^{13}C nuclear magnetic resonance spectral study on the distribution of substituents in relation to the preparation method of partially acetylated dextrans

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^{13}C nuclear magnetic resonance (n.m.r.) spectra of partially modified dextrans with acetyl groups prepared by reaction with acetyl chloride or acetic anhydride in the homogeneous phase were analysed at 75.4 MHz. It was found that the distribution of substituents in the anhydroglucose units of these partially acetylated dextrans can be estimated from their ring carbon spectra. The results showed that for acetylated dextrans prepared by reaction with acetyl chloride, the reactivity of individual secondary hydroxyl groups decreases in the order C-2>C-4>C-3. For those modified dextrans prepared with acetic anhydride, the ease of acetylation was C-2≃C-3>C-4. The results were explained by considering the formation of intramolecular hydrogen bonds as well as by steric considerations.

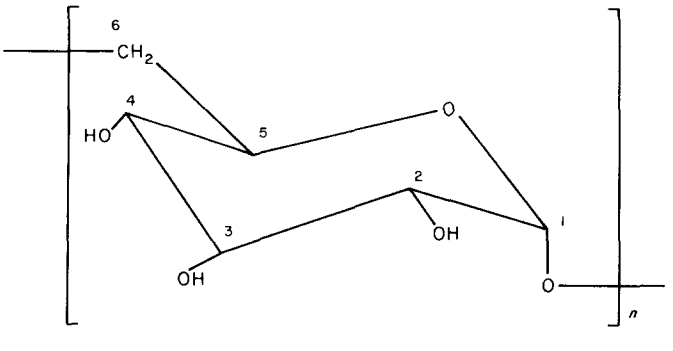
(Keywords: dextran; acetyl chloride; acetic anhydride; degree of substitution; relative reactivity; ^{13}C nuclear magnetic resonance)

INTRODUCTION

Several studies have shown that, although primary hydroxyl groups are normally the most reactive towards acylating agents, secondary hydroxyl groups within a carbohydrate molecule often show significant differences in reactivity[1]. Considerable attention has been focused on this fact in view of theoretical interests and because the distribution of substituents in the anhydroglucose (AHG) units may exert important effects on the physical, chemical and biochemical properties of partially modified polysaccharides.

Recently, ^{13}C n.m.r. has been suggested as an adequate method to evaluate the distribution of substituents in the AHG units[2-6]. This method afforded more accurate knowledge of structures, with a consequent increase in the reliability of deductions based on them.

Dextran is a predominantly α-(1→6)-linked D-glucan elaborated by *Leuconostoc mesenteroides* and related micro-organisms. The repeat AHG units of dextran can be represented schematically as follows, where the numbers denote the positions of the carbon atoms:



The three hydroxyl groups at C-2, C-3 and C-4 positions on the AHG units offer a variety of possibilities for making useful derivatives. Up to now, some attempts have been made to evaluate the distribution of substituents in partially modified dextrans[6-8].

The purpose of this paper is to study by ^{13}C n.m.r. the distribution of *O*-acetyl groups in partially acetylated dextrans prepared by reaction with acetyl chloride and acetic anhydride in the homogeneous phase using pyridine as catalyst.

EXPERIMENTAL

Materials

The dextran was a commercial product (T-70, from Pharmacia Fine Chemicals) with a linear structure (as revealed by ^{13}C n.m.r.[9]) and a weight-average molecular weight (from light scattering) $\bar{M}_w$ of 70000. The polymer was dried in vacuum for a few days at 80°C in the presence of phosphorus pentoxide to constant weight. Acetyl chloride (from C. Erba) and acetic anhydride (from Panreac) were purified prior to use by distillation under normal pressure immediately before use. Pyridine (from C. Erba) and *N,N'*-dimethylformamide (DMF) (from Ferosa) were purified following one of the conventional methods[10,11]. LiCl (from Panreac) was dried in vacuum in the presence of phosphorus pentoxide to constant weight.

Preparation of polymer samples

Partially modified dextrans with acetyl groups were prepared by reaction with acetyl chloride at 25°C or

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acetic anhydride at 50°C. Equimolar concentrations of acetylating agent and pyridine were reacted with 4 g (0.074 mol OH) of dextran in 100 ml of DMF containing 1 g/100 ml LiCl. The polymer was kept soluble throughout the process. The degree of substitution (*DS*) was controlled by the amount of acetylating agent 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 for polymers with low *DS*. All samples were purified by reprecipitation, then dried in vacuum in the presence of phorphorus pentoxide. The *DS* values were determined 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.

