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DISTRIBUTION OF SUBSTITUENTS IN *O*-(2-HYDROXYETHYL)CELLULOSE

BENGT LINDBERG, ULF LINDQUIST*,
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

AND OLLE STENBERG
Berol Kemi AB, Box 851, S-444 01 Stenungsund (Sweden)

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ABSTRACT

The distribution of substituents in four samples of *O*-(2-hydroxyethyl)cellulose has been investigated by methylation analysis. One sample was commercial and the others were prepared under conditions analogous to those used industrially during the hydroxyethylation step in the manufacture of *O*-ethyl-*O*-(2-hydroxyethyl)cellulose. The results indicate that the distribution of substituents is similar to that expected for a homogeneous reaction.

INTRODUCTION

Ethyl(hydroxyethyl)cellulose [*O*-ethyl-*O*-(2-hydroxyethyl)cellulose] is a commercial product that is used, for example, as an adhesive and a thickener. The distribution of the substituents in this product is of interest since this affects the physical properties of its solutions. Several years ago, an attempt to determine this distribution by hydrolysis followed by fractionation and characterisation of the glucose ethers formed[1] was not successful because the complex mixture of products could not be resolved by carbon column chromatography. In the manufacture of ethyl(hydroxyethyl)cellulose, the cellulose is first hydroxyethylated by treatment of "alkali cellulose" with ethylene oxide. The product is then ethylated by treatment with ethyl chloride. Hydrolysis of a (hydroxyethyl)cellulose [*O*-(2-hydroxyethyl)cellulose] thus obtained yielded a mixture of glucose ethers which was resolved by carbon column chromatography[2]. When this work was done, we were not aware that 2-*O*-(2-hydroxyethyl)-D-glucose readily forms bicyclic glycosides on treatment with aqueous acid[3], and the extent of 2-substitution was therefore underestimated. The results demonstrated, however, that, for the sample investigated, the distribution of substituents was surprisingly close to that expected for a homogeneous reaction. Ramnäs and Samuelsson[4] fractionated the mixtures of glucose ethers, obtained on

* Present address: National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.

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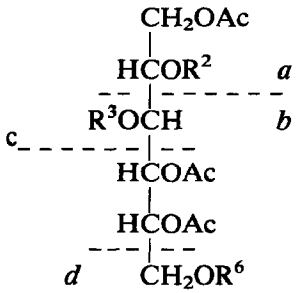
hydrolysis of various (hydroxyethyl)celluloses, by ion-exchange chromatography followed by g.l.c. of their trimethylsilyl derivatives. The analyses included the 1,2-*O*-ethylene-D-glucoses and confirmed that the distribution of substituents produced in the heterogeneous reaction was close to that expected for a homogeneous reaction. It was also found that the relative reactivities at the different positions depended upon the concentration of alkali used. At low concentration, HO-2, the most acidic hydroxyl group, showed the highest reactivity. At higher concentrations of alkali, the most accessible hydroxyl groups, namely, HO-6 and that in the $HOCH_2CH_2$ group, were the most reactive.

G.l.c. has also been used in similar studies of *O*-(2-hydroxyethyl)starch[5], *O*-(2-hydroxypropyl)guaran[6], and *O*-(carboxymethyl)guaran[7]. With the g.l.c. techniques now available, it seemed feasible to reinvestigate ethyl(hydroxyethyl)cellulose and we now report studies of the intermediate (hydroxyethyl)cellulose.

RESULTS AND DISCUSSION

Three samples of (hydroxyethyl)cellulose (HEC 1.3, 2.3, and 3.3) were prepared under conditions similar to those of the first step in the technical procedure for manufacturing ethyl(hydroxyethyl)cellulose, but using different amounts of ethylene oxide. A commercial sample (HEC 2.8) was also investigated. The samples, in methyl sulfoxide, were fully methylated, using the Hakomori procedure[8], the products were hydrolysed, and the resulting glucose derivatives were converted into the alditol acetate derivatives and analysed by g.l.c.–m.s. By methylating the (hydroxyethyl) cellulose, the formation of 1,2-*O*-ethyleneglucoses was avoided, and a mixture consisting exclusively of glucitol derivatives (1) was obtained.



