exposed to air. The principal values of the g tensor for both radicals are 2.0349, 2.0069, and 2.0032 at 77 K. Extra transitions are observed due to motional averaging.

2. The relative intensity of and the lineshapes from these radicals are not sensitive to the degrees of crystallinity of the polymer in the range 0.430–0.817.

3. The more stable peroxy radical is a chain radical whose precursor is the midchain alkyl radical $-CH_2\dot{C}(CH_3)CH_2-$.

4. The temperature dependence of the ESR spectra from the mobile peroxy radical is simulated well by assuming a chain axis rotation with 180° jumps. The angles between the principal values of the g tensor and the chain axis are 50°, 97°, and 41° for g_1, g_2, and g_3, respectively. Likely precursors for this peroxy radical are the propagating radicals $-CH_2\dot{C}H(CH_3)$ and $-CH_2CH(CH_3)CH_2^{\bullet}$.

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^{13}C NMR Study of the Selectivity in the Modification of Dextran with Ethyl Chloroformate

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ABSTRACT: The selectivity of the reaction of dextran with ethyl chloroformate in the homogeneous phase has been studied by ^{13}C NMR. The analysis of spectra of the anhydroglucose, oxymethylene, and carbonyl carbons shows that the hydroxyl group at position C-2 is selectively substituted and that the reactivity of individual secondary hydroxyls decreases in the order C-2 > C-4 > C-3. The results obtained are explained by considering the formation of intramolecular hydrogen bonding between the hydroxyl at C-2 and the axial anomeric oxygen as well as between the hydroxyl at C-4 and the carbonyl group of the ethyl carbonate at the C-2 position on the adjacent anhydroglucose unit.

Introduction

It is well-known that the degree of substitution (DS) of polysaccharides may have important effects on their behavior and properties. However, derivatives with similar DS values may have different substituent distributions. The difference in the relative DS at individual hydroxyl groups arises from the fact that three hydroxyl groups at the anhydroglucose residue may differ in reactivity.[1-3] The physical, chemical, and biochemical properties are considered to depend markedly on the distribution of substituents in the anhydroglucose units.[4-7]

The relative reactivities of these groups have been investigated mainly by chemical methods.[8-10] However, at present ^1H and ^{13}C NMR spectroscopy have afforded more accurate knowledge of structures, with a consequent increase in the reliability of deductions based on them. Recently, the study of the microstructural characterization of cellulose acetates by ^{13}C NMR[7,11,12] has been suggested. The distribution of *O*-acetyl groups can be estimated not only from the *O*-acetyl carbonyl carbon spectra but also from the ring carbon spectra.

In general, the models used to study the substitution reactions in cellulose and other polysaccharides involve the following assumptions:[5,13-15] (a) all the anhydroglucose units in the polysaccharide molecule are equally accesible for reaction; (b) the relative rate constants of reaction of the different hydroxyls remain unchanged throughout the process; (c) substitution within a given unit does not affect the reactivity of the remaining unreacted hydroxyls; and (d) the effects of end groups are negligible.

On the other hand, dextrans are high molecular weight polymers of D-glucopyranose synthesized from sucrose by a number of bacterial species belonging to the family Lactobacillae. Most of the glucosidic linkages are 1→6,

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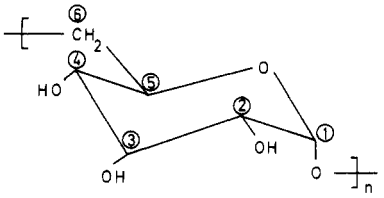
but to a lesser extent 1→2, 1→3, and 1→4 linkages also appear. The repeat anhydroglucose units of dextran can be represented schematically as follows, where the numbers denote the positions of the carbon atoms.



Dextran is used extensively in clinical practice as a blood flow improver and a volume expander; it is also used in a modified form as molecular sieves and a degradable support for sustained delivery of bioactive compounds.[16,17]

The three hydroxyl groups at positions 2, 3, and 4 on the glucopyranosyl units of dextran offer a variety of possibilities for making useful derivatives. Thus, the partial modification of dextran with ethyl chloroformate using pyridine as a catalyst/acceptor system and the *N,N*-dimethylformamide/LiCl system as a solvent[18]

$$D_i\text{–OH} + ClCOOCH_2CH_3 \xrightarrow{k_i} D_i\text{–OCOOCH}_2CH_3$$

yields hydrophilic polymers that are chemically homogeneous[19] and have been tested as supports for sustained-delivery systems.[20]

Our aim has been to characterize by ^{13}C NMR the overall distribution of substitution of the three secondary hydroxyls at positions C-2, C-3, and C-4 of the glucopyranosyl units of hydrophilic polymers prepared by reaction of a linear dextran with ethyl chloroformate in the homogeneous phase.

