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ANALYSIS OF THE CARBON-13 N.M.R. SPECTRUM OF HYDROLYZED *O*-(CARBOXYMETHYL)CELLULOSE: MONOMER COMPOSITION AND SUBSTITUTION PATTERNS

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ABSTRACT

The effects of *O*-carboxymethylation at each of positions 2, 3, and 6 on the ^{13}C chemical shifts of glucose have been used to assign first the ^{13}C-n.m.r. spectra of 2,3-, 2,6-, and 3,6-di-*O*-(carboxymethyl)glucose and 2,3,6-tri-*O*-(carboxymethyl)-glucose and then the spectrum of hydrolyzed *O*-(carboxymethyl)cellulose (CMC). Quantitative analysis of the latter spectrum yields the composition of CMC in terms of the mole fractions of the eight component monomeric residues. The results on the monosaccharide composition for a series of samples having degrees of substitution in the range 0.55–2.17 are described well by Spurlin's statistical kinetic model for the arrangement of substituents (substitution pattern) in cellulose derivatives. The model assumes that the substitution pattern is governed by the relative rate-constants for reaction of the three hydroxyl groups in the glucose residue. The values of the constants determined in this work are $k_2:k_3:k_6 = 2.14:1.00:1.58$.

INTRODUCTION

The potential of ^{13}C-n.m.r. spectroscopy for the characterization of cellulose ethers was demonstrated in 1977 by Parfondry and Perlin[1]. Improved n.m.r. instrumentation has enabled us to realize this potential for complete assignment of the ^{13}C-n.m.r. spectrum of hydrolyzed *O*-(carboxymethyl)cellulose (CMC). Furthermore, we were able to obtain a detailed analysis of the hydrolysis mixture in terms of the mole fractions of the component monosaccharides.

The sodium salt of CMC is the most widely used water-soluble derivative of cellulose, and has applications in the food, cosmetic, pharmaceutical, paper, and petroleum-producing industries. It is made by the reaction of alkali cellulose with chloroacetate[2]. The reaction conditions are usually chosen such that the resulting product has an average degree of substitution (d.s.) in the range 0.4–1.3. Therefore, the three hydroxyl groups on the glucose residue of cellulose are, on the average, only partly substituted. Thus, CMC may be viewed as a copolymer of eight monomers: the residues of glucose, the three *O*-(carboxymethyl)glucoses (2-, 3-, and 6-), the three di-*O*-(carboxymethyl)glucoses (2,3-, 2,6-, and 3,6-), and 2,3,6-tri-*O*-(carboxy-

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methyl)glucose. The distribution of substituents and the monomer composition of such a cellulose derivative have been problems of long-standing interest[3]. In 1939, Spurlin proposed a statistical model that assumes, *inter alia*, that the substitution pattern and the monomer composition are governed by the relative rate-constants for reaction of the three hydroxyl groups on the glucose residue[4]. However, attempts to test the applicability of the model to CMC have resulted in controversial conclusions, in particular with regard to the relative reactivity of the three hydroxyl groups[5-8].

Because of the inherent intra- and inter-chain heterogeneity of polymeric CMC, all analytical procedures for the determination of its composition involve the acid hydrolysis of the material[5-8]. In this way, a mixture of 16 monosaccharide species is formed, taking into account the α and β anomers of each of the eight monomeric sugars. Thus, the aliphatic region of the ^{13}C-n.m.r. spectrum of hydrolyzed CMC is composed of 120 lines of different intensities. For the analysis of this spectrum, we obtained and assigned the spectra of the individual component monosaccharides. Then a series of eleven CMC samples having d.s. in the range 0.55–2.17 were analyzed. The resulting mole fractions of the monomeric sugars and other related quantities were fitted to Spurlin's model. As shown in the last section of this paper, the model affords a very good description of the results.

EXPERIMENTAL

Samples. — Aqueous solutions of the separated *O*-(carboxymethyl)-D-glucoses were kindly provided by Dr. K. B. de Roos. The 2,6- and 2,3-di-*O*-(carboxymethyl)-glucoses were received as a 2:1 mixture. The only treatment of these samples was acidification to pH 1 and addition of a drop of methanol, which served as a chemical-shift reference.

