSO_2Me and $BF_3 \cdot Et_2O$ (as the catalyst). This catalyst mixture had been shown[8] to cleave, reductively, 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[9]. The numbered peaks were identified through comparison to independently synthesized standards[10] by chemical-ionization (NH_3) mass spectrometry (c.i.m.s.), electron-impact mass spectrometry (e.i.m.s.), and g.l.c. retention time. Peaks 1 and 8 were identified as the tri-*O*-methyl (**1**) and tri-*O*-ethyl (**8**) derivatives, respectively. Peaks 2–4 were those of the isomeric *O*-ethyl-di-*O*-methyl derivatives, namely, **3** (Peak 2), **2** (Peak 3), and **4** (Peak 4), whereas Peaks 5–7 were those of the isomeric di-*O*-ethyl-*O*-methyl derivatives, namely, **5** (Peak 5), **7** (Peak 6), and **6** (Peak 7). In addition to these expected products, traces of components were present which arose as a result of incomplete permethylation or perethylation. In the case of samples of per-*O*-ethylated *O*-methylcellulose, only traces of under-ethylated products were observed. For the permethylated *O*-ethyl-

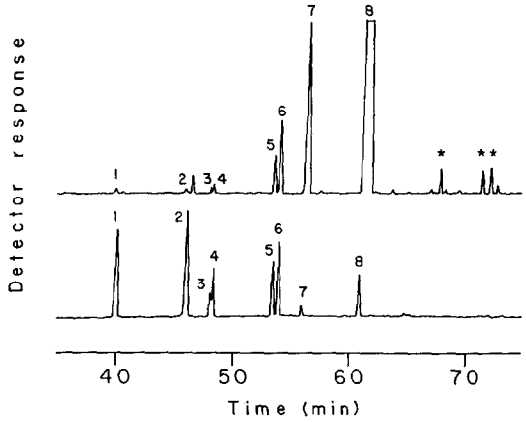


Fig. 1. Gas–liquid chromatograms of the anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated *O*-ethylcellulose, sample C (upper) and per-*O*-ethylated *O*-methylcellulose, sample B (lower). The numbered peaks were identified as follows: (1) **1**; (2) **3**; (3) **2**; (4) **4**; (5) **5**; (6) **7**; (7) **6**, and (8) **8**. The peaks indicated with an asterisk were the isomeric di-*O*-acetyl-1,5-anhydro-di-*O*-ethyl-D-glucitol derivatives arising as a result of incomplete methylation. Unnumbered peaks were not of carbohydrate origin.

cellulose sample, however, three uncharacterized di-*O*-acetyl-1,5-anhydro-di-*O*-ethyl-D-glucitol derivatives were observed in approximately equal proportions at retention times of 67.7, 71.4, and 72.1 min, but these accounted for only 4.1 mole % of the product.

Integration of all peaks, and correction for molar response[11,12] gave the mole fraction of each product (**1–8**) in each sample of *O*-methyl- and *O*-ethyl-cellulose

TABLE I

MOLE FRACTIONS OF PRODUCTS (COMPOUNDS **1–8**) DERIVED BY REDUCTIVE CLEAVAGE OF PER-*O*-ETHYLATED *O*-METHYLCELLULOSE AND PER-*O*-METHYLATED *O*-ETHYLCELLULOSE

Compound	*Sample*[a]		
	A	*B*	*C*
	Mole fraction[b]		
1	0.194	0.275	0.004
2	0.096	0.096	0.006
3	0.250	0.265	0.002
4	0.047	0.051	0.004
5	0.123	0.101	0.026
6	0.024	0.020	0.158
7	0.168	0.130	0.040
8	0.099	0.061	0.719
d.s.	1.68	1.96	2.66

[a]Sample A: *O*-methylcellulose, Henkel MC3000; B: *O*-methylcellulose, Henkel MC4000; C: *O*-ethylcellulose, Hercules 8659-C. [b]±0.004.

analyzed (see Table I). As expected, components that were derived as major products from per-*O*-ethylated *O*-methylcellulose were derived as minor products from per-*O*-methylated *O*-ethylcellulose, and *vice versa*. Qualitatively, the results obtained by this method (see Table I) are in full agreement with the results of other methods[1,2,4,5]; *i.e.*, the reactivities of the hydroxyl groups of cellulose toward methylation are OH-2 > OH-6 > OH-3, whereas toward ethylation, the reactivities are OH-6 > OH-2 > OH-3. Quantitatively, the results obtained by this method for *O*-methylcellulose (sample B) are in excellent agreement with those obtained for the same sample by Reuben[4] by n.m.r. spectroscopy. From the results obtained by Reuben, the mole fraction (in parentheses) predicted for compounds **1–8** are as follows: **1** (0.295); **2** (0.109); **3** (0.252); **4** (0.054); **5** (0.099); **6** (0.023); **7** (0.118); and **8** (0.054). With the exception of compound **1**, the mole fractions of these monomers are in agreement with these predicted values within one mole-percent. Incidentally, the two methods gave the same value (1.96) for the degree of substitution of sample B.