Nuclear magnetic resonance measurements

^{13}C n.m.r. spectra were obtained from a Varian model XL-300 n.m.r. spectrometer operating at 75.4 MHz in the proton-noise-decoupled mode by using a 10 mm probe and deuterated dimethylsulphoxide (DMSO-d_6) as solvent at 80°C. Chemical shifts were measured with respect to that of the central peak of the methyl carbon of DMSO-d_6, which was taken as 39.7 ppm downfield from tetramethylsilane. The spectral measurement conditions were similar to those of the structural analysis of cellulose derivatives[4]. The spectra were accumulated for about 16 000 scans with a repetition time of 3 s. The resonance areas were measured by electronic integration as well as by planimetry and the weights cut out from Xerox copies.

RESULTS AND DISCUSSION

The ^{13}C n.m.r. spectra of modified dextrans with *O*-acetyl groups show three spectral regions at 29–31, 60–101 and 168–171 ppm, which arise from the methyl carbon atoms in the acetyl groups, the ring carbons of AHG units and the carbonyl carbon atoms, respectively. However, the analysis of the signals of both the methyl and the carbonyl carbon atoms in partially modified dextrans involves great difficulties. which are due to the fact that these regions usually yield a complicated resonance pattern. Therefore, we have considered that only the signals of the ring carbon atoms can give adequate analytical information.

The ^{13}C n.m.r. spectra of the ring carbon region of the original dextran and several samples of partially modified dextrans prepared by reaction with acetyl chloride in the presence of pyridine (*DS* ranging from 0.41 to 2.50) are shown in *Figure 1*. In *Figure 2* are presented the ^{13}C resonances of the C-1 to C-6 carbons for samples prepared by reaction of dextran with acetic anhydride using pyridine as catalyst (*DS* ranging from 0.45 to 3.0).

According to data reported for linear dextrans[12,13], we have assigned the corresponding signals in the spectrum of dextran to carbons of the AHG units as quoted in *Table 1*. 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. *Table 1* also contains the assignments corresponding to the signals of 2,3,4-tri-*O*-acetyldextran according to the literature[13].

Analysis of the spectra of the ring carbon region of

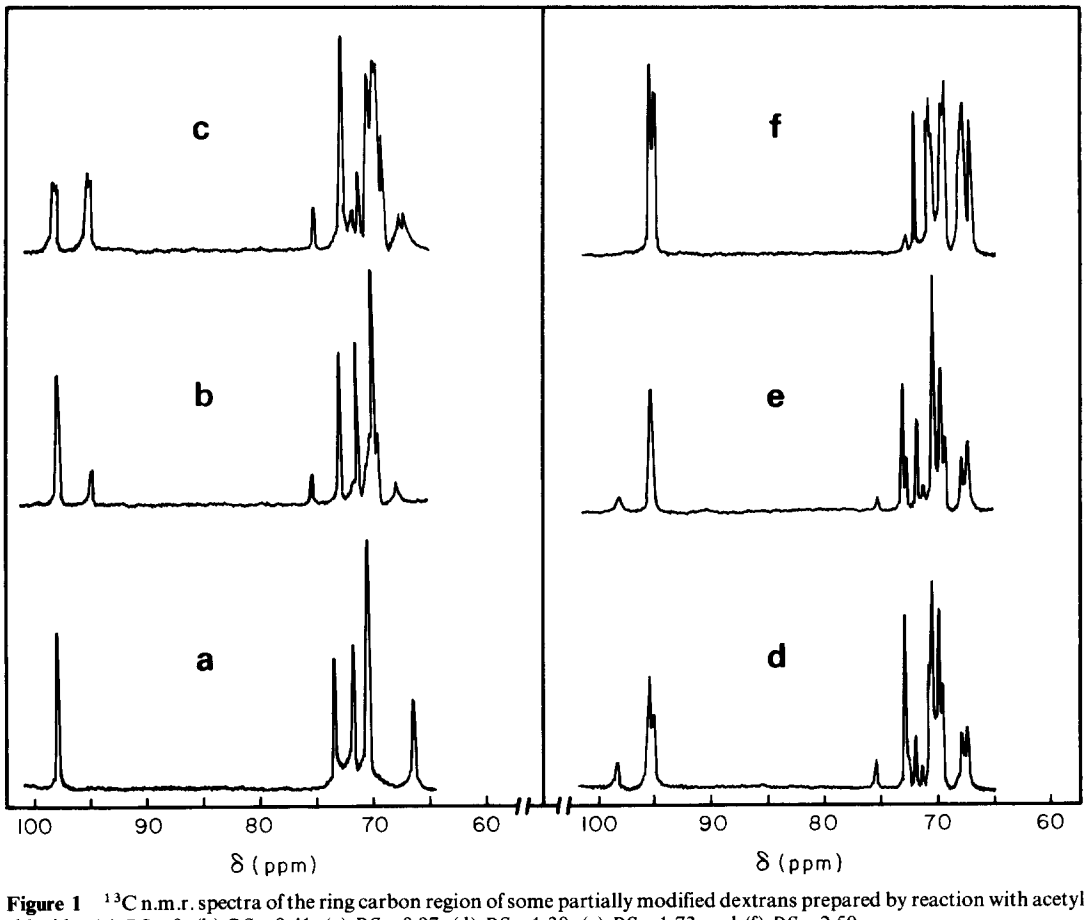


Figure 1 ^{13}C n.m.r. spectra of the ring carbon region of some partially modified dextrans prepared by reaction with acetyl chloride: (a) *DS* = 0, (b) *DS* = 0.41, (c) *DS* = 0.97, (d) *DS* = 1.38, (e) *DS* = 1.73 and (f) *DS* = 2.50