The choice of acid for hydrolysis of CMC was dictated by the desire to minimize the electrolyte content of the samples, as an excess of electrolyte interferes with the acquisition of ^{13}C-n.m.r. spectra[9,10]. Hydrolysis was achieved by incubating a solution in 2.4M perchloric acid for about a week at 65°. The resulting solution was then made neutral with dilute potassium hydroxide and the precipitate of potassium perchlorate removed by filtration. The water was removed in a rotary evaporator and the residue dissolved in D_2O to a concentration of ~5% w/w. The pH was brought to 1 by using DCl. A small amount of methanol was added, its methyl resonance served as an internal reference for ^{13}C chemical-shift measurements. It was assigned a chemical shift of 49.00 p.p.m. relative to tetramethylsilane.

Spectra. — Carbon-13 n.m.r. spectra were acquired from samples in 12-mm tubes at a probe temperature of 27° on a Nicolet NT-360WB spectrometer operated at 90.55 MHz in the Fourier-transform mode. Spectra of hydrolyzed CMC, which required accurate quantitation[11], were obtained by using a flip angle of 60°, and a repetition delay-time of 3.5 s, with the proton broad-band decoupler switched on only during acquisition. Gated decoupling was necessary in order to minimize line-

broadenings due to inhomogeneous heating of the samples[9,10,12]. Overnight spectral accumulations of ~9000 transients were usually required in order to obtain satisfactory spectra with 5% (w/w) solutions.

The spectra were deconvoluted by using the supplied Nicolet program NTCCAP. In this process, Lorentzian lines were constructed and matched with the experimental ones until the difference between the two spectra was minimized. The r.m.s. deviations between the experimental and calculated spectra were <1%. The integrals of the curve-resolved spectrum were then printed out. The accuracy of this approach may be estimated from the integrated areas of the monoprotonated carbons 1, 2, 3, 4, and 5 of glucose. These were found to be in the ratio 1.00:0.99:1.03:1.01:0.97, that is, the accuracy is ±3%.

RESULTS AND DISCUSSION

Spectra of the monosaccharides. — The assignments for all of the monosaccharide derivatives are listed in Table I. The glucose assignments were made according to the literature[1]. The spectra of the mono(carboxymethyl)glucoses were assigned according to the expected effects of *O*-substitution on ^{13}C chemical shifts: large (7–11 p.p.m.) downfield shifts for the carbon atoms α to the substituent and smaller (1–2 p.p.m.) upfield shifts for the carbon atoms β to the substituent[13]. These effects are clearly seen by comparing the shifts of 2-*O*-(carboxymethyl)glucose with those of glucose. The signals of C-2 of both anomers are now in the 79–86 p.p.m. spectral region, and the signals of C-3 are shifted slightly upfield. In addition, signals from the CH_2 carbon atom of the substituent are apparent in the 68–70 p.p.m. region. The signals for the carboxyl group usually appear at ~180 p.p.m. and were outside the spectral region of interest. Except for minor discrepancies (reversal of C-3 and C-5 resonance positions of 3-*O*-carboxymethyl-α-glucose and the relative positions of the CH_2 peaks) attributable to the low pH employed in this work, our assignments are in agreement with those of Parfondry and Perlin[1].

The effects of monocarboxymethylation on the ^{13}C chemical shifts of glucose are summarized in Table II. The magnitudes of these effects were used in the assignment of the spectra of the di- and tri-*O*-(carboxymethyl)glucoses. It was assumed that the effects are additive. Additivity of substituent effects is a general phenomenon in ^{13}C-n.m.r.[14]. The chemical shifts calculated from the shift of glucose and the appropriate sum of values from Table II are listed in Table I in parentheses under the experimental values. The standard deviation between the two sets of values is 0.09 p.p.m.

The power of our method of assignment was demonstrated by the spectrum of a sample labeled originally as 2,6-di-*O*-(carboxymethyl)glucose. The spectrum showed a set of major peaks as well as a number of smaller signals. However, a signal was also observed at 60.5 p.p.m., which arises from unsubstituted C-6. In the 79–86 p.p.m. region there were four minor peaks, in addition to the two major peaks attributable to C-2 of the α and β anomers of 2,6-*O*-(carboxymethyl)glucose. One