Experimental Section

Preparation of Modified Polymers. Partially modified dextran polymers with ethyl carbonate groups were obtained by reaction of ethyl chloroformate with dextran (T-70, from Pharmacia Fine Chemicals) with a linear structure (as revealed by ^{13}C NMR[21]) and a weight-average molecular weight (from light scattering) ($\bar{M}_w$) of 70 000, using *N,N*-dimethylformamide containing 2 g/100 mL of LiCl as solvent at 5 °C.[18] The degree of substitution (DS) was controlled by the amount of ethyl chloroformate used. Different precipitants were used to isolate the polymer depending on the DS. Distilled water appeared to be best for the highly modified polymers, and isopropyl alcohol was best for polymers with low DS.

All samples were purified by reprecipitation, and then they were dried in vacuo over P_2O_5 at 40 °C. The amount of carbonate groups of modified polymers was determined by ^1H NMR. The DS was also determined in some cases by means of alkaline hydrolysis at 60 °C, using a standard solution of sodium hydroxide and titrating back the unreacted base with 0.1 M hydrochloric acid in the presence of phenolphthalein. Very close values were obtained by both methods.

NMR Measurements. ^{13}C NMR spectra were recorded with a Bruker WP80SY spectrometer at 20.15 MHz in the proton-noise-decoupled mode, using 10% (w/v) solutions in Me_2SO-d_6 at 80 °C and hexamethyldisiloxane (HMDS) as internal reference standard. The spectral measurement conditions were similar to those of the structural analysis of cellulose derivatives.[12] About 40 000 scans were accumulated for each spectrum with a repetition time of 3 s. The resonance areas were measured by electronic integration as well as by triangulation and planimetry.

Results and Discussion

Analysis of the Anhydroglucose Carbon Resonance Signals. In view of the relatively poor resolution of the ^1H NMR spectra we have considered the utility of the corresponding ^{13}C spectra. In Figure 1 are presented the 20.15-MHz proton-decoupled ^{13}C resonances of the C-1 to C-6 carbons for dextran and modified products with low

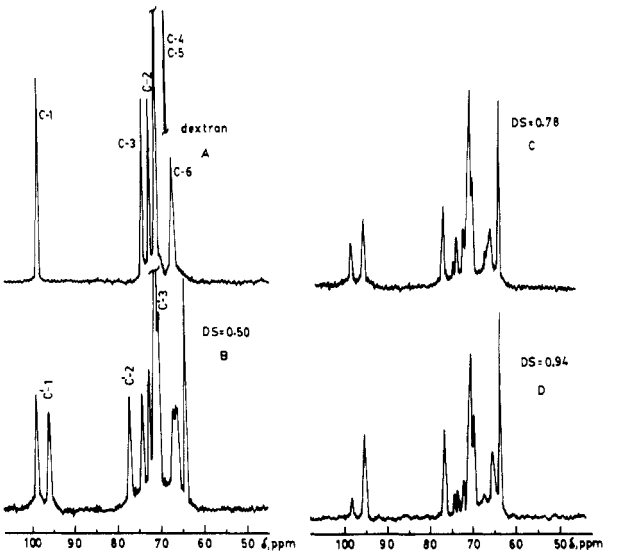


Figure 1. Expanded ^{13}C NMR spectra of anhydroglucose carbons of dextran and modified dextrans at low substitution degrees.