$R = (CH_2CH_2O)_nMe$

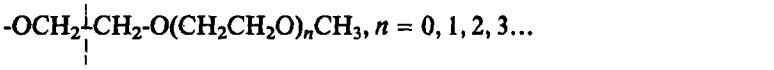
$n = 0, 1, 2, 3...$

(1)

The fragmentation of the glucitol ethers (1) in e.i.—m.s. follows the principles outlined for partially methylated alditol acetates[9]. Substitution at position 2 is determined from the major primary fragment *a*, m/z 117 + $n_2 \times 44$, where $n_2 = 1, 2, 3\cdots$, and n_2 is the number of CH_2CH_2O residues in R^2. The primary fragment *b*, m/z 233 + $n_3 \times 44$ + $n_6 \times 44$, gives the sum of such residues in R^3 and R^6, and gives a strong secondary fragment, $b - 120$, which is also of diagnostic value. The sum $n_2 + n_3$ is

determined from the rather weak primary fragment *c*, m/z 161 + $n_2 \times 44 + n_3 \times 44$, and the stronger secondary fragment, $c - 60$.

Fragment *d*, m/z 45 + $n_6 \times 44$, is obtained as indicated in **1**, but an identical fragment may be obtained from the structural element **2**, which gives a strong secondary fragment, $d - 30$, by elimination of formaldehyde. Despite the uncertain origin, fragment *d* gives some information on the number of consecutive (CH_2CH_2O) residues. Thus, the occurrence of ions with m/z 133 and 103 indicates at least two such residues at position 6 or at least three such residues at positions 2 or 3. Reduction of the glucose ethers by sodium borodeuteride, instead of sodium borohydride, produced alditols in which the *a*- and *c*-fragments were one mass unit larger and thus even. The use of this labelling sometimes facilitated the interpretation of the mass spectra.



(2)

When it was difficult to distinguish between two components from their mass spectra, some regularities in the retention times in g.l.c. were helpful. Ethers with 1–4 (CH_2CH_2O) residues appeared in well separated groups. For substances within a group, differing in the position of a single substituent, those with this substituent at position 3 or 6 had the lowest and the highest retention time, respectively. This observation, and the fact that position 3 had by far the lowest reactivity, facilitated the identification of some of the higher ethers.

The number of isomeric hydroxyethyl ethers increases with increase in the degree of substitution. For ($n_2 + n_3 + n_6$) = 0, 1, 2, 3, 4, and 5, the theoretical number of isomers is 1, 3, 6, 10, 15, and 21, respectively. All possible mono- and di-substituted derivatives were observed but, for the most highly substituted (hydroxyethyl)celluloses, at most eight trisubstituted and seven tetrasubstituted ethers were observed (see Table I). The presence of pentasubstituted derivatives was indicated, but they could not be fractionated and identified. An ether may escape detection because it occurs in low concentration and possibly also because it is not well separated from a component occurring in higher concentration. Among the ethers not observed, those with a substituent at position 3 preponderate. This means that the relative number of substituents at position 3 is underestimated and also that the degree of substitution of the (hydroxyethyl)cellulose, as calculated from the composition of the glucitol ethers, is too low.

The glucitol ethers observed, their retention times in g.l.c., and their molar percentages for the four different samples analysed are given in Table I. The molar percentages were calculated from the responses of the flame-ionisation detector and the relative molar response factors[10]. The factor for 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol was 0.74, and 0.10 was added for each CH_2CH_2O residue.