TABLE I

CARBON-13 CHEMICAL SHIFTS[a] OF GLUCOSE AND THE *O*-(CARBOXYMETHYL)GLUCOSES IN ACIDIFIED AQUEOUS SOLUTIONS[b]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_2-2	CH_2-3	CH_2-6
α-Glucose	92.13	71.57	72.86	69.78	71.50	60.78			
β-Glucose	95.96	74.24	75.85	69.73	75.96	60.89			
2-*O*-Carboxymethyl-α-glucose	90.09	80.37	72.12	69.77	71 31	60.74	67.54		
2-*O*-Carboxymethyl-β-glucose	95.58	83.37	75.08	69.77	75.85	60.78	68.97		
3-*O*-Carboxymethyl-α-glucose	92.06	71.13	83.02	69.36	71.41	60.60		69.25	
3-*O*-Carboxymethyl-β-glucose	95.86	73.74	85.52	69.36	75.68	60.71		69.25	
6-*O*-Carboxymethyl-α-glucose	92.09	71.54	72.78	69.79	70.32	70.32			68.37
6-*O*-Carboxymethyl-β-glucose	96.01	74.20	75.75	69.79	74.74	70.32			68.37
2,3-Di-*O*-carboxymethyl-α-glucose	90.25	79.96	82.24	69.82	71.39	60.65	68.78	69 09	
	(90.02)	(79.93)	(82.28)	(69.37)	(71.23)	(60.56)			
2,3-Di-*O*-carboxymethyl-β-glucose	95.67	82.97	84.88	69.82	75.64	60.77	68 78	69.09	
	(95.48)	(82.87)	(84.75)	(69.40)	(75.57)	(60.60)			
2,6-Di-*O*-carboxymethyl-α-glucose	90.06	80.28	71.90	69.82	70 12	70.12	68.78		68.31
	(90.05)	(80.34)	(72.04)	(69.80)	(70.13)	(70.28)			
2,6-Di-*O*-carboxymethyl-β-glucose	95.67	83.31	74.93	69.82	74.63	70.12	68.78		68.31
	(95.63)	(83.33)	(74 98)	(69.83)	(74.63)	(70.21)			
3,6-Di-*O*-carboxymethyl-α-glucose	92.08	71.03	82.86	69.42	70.21	70.21		69.28	68.13
	(92.02)	(71.10)	(82.94)	(69.37)	(70.23)	(70.14)			
3,6-Di-*O*-carboxymethyl-β-glucose	95.86	73.64	85.36	69.42	74.47	70.21		69.28	68 13
	(95.91)	(73.70)	(85.42)	(69.42)	(74.46)	(70.14)			
2,3,6-Tri-*O*-carboxymethyl-α-glucose	90 21	79.91	82.10	69.22	70.18	70 18	68 09	69.94	68 56
	(89.98)	(79.90)	(82.20)	(69.48)	(70.05)	(70.10)			
2,3,6-Tri-*O*-carboxymethyl-β-glucose	95.64	82.89	84.69	69.52	74.43	70.18	69.22	69 94	68.56
	(95.53)	(82.83)	(84.65)	(69.46)	(74 35)	(70.03)			

[a]The peak of methanol served as internal standard and was assigned a chemical shift of 49.00 p p m. [b]Values in parentheses were calculated assuming additivity of substituent effects (see Table II)

TABLE II

SUBSTITUENT EFFECT OF CARBOXYMETHYLATION ON THE CARBON-13 CHEMICAL SHIFTS[a] OF GLUCOSE IN ACIDIFIED AQUEOUS SOLUTIONS

Position	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
2α	−2.04	8.80	−0.74	0.01	−0.19	−0.04
2β	−0.38	9.13	−0.77	0.04	−0.11	−0.11
3α	−0.07	−0.44	10.16	−0.42	−0.09	−0.18
3β	−0.10	−0.50	9.67	−0.37	−0.28	−0.18
6α	−0.04	−0.03	−0.08	0.01	−1.18	9.54
6β	+0.05	−0.04	−0.10	0.06	−1.22	9.43

[a]Positive values indicate downfield shifts.

of the monosaccharides expected to show four peaks in this region is 2,3-di-*O*-(carboxymethyl)glucose. The positions of these four peaks are different from those of 2,3,6-tri-*O*-(carboxymethyl)glucose. Thus, the identification is positive. This was a fortunate finding, as a sample of pure 2,3-di-*O*-(carboxymethyl)glucose was not available.