Table I
^{13}C NMR Chemical Shifts (δ) of Dextran and Trisubstituted Dextrans

C-1	C-2	C-3	C-4	C-5	C-6
		Dextran			
98.1[a]	71.8[a]	73.3[a]	70.2[a]	70.2[a]	66.1[a]
97.85[b]	71.50[b]	73.50[b]	70.00[b]	70.35[b]	66.15[b]
98.40[c]	71.85[c]	73.34[c]	70.46[c]	70.46[c]	66.45[c]
		2,3,4-Tri-*O*-acetyldextran			
95.55[b]	70.80[b]	70.15[b]	68.75[b]	68.65[b]	65.80[b]
		2,3,4-Tri-*O*-ethoxycarbonyldextran			
95.13[c]	73.43[c]	71.61[c]	72.80[c]	67.60[c]	65.30[c]

[a] Reference 22. [b] Reference 23. [c] This work.

degrees of substitution. According to several authors,[21,23] in the spectrum of dextran (Figure 1A) we can assign the peak in the lowest field region at 98.40 ppm from HMDS to the hemiacetal carbon C-1 and the highest field peak at 66.45 ppm to the methylene carbons C-6 of the bridge between glucopyranoside units. The complex resonance pattern in the 70–80 ppm range arises from the four remaining ring carbons, and the corresponding signals have been assigned to carbons as quoted in Table I, together with those given in the bibliography for linear dextrans. It can be considered that branching of the dextran used could be neglected since the glucopyranosyl units are almost entirely connected by α-D-(1→6) linkages.

The ring carbon region for samples with DS = 0.50 (Figure 1B) shows a decrease in the intensity of signals assigned to the C-1, C-2, and C-3 carbons, with respect to dextran, together with the appearance of three new signals at 95.40, 76.40, and 69.82 ppm, whereas the signals assigned to C-4 and C-5 seem to be not affected. In this sense, it is well-known that the modification of a hydroxyl group (etherification or esterification) of glucopyranosyl compounds, including celluloses, causes an upfield shift of the resonance of the adjacent carbons[11,24-28] and that the resonance of the carbon directly linked to a modified hydroxyl group is shifted downfield with respect to the chemical shift of the carbon bearing an unsubstituted hydroxyl group. Therefore, according to data reported for oligosaccharides[24] and cellulose,[25,29,30] the peak at 76.40 ppm (C′-2) must be assigned to carbon-2, bearing a substituted hydroxyl group, the peak at 95.40 ppm (C′-1) can be assigned to the hemiacetal carbon, and the peak at 69.80 ppm can be assigned to C′-3 carbons adjacent to the

Table II
Calculation of Carbon Chemical Shifts of Modified Dextrans Considering $\Delta\delta_\alpha$ = 4.6 ppm, $\Delta\delta_\beta$ = −3.0 ppm, and $\Delta\delta_\gamma$ = 0 ppm

substituent position[a]			chem shift, ppm				
2	3	4	C_1	C_2	C_3	C_4	C_5
OH	OH	OH	98.41	71.85	73.34	70.46	70.46
OR	OH	OH	95.41	76.45	70.34	70.46	70.46
OR	OH	*OR*	95.41	76.45	67.34	75.06	67.46
OR	*OR*	OH	95.41	73.45	74.94	67.46	70.46
OR	*OR*	*OR*	95.41	73.45	71.94	72.06	67.46

[a] R = $COOCH_2CH_3$.

modified C′-2, considering that the shielding of these carbons may be sensitive to the modification of its neighboring secondary hydroxyl groups. The analysis of results indicates that at this substitution degree (DS = 0.50) the secondary hydroxyl group linked to carbon-2 is selectively modified by the esterification reaction.

The spectra of samples with DS = 0.78 and DS = 0.94 (Figure 1C,1D) clearly show decreased intensities of signals C-1, C-2, and C-3 and corresponding increase in the intensities of signals C′-1, C′-2, and C′-3, which supports the high selectivity of the esterification reaction toward the secondary hydroxyl group at C-2.

From the spectral analysis of carbons C-1 to C-5 at low DS values we may consider that the possible shielding effect of substituents located farther than three bonds away can be neglected. Therefore, the changes in chemical shift of carbons in positions α and β as a consequence of replacing the hydroxyl group by an ethyl carbonate group amount to +4.6 and −3.0 ppm, respectively. Spectral assignments can be made by using the balance of inductive deshielding (α effect) and the shielding due to nonbonded interactions (β effect) for the different possibilities of substitution at the three secondary hydroxyls, but considering that the hydroxyl group linked to C-2 is selectively substituted at low DS values. On this basis, the chemical shifts calculated for resonances of carbons C-1 to C-5 are given in Table II and can be useful as a qualitative guide to assign the resonance signals of samples with higher DS values.