The average number (MS) of CH_2CH_2O residues per glucosyl residue in the (hydroyethyl)cellulose can be calculated from the composition of the mixture of

TABLE I

GLUCOSE ETHERS OBTAINED ON HYDROLYSES OF FULLY METHYLATED (HYDROXYETHYL)CELLULOSE SAMPLES

Ether[a]	T[b]	*Mole %*			
		HEC 1.3	*HEC 2.3*	*HEC 3.3*	*HEC 2.8*
H_0	1.00	62.8	44.0	31.2	5.1
H_3	1.66	1.4	1.6	1.4	0.8
H_2	1.70	9.4	10.6	8.0	4.8
H_6	1.81	14.2	15.7	13.2	5.8
H_{23}	2.41	1.5	2.1	2.1	4.0
H_{36}	2.55	0.8	1.0	1.2	1.6
H_{26}	2.60	2.4	3.7	3.8	7.5
H_{33}	2.62		0.5	0.6	0.8
H_{22}	2.67	2.4	3.2	3.2	4.4
H_{66}	2.81	4.0	8.0	9.6	3.7
H_{236}	3.28	0.4	0.9	1.7	9.1
H_{223}	3.35		0.9	1.7	4.5
H_{336}	3.37		0.9	2.9	1.5
H_{333}	3.53		0.3	0.7	0.6
H_{226}	3.55		1.3	1.6	9.1
H_{266}	3.58	0.5	2.0	2.4	4.3
H_{222}	3.65		1.1	1.5	2.3
H_{666}	3.80	0.2	2.2	4.7	1.6
H_{2336}	4.20			0.9	7.8
H_{2236}	4.25			1.1	5.0
H_{2366}	4.36			0.8	5.8
H_{2266}	4.64			2.0	4.9
H_{2226}	4.71			1.2	3.1
H_{2666}	5.13				1.9
H_{2222}	5.86			0.5	
H_{6666}	6.04			2.0	

[a] Subscripts: 2 connotes a $MeOCH_2CH_2$ substituent at position 2 in 1, 22 connotes $MeOCH_2CH_2OCH_2CH_2$ at position 2, 222 connotes $MeOCH_2CH_2OCH_2CH_2OCH_2CH_2$ at position 2, *etc.*
[b] Retention time of the corresponding alditol acetate relative to that of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol in g.l.c. (see Experimental).

glucose ethers and is given in Table II, together with the analytically determined value.

The results in Table I lend themselves to a simple, kinetic analysis. It is assumed that all residues in the "alkali cellulose" are equally accessible and that the relative reactivities at positions 2, 3, and 6 and of the 2-hydroxyethyl groups are constant and unaffected by substituents in adjacent positions. The fractions S_2 and S_{23} of the glucosyl residues in the (hydroxyethyl)cellulose substituted only at position 2 and at positions 2 and 3 are defined, respectively, by

$$S_2 = H_2 + H_{22} + H_{222}\cdots$$

$$S_{23} = H_{23} + H_{223} + H_{233}\cdots \textit{ etc.}$$ (see footnote to Table I),

TABLE II

RELATIVE RATE CONSTANTS FOR THE REACTIONS AT DIFFERENT POSITIONS DURING THE HYDROXYETHYLATION OF CELLULOSE

Sample	*MS*[a] (calc.)	*MS* (found)	k_2t	k_3t	k_6t	k_Ht	k_2:k_3:k_6:k_H
HEC 1.3	0.51	0.54	0.182	0.042	0.255	0.47	1:0.23:1.40:2.6
HEC 2.3	0.94	1.12	0.300	0.086	0.445	0.83	1:0.29:1.48:2.7
HEC 3.3	1.49	1.69	0.393	0.164	0.675	1.13	1:0.42:1.72:2.9
HEC 2.8	2.68	2.81	1.54	0.536	1.30	0.65	1:0.35:0.84:0.42

[a] Average number of CH_2CH_2O residues per glucosyl residue.

and the relative reactivities at positions 2, 3, and 6, and at the 2-hydroxyethyl groups are defined as k_2, k_3, k_6, and k_H. The rates decrease with time, as the amount of ethylene oxide decreases, but the concentration of alkali is constant, and the relative rates should therefore be unaffected. Thus, the alkylation may be treated as a first-order reaction and the "time" t and kt reflects the degree of alkylation achieved in each experiment. The values for k_2t, k_3t, and k_6t were calculated from

$$S_2 + S_{23} + S_{26} + S_{236} = (1 - e^{-k_2t})\ etc.$$

For the calculation of k_Ht, the equation for two consecutive first-order reactions gives

$$H_6 + H_{26} + H_{36} + H_{236} = \frac{k_6}{k_H - k_6}(e^{-k_6t} - e^{-k_Ht})$$

This value is given only for the further reaction at 6-*O*-(2-hydroxyethyl) groups, except for HEC 2.8, for which the reaction at the 2-*O*-(2-hydroxyethyl) groups is given. The reason for this is that the values calculated for the other positions are uncertain because of the lower degree of substitution at these positions.