The spectrum of hydrolyzed CMC. — The ^{13}C-n.m.r. spectrum of the hydrolyzate of a CMC sample having d.s. 1.26 is shown in Fig. 1. The spectral region 79–86 p.p.m. is the richest in analytical information. Twelve of the 16 monosaccharide species

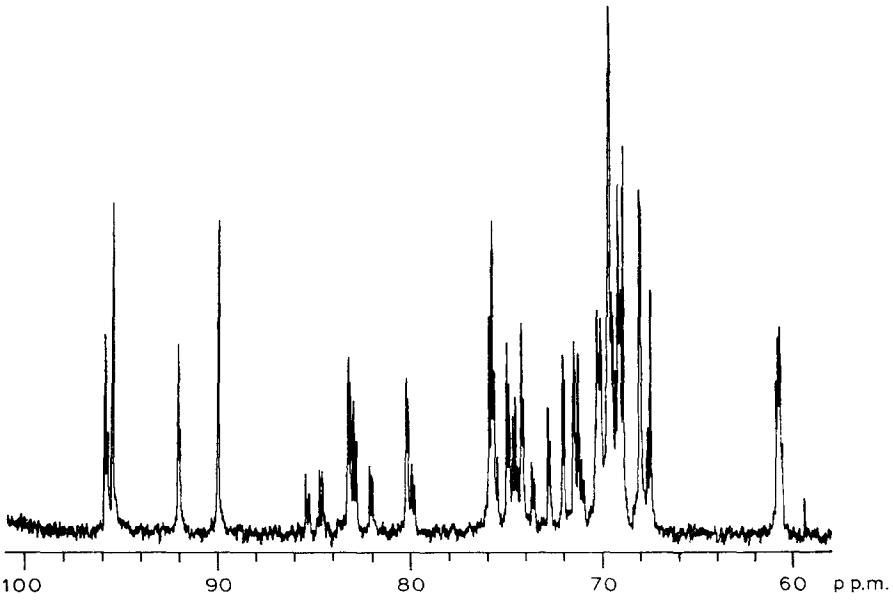

Fig. 1. The ^{13}C-n.m.r. spectrum of a hydrolyzed sample of *O*-(carboxymethyl)cellulose having d.s. 1.26. The small peak at 59.4 p.p.m. was caused by a glycolic acid impurity.

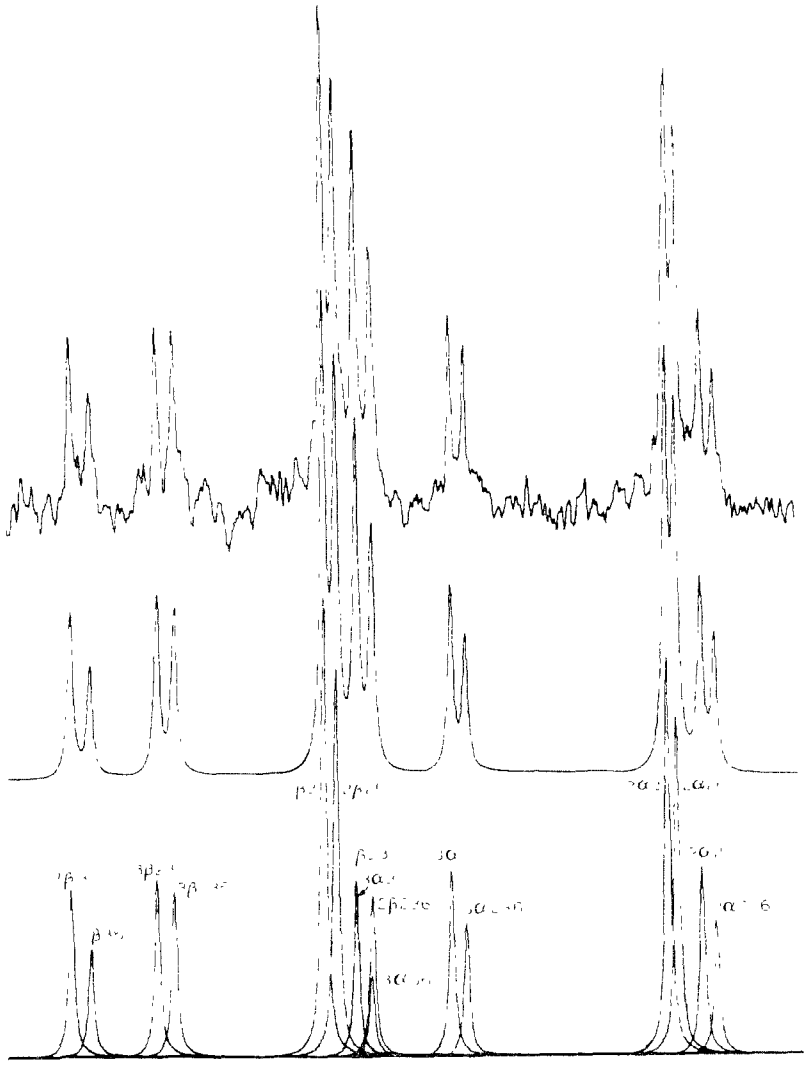

Fig. 2. Expanded 79–86 p.p.m. spectral region of the ^{13}C-n.m.r. spectrum of hydrolyzed CMC (see Fig. 1), experimental (top), calculated (middle), and curve-resolved (bottom).