The spectra of samples with DS = 0.94 (Figure 1D) contain only weak signals at 74.50 and 67.30 ppm together with a decrease in the intensity of the sharp signal assigned to C-4 and C-5 in the spectrum of dextran. According to calculated values given in Table II, these signals can be assigned to C-4 and C-5 in glucopyranosyl units with disubstituted hydroxyls at positions C-2 and C-4. Also it is noteworthy that the signal assigned to C-6 in dextran is shifted to higher field even for samples with low DS values as a consequence of the substitution of the hydroxyl group linked to C-2. From the study of molecular models, this shielding can be attributed to a possible steric effect of the ethyl carbonate groups at position C-2 on the methylene group at C-6 of the neighboring glucopyranosyl unit. This fact is supported by the broadening observed for the corresponding signal, which might be ascribed to a restriction of the motion of the C-6 carbon or simply a splitting of the signal by the substitution of the hydroxyl group at C-2. Moreover, this new signal is not affected by the substitution reaction at DS values higher than unity because of the high selectivity of the esterification reaction at position C-2.

Figure 2 shows the resonances of the ring carbon region for samples with DS values of 1.41, 1.92, 2.40, and 3.00. The evolution of resonance signals follows approximately the balance of contributions of α and β effects for the

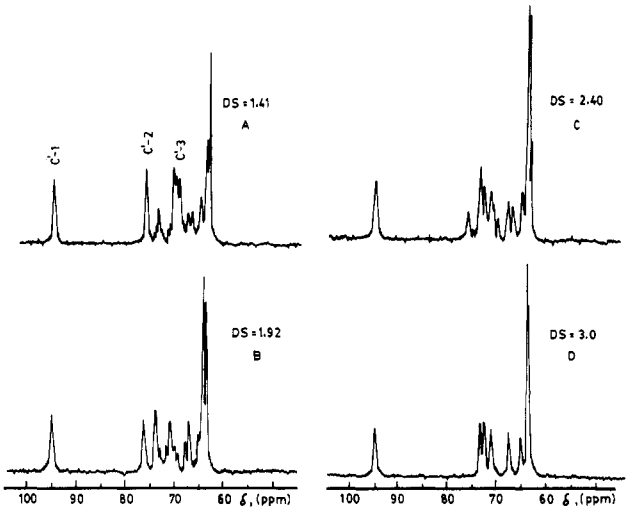


Figure 2. Expanded ^{13}C NMR spectra of anhydroglucose carbons of modified dextrans at high substitution degrees.

different secondary hydroxyl groups at C-2, C-3, and C-4 according to the values reported in Table II. However, it is difficult to distinguish the different resonance signals in the interval 69–75 ppm because resonance signals from nine possible structures can be expected. However, it is possible to study the course of the modification reaction through the evolution of peaks perfectly identified in the spectra of samples with low DS values (i.e., peaks at 95.40, 76.40, and 69.80 ppm, assigned to C-1, C-2, and C-3 in monosubstituted glucopyranosyl units) and the comparison of the whole bands in this interval, taking into account the corresponding contribution of different structures according to Table II. It is necessary to indicate two anomalies observed with respect to the general additivity of α and β effects. The comparison of the integral intensities of the peaks in the interval 69–75 ppm with those at 95.40 and 76.40 ppm in the spectra of samples with DS values of 1.92 and 2.40 seems to indicate that the C-3 resonance in disubstituted rings at positions C-2 and C-4 is not shifted to 67.34 ppm as expected from the calculated contributions quoted in Table II. On this basis, we assume that the C-3 resonance remains in the group of signals about 70 ppm. The second anomaly is the observed evolution of peaks assigned to C-5, which seems to be somewhat shifted to lower field (67.60 ppm) for trisubstituted samples (completely modified rings) than for disubstituted (at C-2 and C-4) samples (67.30 ppm). This behavior is supported by the fact that the ratio between the integral intensities of the two peaks is similar to the ratio between disubstituted anhydroglucose units at C-2 and C-4 and trisubstituted units.