The kt values, and the relative values, taking $k_2 = 1$, are given in Table II. The relative rate constants for HEC 1.3, HEC 2.3, and HEC 3.3 are fairly constant, considering the severe approximations used in the calculations. However, the values for the commercial sample, HEC 2.8, are dissimilar and indicate that it has been prepared under different conditions, most probably at a lower concentration of alkali.

In Table III, the observed values for S_0, S_2···are compared with those calculated, using the values for k_2t, k_3t, and k_6t given in Table II. There is reasonably good agreement between the observed and the calculated values, indicating that the accessibilities of the different glucosyl residues in the "alkali cellulose" used are fairly similar. If some of these residues were less accessible than the others, a different distribution of substituents would have been expected. Thus, the percentages of unsubstituted and trisubstituted glucosyl residues would have been considerably higher than the values calculated assuming a homogeneous reaction. This behaviour

TABLE III

OBSERVED AND CALCULATED VALUES FOR THE DISTRIBUTION OF SUBSTITUENTS[a] IN THE (HYDROXYETHYL)CELLULOSE SAMPLES

	HEC 1.3		*HEC 2.3*		*HEC 3.3*		*HEC 2.8*	
	Calc.	*Found*	*Calc.*	*Found*	*Calc.*	*Found*	*Calc.*	*Found*
S_0	61.9	62.8	43.6	43.8	29.3	31.2	3.4	5.1
S_2	12.3	11.8	15.2	14.9	14.0	13.3	12.5	11.5
S_3	2.7	1.4	3.9	2.4	5.2	2.7	2.4	2.2
S_6	18.0	18.4	24.4	26.1	28.1	29.5	9.1	11.1
S_{23}	0.5	1.5	1.4	3.0	2.5	3.8	8.9	8.5
S_{26}	3.6	2.9	8.5	7.0	13.5	11.0	33.5	30.8
S_{36}	0.8	0.8	2.2	1.9	5.0	4.1	6.5	3.1
S_{236}	0.2	0.4	0.8	0.9	2.4	4.5	23.7	27.7

[a] S_2 connotes the fraction of glucosyl residues substituted at position 2, *etc.*

was observed on heterogeneous methylation of "alkali cellulose" with methyl sulfate[11], when considerable differences in accessibility were observed.

EXPERIMENTAL

Preparation of O-(2-hydroxyethyl)cellulose. — Cotton-linters cellulose was ground to a particle size of <0.5 mm. A reactor was charged with cellulose (1 part by wt.), then evacuated (40 mmHg), and filled with nitrogen. This procedure was repeated three times. Aqueous sodium hydroxide (0.54 part, 0.457 g of sodium hydroxide/mL), corresponding to 1 mol of sodium hydroxide per glucosyl residue, was added with stirring. Stirring was continued for 5 min and ethyl chloride (2 parts) was added. After stirring for a further 30 min, ethylene oxide (0.362, 0.614, or 0.904 part, corresponding, respectively, to 1.33, 2.26, and 3.33 mol per glucosyl residue) was added. Stirring was continued, and the temperature was raised to 43° and kept thereat for 1, 1.5, or 2 h. The ethyl chloride was then distilled off, and the product was suspended in acetone–water and neutralised with acetic acid. The (hydroxyethyl)cellulose was isolated by centrifugation and ground to a particle size of <2 mm.

In this procedure, the ethyl chloride acts as solvent and no ethylation occurs, as evident from the analysis of the methylated, hydrolysed product. In the preparation of ethyl(hydroxyethyl)cellulose, the temperature was raised to 105° after completion of the hydroxyethylation, and kept thereat for 1 h. The product was then isolated as described above.