(see Table I) present in the mixture have either their C-2 or C-3 (or both) resonances in this region. Only glucose and 6-*O*-(carboxymethyl)glucose are missing. With the exception of two overlaps (C-3 of 3-*O*-carboxymethyl-α-glucose, C-2 of 2,3-di-*O*-carboxymethyl-β-glucose and C-3 of 3,6-di-*O*-carboxymethyl-α-glucose, and C-2 of 2,3,6-tri-*O*-carboxymethyl-β-glucose), the lines are resolved sufficiently for accurate quantitation. The other spectral region used in the quantitative analysis is 71.9–76 p.p.m., which includes resonances of all of the monosaccharides. The expanded forms of these two spectral regions are shown in Figs. 2 and 3, respectively. Also shown in these Figs. are the calculated spectra and the curve-resolved lines, together with their

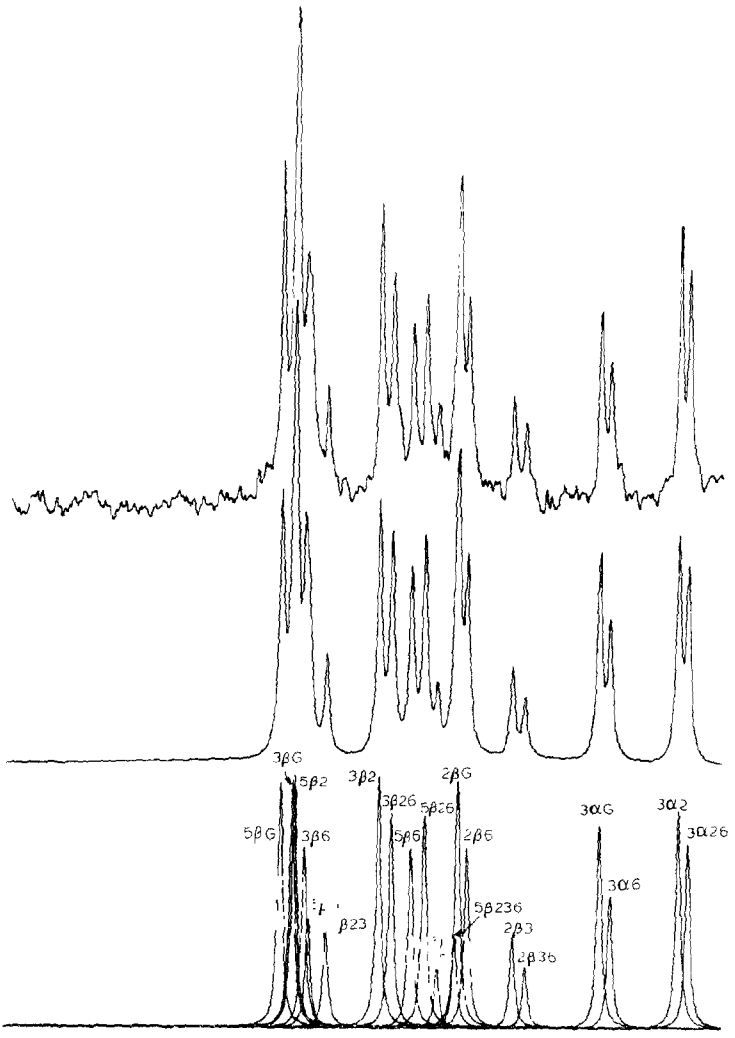

Fig. 3. Expanded 71.9–76 p.p.m. spectral region of the ^{13}C-n.m.r. spectrum of hydrolyzed CMC (see Fig. 1): experimental (top), calculated (middle), and curve-resolved (bottom).

assignments. The integral of either C-2 or C-3 of each species was used to calculate the respective mole fraction in the mixture.