Although it is difficult give a definitive interpretation of the two mentioned anomalies, it must be taken into consideration that steric factors and possibly polar effects between unsubstituted groups and ethyl carbonate functions in samples with relatively high DS values play an important role not only in the course of the modification reaction but also in the magnetic character of the different nuclei. In this sense, it has been stated in the study of *O*-(2-hydroxypropyl)cellulose[31] that for substituents at positions C-4 and C-6, the inductive deshielding effect is progressively offset as the steric factor increases.

However, the assignment made by using the chemical shift changes as reported in Table II permits us to identify the different structures for quantitative analysis from the spectral integration of the proton-decoupled ^{13}C NMR spectra of modified dextran at different DS values. It has been considered that under the experimental conditions

Table III
Quantitative Structural Analysis of Substitution at Individual Hydroxyls by Ethyl Carbonate Groups

DS[a]	signal	degree of substitution at position C-2	C-3	C-4	total
0.50	^{13}C(AHU)[b]	0.48	0	0	0.48
	^{13}C(C=O)				
	^{13}C(OCH_2)				
0.78	^{13}C(AHU)	0.66	0	0.09	0.75
	^{13}C(C=O)	0.68	0	0.07	
	^{13}C(OCH_2)	0.69	0	0.06	
0.94	^{13}C(AHU)	0.81	0	0.18	0.99
	^{13}C(C=O)	0.82	0	0.17	
	^{13}C(OCH_2)	0.80	0	0.19	
1.41	^{13}C(AHU)	0.99	0	0.39	1.38
	^{13}C(C=O)	0.99	0	0.39	
	^{13}C(OCH_2)	0.95	0	0.43	
1.92	^{13}C(AHU)	1.00	0.21	0.72	1.93
	^{13}C(C=O)	1.00	0.22	0.73	
	^{13}C(OCH_2)	1.00	0.20	0.72	
2.40	^{13}C(AHU)	1.00	0.60	0.81	2.41
	^{13}C(C=O)	1.00	0.62	0.78	
	^{13}C(OCH_2)	1.00	0.65	0.80	

[a] Degree of substitution from chemical analysis and ^1H NMR [b] AHU = anhydroglucose units.

used in the present work, the spin–lattice relaxation times and the nuclear Overhauser effect factors of all six carbons of the anhydroglucose units may be very similar. Consequently, the spectral integration gives reliable values, and the relative DS values of individual hydroxyl groups attached to the C-2, C-3, and C-4 carbons have been estimated from ratios of the integral intensities of the resonance signals assigned to the carbons C-1 to C-5 of the anhydroglucose units according to the scheme of Table II. The results obtained are listed in Table III.

Analysis of the Oxymethylene Carbon Resonance Signals. ^{13}C NMR spectra of substituted dextran samples at different DS values contain resonances for the oxymethylene carbon of the ethyl carbonate side groups which are much narrower than those of the anhydroglucose residues. As shown in Figure 3, the pattern of the oxymethylene resonances is drastically affected by the extent of the modification and by the position of ethyl carbonate side groups in the glucosyl residues. It may be noted that for samples with low DS values (Figure 3A), only one sharp peak at 63.50 ppm is observed, which makes it evident that an essentially quantitative substitution at the hydroxyl C-2 position occurs at low modification levels.

However, it is noteworthy that two new signals appear at 64.10 and 64.30 ppm for samples with higher DS values, as shown in Figure 3B,C; the intensities of these signals increase with increasing degree of substitution whereas the intensity of the signal at 63.50 ppm decreases progressively. These resonance signals appear to be related to oxymethylene carbons of the ethyl carbonate groups appended to positions C-2, C-3, and C-4 in di- and trisubstituted glucosyl residues, since they remain in the spectrum of trisubstituted dextran (Figure 3D). On the basis of the evolution of resonance signals assigned to carbons C-1 to C-5 of the glucosyl residues and taking into account the variation of intensities of the oxymethylene carbon resonances, the signal at 63.50 ppm may be attributed exclusively to ethyl carbonate substituents at C-2 in monosubstituted and C-2,C-4-disubstituted glucosyl residues. The signal at 64.10 ppm can be attributed to ethyl carbonate substituents at the C-4 position in C-2,C-4-disubstituted and trisubstituted anhydroglucose units, whereas the signal at 64.30 ppm represents the contribution of resonances of ethyl carbonate groups at the C-2 and C-3 positions in