In spite of the excellent agreement between the two methods, the method reported herein offers some distinct advantages over the ^{13}C-n.m.r.-spectral method, wherein hydrolyzed or methanolyzed samples are analyzed. The primary advantage of the method reported herein is its simplicity, *i.e.*, the identities and mole fractions of the monomers are obtained simply by integration of the g.l.c. profile rather than by chemical-shift analysis and curve fitting of ^{13}C-n.m.r. spectra. The present method also offers the advantage that the positions of substitution of *O*-alkyl groups in both *O*-methylcellulose and *O*-ethylcellulose can be established by analysis of only 8 compounds, rather than 32 as in the ^{13}C-n.m.r.-spectral method. The sole disadvantage of the present method is that permethylation or perethylation of the sample, as appropriate, is required. Some difficulty was encountered in achieving complete methylation of the *O*-ethylcellulose sample, but even in the worst case, undermethylated products accounted for only 4% of the product. Although under-alkylation introduces a small error in the determination, this error could be eliminated by identification of the appropriate, reductive-cleavage products.

EXPERIMENTAL

General. — Reductive cleavage was performed by a modification of the procedure of Jun and Gray[8]. A 5-mg sample of per-*O*-alkylated polysaccharide was dissolved in 450 μL of a reagent solution (prepared immediately prior to use) consisting of 0.5M Et_3SiH, 0.5M Me_3SiOSO_2Me, and 0.1M $BF_3 \cdot Et_2O$ in dry CH_2Cl_2. The reaction was allowed to proceed for 4 h, and the products were isolated and acetylated in the usual way[8]. 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 25 min at 100°, and then programmed to 300° at 1°/min.

Preparation of methylsulfinyl carbanion. — Lithium methylsulfinyl carbanion (lithium dimsylate) was prepared by a modification of the procedure of Blakeney and Stone[13]. Cold (4°) methyllithium (10 mL, 1.4M in diethyl ether, Aldrich) was added in a stream of nitrogen *via* syringe to an equal volume of dry dimethyl sulfoxide (Me_2SO) contained in a small, round-bottomed flask. The mixture was stirred at room temperature until the ether had evaporated and the solution cleared (15 min), at which time the reaction was judged to be complete. The flask was capped with a rubber septum, evacuated, and purged (3 times) with N_2. The clear, pale-yellow lithium dimsylate solution (1.4M) was used immediately.

Per-O-alkylation of O-alkylcellulose samples. — Prior to alkylation, *O*-methylcellulose samples were dissolved in water (~1 mg/mL) by sonication using a Sonifer cell-disrupter (Heat Systems–Ultrasonics, Inc.), and the solutions lyophilized. *O*-Ethylcellulose samples were suspended in methanol (~1 mg/mL) by sonication, diluted with water to 0.2–0.3 mg/mL, and the solutions lyophilized.

Per-*O*-alkylation of the *O*-alkylcellulose samples was carried out by a modification of the procedure of Harris *et al.*[14]. The lyophilized polysaccharides were added to a small round-bottomed flask and dissolved, with stirring, in the minimal volume (usually 5 mg/mL) of dry Me_2SO. The flask was capped with a rubber septum, evacuated, and purged 3 times with N_2. Freshly prepared lithium dimsylate (6 equiv./D-glucosyl residue) was added *via* syringe at room temperature, and the solution was stirred for 40 min. A 0.1-mL aliquot of a solution of triphenylmethane in Me_2SO (1 mg/mL) was then added *via* syringe, and stirring was continued for 20 min to ensure that deprotonation was complete (as determined by the persistence of the red color of triphenylmethyl anion). Cold (0–4°) methyl iodide or ethyl iodide (6 equiv./D-glucosyl residue) was then added, and stirring was continued for 2 h. The excess of alkyl iodide was removed by bubbling N_2 through the reaction mixture for at least 15 min, and the deprotonation–alkylation cycle was repeated. A third deprotonation cycle was then performed as just described, with the exception that stirring was continued for 45 min after addition of the triphenylmethane solution. Final methylations and ethylations were allowed to proceed for 8–12 h, and 2 days, respectively. The reaction mixtures were then diluted with one volume of acetone, and dialyzed against running water for 12–18 h. The contents of the dialysis bag were filtered through paper, and the products were air-dried. Products were recovered from the filter paper by dissolution in CH_2Cl_2, and evaporation of the solution to dryness.

ACKNOWLEDGMENT

The authors thank Jacques Reuben (Hercules, Inc.) for providing a sample of *O*-ethylcellulose.

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