Monomer composition and substitution patterns. — The quantitative spectral-analysis of hydrolyzed CMC samples yielded the mole fractions $s_0 = C_0$ (glucose), s_i (monosubstituted glucoses), s_{ij} (disubstituted glucoses), and $s_{236} = C_3$ (the trisubstituted glucose). The results obtained with 11 samples are graphically displayed as a function of d.s. in Figs. 4, 5, and 6. Fig. 4 shows also the fractions of total monosubstituted glucoses, $C_1 = s_2 + s_3 + s_6$, and disubstituted glucoses, $C_2 =$

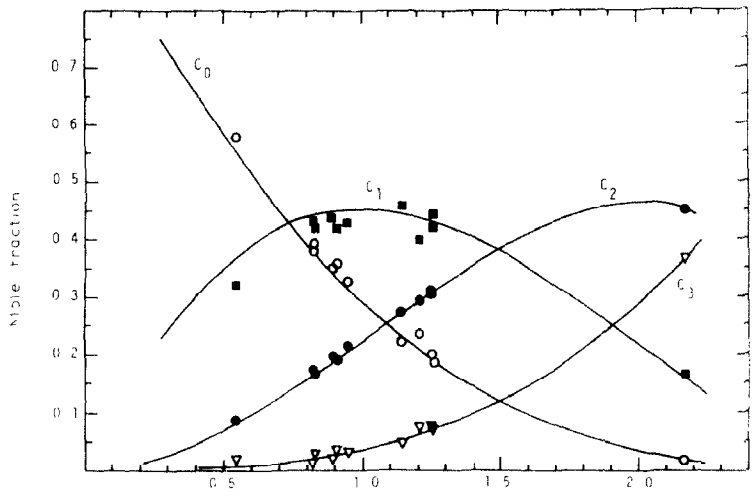

Fig. 4. The mole fractions of glucose (C_0), the *O*-(carboxymethyl)glucoses (C_1), the di-*O*-(carboxymethyl)glucoses (C_2), and the 2,3,6-tri-*O*-(carboxymethyl)glucose (C_3) in hydrolyzed CMC plotted as a function of d.s. The curves are calculated (see text).

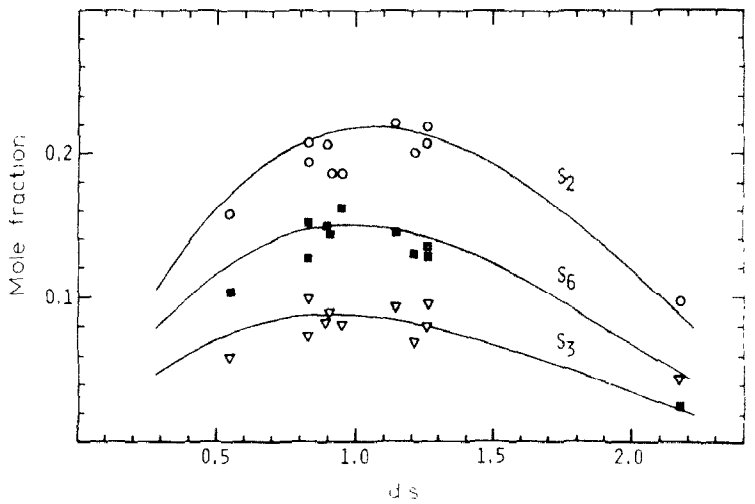

Fig. 5. The mole fraction of 2-, 3-, and 6-*O*-(carboxymethyl)glucose in hydrolyzed CMC plotted as a function of d.s. The curves are calculated (see text).

$s_{23} + s_{26} + s_{36}$. The fractional degree of substitution, x_i, at each of the three positions on the glucose residue and the average degree of substitution, (d.s.) were calculated from the following defining equations: $x_2 = s_2 + s_{23} + s_{26} + s_{236}$, $x_3 = s_3 + s_{23} + s_{36} + s_{236}$, and $x_6 = s_6 + s_{26} + s_{36} + s_{236}$; where d.s. $= x_2 + x_3 + x_6$. The x_i values are plotted as a function of d.s. in Fig. 7.

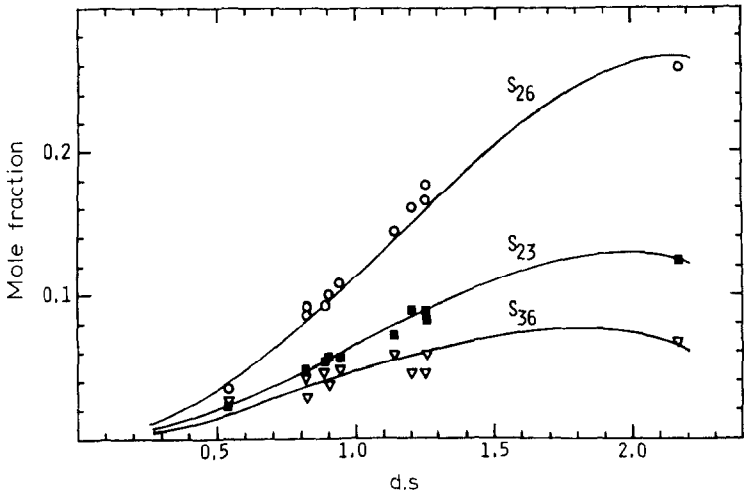

Fig. 6. The mole fraction of 2,3-, 2,6-, and 3,6-di-*O*-(carboxymethyl)glucose in hydrolyzed CMC plotted as a function of d.s. The curves are calculated (see text).