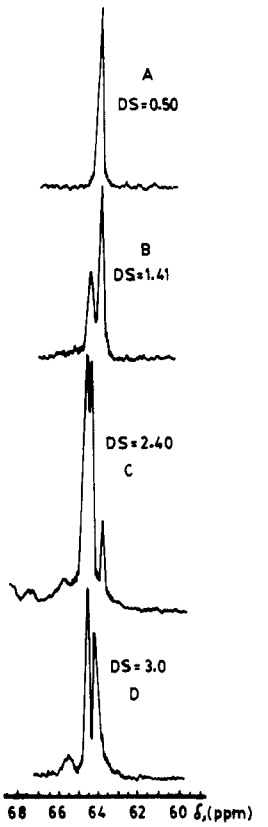


Figure 3. Expanded ^{13}C NMR spectra of oxymethylene carbons of dextrans modified with ethyl chloroformate.

C-2,C-3-disubstituted and trisubstituted glucopyranosyl residues. This behavior can be satisfactorily explained by means of molecular models, which suggest that the nonbonded interactions between ethyl carbonate substituents at positions C-2 and C-3 may be different from that of substituents at C-3 and C-4. Moreover, ethyl carbonate groups at positions C-2 and C-4 of a given glucosyl residue can be neglected because of the spatial orientation of both groups.

According to the assignment suggested and taking into consideration the variation of intensities of resonance signals with the degree of substitution, it appears that once an ethyl carbonate group is introduced exclusively at position C-2, the reactivity of the hydroxyl at position C-4 is higher than that at position C-3. The integration of the proton-decoupled ^{13}C NMR spectra of modified dextran at different DS values makes possible an accurate quantitative structural analysis. Results obtained are summarized in Table III, and the good agreement with the results obtained from the spectral analysis of the anhydroglucose carbons is suggestive of the validity of the present assignments.

Analysis of the Carbonyl Carbon Resonance Signals. In order to confirm the validity of the assignments suggested for the resonance signals of the anhydroglucose units as well as those of the oxymethylene carbons of the ethyl carbonate substituents, we have examined the systematic variation of the carbonyl carbon resonances with the degree of substitution.

Figure 4 shows the expanded proton-decoupled ^{13}C NMR spectra of carbonyl groups for several modified dextrans. As in the case of the oxymethylene carbon resonances, samples with low DS values give only a sharp signal at 154.20 ppm (Figure 4A), which logically has been assigned to the carbonyl carbon of ethyl carbonate substituents C-2 in the anhydroglucose units. Dextrans modified with DS values higher than unity present one or

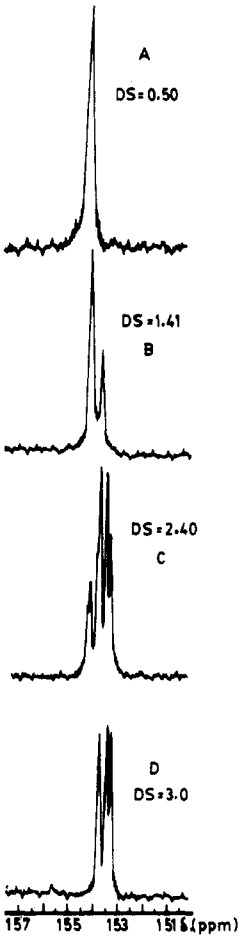


Figure 4. Expanded ^{13}C NMR spectra of carbonyl carbons of dextrans modified with ethyl chloroformate.

three new resonance signals at 153.70, 153.40, and 153.20 ppm, whose intensities change with increasing DS, together with a systematic decrease of the signal at 154.20 ppm, which is absent from the spectrum of the completely modified dextran. According to the results obtained by the structural analysis of the resonances of anhydroglucose carbons and oxymethylene carbons, the signal at 154.20 ppm can be assigned to ethyl carbonate groups at C-2 in monosubstituted and C-2, C-4-disubstituted anhydroglucose units.