Analysis of ether groups. — The (hydroxyethyl)- or ethyl(hydroxyethyl)-cellulose was extracted with acetone in a Sohxlet extractor for 4 h, in order to remove any oligo- or poly-(ethylene glycol). A mixture of the sample (15 mg) and hydrogen

bromide in acetic acid (0.5 mL, 0.4 mg of HBr/mL) was kept in a sealed glass tube for 3 h at 150°. The tube was cooled (solid CO_2) and the contents were mixed with carbon disulfide (2 mL, containing 1.2 mg of 1-bromohexane as the internal standard). The phases were separated, and the proportions of ethyl bromide, 1-bromohexane, and 1,2-dibromoethane were determined by g.l.c. on a column of 10% Apeizon M on Chromosorb W; temperature programme: 40° for 2 min, → 130° at 3°/min.

Methylation analysis[12]. — The samples of (hydroxyethyl)cellulose were not readily soluble in methyl sulfoxide. In order to improve the solubility, dilute aqueous solutions were freeze-dried, and each residue (1–2 mg) was mixed with methyl sulfoxide (2–4 mL) in a serum vial containing a small magnet. The vial was closed and flushed with dry nitrogen *via* two injection needles, and the mixture was stirred for 8 days at room temperature. Each day it was sonicated and kept for 30 min at 40°. 2M Sodium methylsulfinylmethanide in methyl sulfoxide (1–2 mL) was then added and the mixture was stirred overnight at room temperature. Triphenylmethane was added to an aliquot of the reaction mixture in order to confirm an excess of carbanion[13]. The solution was cooled in a refrigerator, methyl iodide (1–2 mL) was added slowly, and the mixture was stirred overnight at room temperature. The serum vial was opened and excess of methyl iodide removed by flushing with air at room temperature. The methylated product was isolated by reversed-phase h.p.l.c. on a Sep-Pak C_{18}-cartridge[14].

A solution of the methylated product in 2M trifluoroacetic acid (3 mL) was kept in a sealed tube for 2 h at 120°. The acid was then evaporated, the residual mixture of glucose ethers was reduced with sodium borohydride, and the products were acetylated with acetic anhydride–pyridine, following standarad procedures, and subjected to g.l.c. and g.l.c.–m.s.

G.l.c. was performed on a Hewlett–Packard 5830A instrument, fitted with a flame-ionisation detector, with hydrogen as the carrier gas. The injection port and f.i.d. temperatures were 250°. All separations were performed on a Hewlett–Packard Ultra 2 (cross-linked 5% phenyl methyl silicone) fused silica, capillary column (25 m × 0.20 mm i.d.). Temperature programme: 2 min at 150°, → 230° at 3°/min, 230° for 20 min.

G.l.c.–m.s. was performed on a Hewlett–Packard 5790-5970 system with helium as the carrier gas; temperature programme: 3 min at 150°, → 250° at 3°/min, 250° for 20 min.

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REFERENCES

1 I. CROON AND B. LINDBERG, *Sven. Papperstidn.*, 59 (1956) 794–799.
2 I. CROON AND B. LINDBERG, *Sven. Papperstidn.*, 60 (1957) 82–84.
3 J. E. HÖÖK AND B. LINDBERG, *Acta Chem. Scand.*, 22 (1968) 2157–2160.
4 O. RAMNÄS AND O. SAMUELSSON, *Sven. Papperstidn.*, 71 (1968) 674–678.
5 O. LARM, K. LARSSON, AND O. THEANDER, *Staerke*, 33 (1981) 240–244.
6 M. MCNEIL AND P. ALBERSHEIM, *Carbohydr. Res.*, 131 (1984) 131–137.
7 M. MCNEIL, W. SZALECKI, AND P. ALBERSHEIM, *Carbohydr. Res.*, 131 (1984) 139–148.
8 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
9 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 5 (1967) 433–440.
10 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.*, 40 (1975) 217–225.
11 I. CROON AND B. LINDBERG, *Sven. Papperstidn.*, 60 (1957) 843–849.
12 P.-E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–75.
13 H. RAUVALA, *Carbohydr. Res.*, 72 (1979) 257–260.
14 T. J. WAEGHE, A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 123 (1983) 281–304.