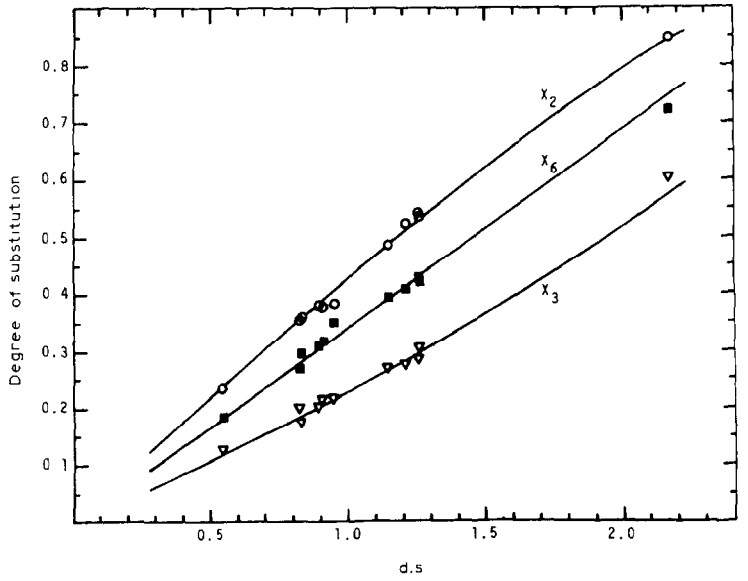

Fig. 7. The average degree of carboxymethylation at each of the three positions in the glucose residue plotted as a function of d.s. The curves are calculated (see text).

Spurlin proposed two statistical models for the arrangement of substituents in cellulose derivatives[4]. The models assumed that the relative reactivities of the three hydroxyl groups in the glucose residue are independent of the d.s. of the cellulose chain as a whole or of the state of substitution at other positions within the same residue. For ether formation in particular, it is further assumed that the extent of

substitution is governed by the relative rates of reaction of the hydroxyl groups in a first-order process*. With these assumptions, a set of simple rate-equations has been derived[4]. From them the monomer composition of CMC may be calculated as a function of a time parameter, B, related to the duration of the reaction. Of course, the relative values of the first-order rate constants, k_2, k_3, and k_6, which are characteristic of the process as a whole, must be determined.

In order to summarize conveniently Spurlin's equations[4], we define the operative quantities:

$$p_i = e^{-Bk_i}, \qquad i = 2, 3, \text{ or } 6, \tag{1}$$

This equation defines the probability of having an unsubstituted hydroxyl group in position i. The probability of having a substituent at the same position is the fractional degree of substitution. It is given by

$$x_i = 1 - p_i \tag{2}$$

The other quantities of interest may now be derived from p_i and x_i on the basis of simple statistical considerations. Thus, the mole fraction of unsubstituted glucose residues is given by the product of the probabilities of having unsubstituted hydroxyl groups at each of the three positions:

$$s_0 = p_2 p_3 p_6. \tag{3}$$

The mole fraction of residues monosubstituted at position i is given by the product of the probabilities of having a substituent at that position (x_i) and the probabilities of having unsubstituted hydroxyl groups at the remaining positions:

$$s_i = x_i p_j p_k. \tag{4}$$

Similarly, for the disubstituted residues:

$$s_{ij} = x_i x_j p_k. \tag{5}$$

Finally, the mole fraction of trisubstituted glucosyl residues is given by the product of the probabilities of having a substituent at each one of the three positions:

$$s_{236} = x_2 x_3 x_6. \tag{6}$$

It is convenient to reduce equations 2 and 3 to the following linear forms:

$$\ln(1 - x_i) = Bk_i, \tag{7}$$

and

$$-\ln s_0 = B(k_2 + k_3 + k_6) \tag{8}$$

*A parallel model describing the products of esterification assumes that the extent of substitution is governed by an equilibrium. This model has recently been applied successfully to analyze ^{13}C-n.m.r. data on nitrocellulose[15]. It can be shown that the equilibrium model is inadequate in our case.