More complex is the assignment of the three remaining peaks; however, the evolution of signals with the degree of substitution indicates that the peak at 153.7 ppm may be assigned to ethyl carbonate groups at C-4 in disubstituted and trisubstituted glucopyranosyl units and to ethyl carbonate groups at C-2 in C-2,C-3-disubstituted rings. The signal at 153.40 pm is assigned to substituted groups at C-3 in C-2,C-3-disubstituted and trisubstituted glucopyranosyl units, and finally, the signal at 153.20 ppm is assigned to substituents at C-2 in completely modified dextran.

Quantitative structural analysis from the integrated intensities of these peaks gives results that are summarized in Table III. These appear to be in fairly good agreement with those obtained from the spectral analysis of the anhydroglucose carbons and oxymethylene groups.

It is noteworthy that the introduction of ethyl carbonate substituents in the glucopyranosyl residues causes deshielding of the resonances of oxymethylene carbons. This is a consequence of nonbonded interactions between the different substituents, whereas shielding effects are observed for the carbonyl carbons of the same groups.

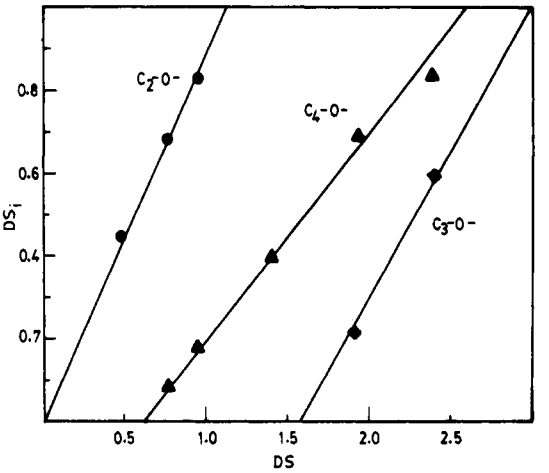


Figure 5. Variation of the substitution degree at individual hydroxyl groups (DS_i) with the total degree of substitution.

However, this is a general trend for ^{13}C NMR resonance of groups with interactions that depend on the spatial orientation of such functional groups.[32] We stress here that the effects of substitution on the carbonyl carbon resonance signals are rather similar to those observed for the oxymethylene carbons, although the carbonyl carbons of substituents at the C-2 and C-3 positions of the trisubstituted anhydroglucose units are not magnetically equivalent. However, the chemical shifts of the carbonyl signals make clear that the nonbonded interactions between ethyl carbonate substituents at positions C-2 and C-3 are different from those of substituents at C-3 and C-4, in a way similar to that observed for the oxymethylene carbons: the chemical shift of the carbonyl carbon of the ethyl carbonate group substituted at C-4 is rather different from those of the substituents at C-2 and C-3 in completely modified dextran. The results quoted in Table III reflect clearly that the relative reactivity of hydroxyl groups at C-2 is much higher than at C-3 and C-4 as has been mentioned above and that the reactivities of the hydroxyl groups toward ethyl chloroformate decrease in the order C-2 > C-4 > C-3.

The high reactivity of the hydroxyl group at C-2 position is consistent with previous studies on substitution reactions of cellulose[5,6,12] and dextrans.[8,9,33] However, the reactivities of hydroxyl groups at the C-3 and C-4 positions seem to be modified with the degree of substitution. This is better illustrated in Figure 5, which shows the relative degree of substitution of individual hydroxyl groups at the C-2, C-3, and C-4 positions as a function of the total DS value. Linear variations of DS for individual groups with the overall DS is observed, but the slope of the straight line corresponding to the substitution of hydroxyl group at the C-3 position seems to be higher than that of the corresponding to the hydroxyl group at C-4. This means that the relative reactivity of the hydroxyl at C-3 with respect to that of the hydroxyl at C-4 is enhanced in the course of the modification of dextran chains.

Figure 6 shows the distribution of substituents along the dextran chains calculated from the ^{13}C NMR spectra. The diagrams obtained reveal that the molar fraction of anhydroglucose units modified at C-2 (A_2) reaches a maximum for a total degree of substitution (DS) = 1.0 and the fraction of anhydroglucose units disubstituted at C-2 and C-4 ($A_{2,4}$) reaches a maximum for (DS) = 2, whereas the formation of anhydroglucose units disubstituted at C-2 and C-3 ($A_{2,3}$) is less favored and the corresponding molar fraction reaches a maximum at (DS) = 2.5. A_0 and A_T represent in the diagram of Figure 6 the molar fraction of