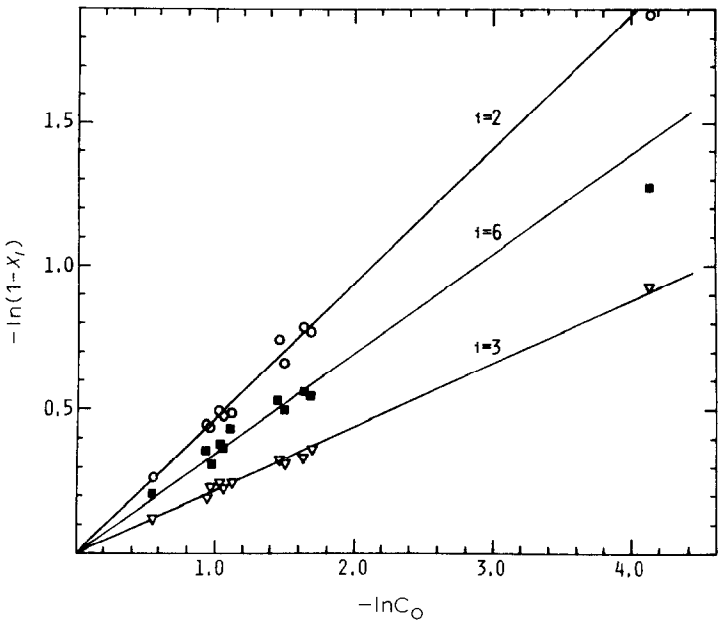

Fig. 8. Logarithmic plots of the mole fractions of unsubstituted hydroxyl groups against the mole fraction of unsubstituted glucose (see Eqn. *7* and *8*). The slopes of the lines are summarized in Table III.

TABLE III

RELATIVE FIRST-ORDER RATE CONSTANTS OF CARBOXYMETHYLATION OF CELLULOSE

i	$k_i/(k_2 + k_3 + k_6)$	k_i/k_2	k_i/k_3	k_i/k_6
2	0.470 ±0.030	1.00	2.14	1.35
3	0.220 ±0.008	0.47	1.00	0.63
6	0.348 ±0.041	0.74	1.58	1.00

TABLE IV

COMPARISON OF RELATIVE RATE-CONSTANTS FOR CARBOXYMETHYLATION OF CELLULOSE

Reference	$k_2:k_3:k_6$
This work	2.14:1.00:1.58
Ho and Klosiewicz[8]	2.0:1.0:1.5
Buytenhuys and Bonn[7]	2.5:1.0:1.8
Croon and Purves[6]	2:1:2.5
Timell and Spurlin[5]	1:1:2

The experimental data may now be plotted as $\ln(1 - x_t)$ versus $\ln s_w$. Provided the model holds, the plots should be linear with a slope of $k_t/(k_2 + k_3 + k_6)$. Such plots are shown in Fig. 8. As may be seen in Fig. 8 the plots are linear, indicating that the model is closely obeyed. The slopes and the relative first-order rate constants obtained from the ratios of the slopes are summarized in Table III. The uncertainties were calculated from the standard deviations.

The procedure used to construct theoretical curves for the monosaccharide fractions and related quantities is as follows. First, d.s. values were calculated for a set of B values and a plot of B against d.s. was constructed. Then, this plot was used to read the value of B corresponding to a given d.s. value of interest. The curves in Figs. 4–7 were calculated in this manner by using the relative rate-constants given in Table III. These plots convincingly demonstrate the excellent conformity of the data to the model. In particular, there is no indication of modification of the reactivity of one hydroxyl group upon substitution of another one in the same glucose residue. Such modifications would have markedly affected the behavior of the data for the disubstituted glucoses (C_2 in Fig. 4, s_{t_1} in Fig. 6) and for the trisubstituted glucose (C_3 in Fig. 4).

The relative rate-constants determined in this work are compared in Table IV with published results. Our findings are in good agreement with the recent results of Ho and Klosiewicz, obtained by proton n.m.r. spectroscopy[8] and with those of Buytenhuys and Bonn, obtained by gas chromatography and mass-spectrometric identification of the silylated monosaccharides[7]. We have not attempted to investigate the origin of the discrepancies with the other two works[5,6]. It should be pointed out, however, that in both of these cases the calculations assumed interference of the substitutent at one position with the reactivity of the neighboring hydroxyl group.

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