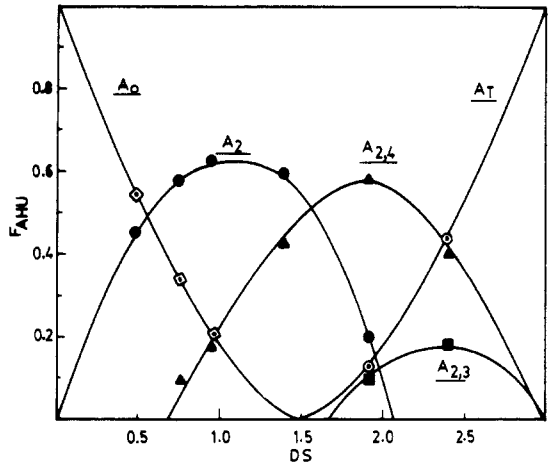


Figure 6. Molar fraction of anhydroglucose units (F_{AHU}) as a function of the total degree of substitution. A_0 = unsubstituted AHU; A_2 = AHU substituted at C-2; $A_{2,4}$ and $A_{2,3}$ disubstituted AHU at C-2,C-4 and C-2,C-3, respectively; A_T = trisubstituted AHU.

anhydroglucose units unsubstituted and trisubstituted, respectively.

It is apparent from Figure 5 that the substitution of hydroxyl groups at the C-2, C-3, and C-4 positions by ethyl carbonate groups cannot be considered a random process, since the hydroxyl group at position C-2 is selectively modified in the first steps of the modification reaction. Once the anhydroglucose units have been modified at C-2, the reactivity of the hydroxyl group at C-4 is higher than that at the C-3 position. In this sense, differences in the reactivity of secondary hydroxyl groups have also been found in the acetylation of monosaccharides with acid chlorides,[1,2] the hydroxyl group at the C-2 position being the most reactive. Doane et al.[34] in the study of the reaction of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with ethyl chloroformate in the presence of pyridine reported the selectivity of the raction at C-2. They found the substitution molar ratio at positions C-2 and C-3 to be equal to 24. On the other hand, de Belder and Norrman[9] reported a difference in the substitution patterns of partially acetylated dextrans depending upon the reaction medium. All three hydroxyls show similar reactivities for the acetylation reaction in aqueous alkali, but the reactivity of the hydroxyl group at C-2 is sensibly enhanced when the acetylation takes place in the presence of pyridine. This has been explained in terms of hydrogen bonding between the hydroxyl group and the oxygen in the axial methoxyl group by Richardson et al.[3,35] for the reaction of methyl α-D-glucopyranoside with benzoyl chloride in pyridine. Previously, Buck et al.[36,37] indicated the possibility of correlating intramolecular hydrogen bonding and the enhancement of esterification rates. Polar and steric effects also have been considered to explain the differences in reactivities of hydroxyl groups in carbohydrates.[1]

The highest reactivity of the hydroxyl group at C-2 of dextran in the reaction with ethyl chloroformate may be correlated with the cis character of the anomeric oxygen and the hydroxyl at C-2, which gives the most favorable orientation for the formation of hydrogen bonding between both groups, increasing the basicity of the alcoholic residue. Similarly, the higher reactivity of the hydroxyl group at C-4 relative to C-3 can also be explained in view of molecular models if it is considered that once the anhydroglucose units have been modified at position C-2, the hydroxyl group at position C-4 of a particular anhydroglucose unit presents a spatial orientation with respect to the ethyl carbonate group at C-2 of the adjacent anhydroglucose unit which favors the formation of intramolecular hydrogen bonding between the hydroxyl and the carbonyl group of the substituent, enhancing the reactivity of the corresponding hydroxyl. This situation is not so clear for the hydroxyl at C-3, which only could form intramolecular hydrogen bonding with the carbonyl group of ethyl carbonate substituents at the C-2 or C-4 positions of the same anhydroglucose unit.

In conclusion, the analysis by ^{13}C NMR spectroscopy of dextran and modified dextrans provides detailed structural information that reveals the reactivity of the three secondary hydroxyls toward the ethyl chloroformate and the mechanism of the substitution reaction.

Registry No. Dextran, 9049-32-5; ethyl chloroformate, 541-41-3.

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