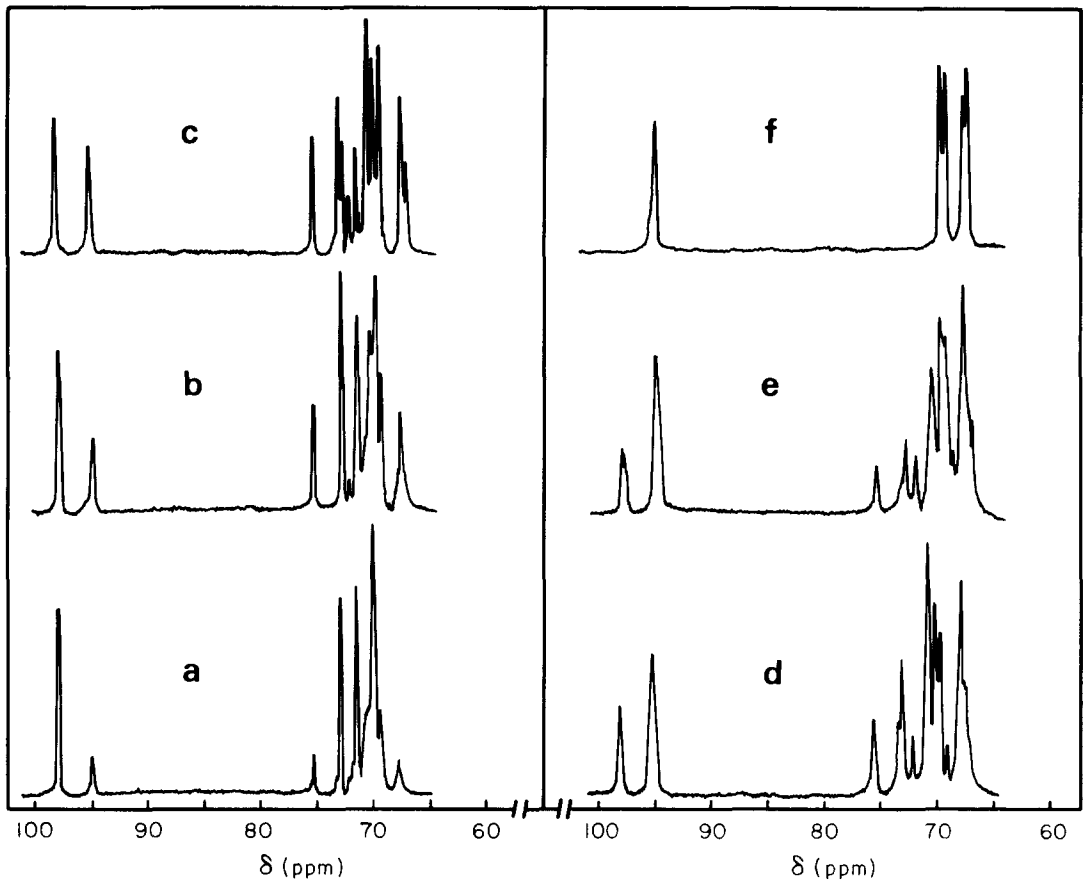


Figure 2 ^{13}C n.m.r. spectra of the ring carbon region of some partially modified dextrans prepared by reaction with acetic anhydride: (a) $DS = 0.45$, (b) $DS = 0.88$, (c) $DS = 1.37$, (d) $DS = 1.82$, (e) $DS = 2.18$ and (f) $DS = 3.00$

Table 1 ^{13}C n.m.r. chemical shifts (ppm) of dextran and triacetylated dextran

C-1	C-2	C-3	C-4	C-5	C-6	Source
Dextran						
98.4	71.8	73.3	70.4	70.4	66.4	This work
98.1	71.8	73.3	70.2	70.2	66.1	Ref. 12
97.8	71.5	73.5	70.0	70.3	66.1	Ref. 13
2,3,4-Tri-*O*-acetyldextran						
95.2	69.9	69.6	68.1	67.8	65.2	This work
95.5	70.8	70.1	68.7	68.6	65.8	Ref. 13

AHG (*Figures 1* and *2*) shows that the spectral intervals at 71–100 ppm as well as at 67–69 ppm are the richest in analytical information. In the range 69–71 ppm there are several peaks heavily overlapped from which no quantitative information on the relative *DS* can be directly estimated.

As can be seen from *Figures 1* and *2* the relative intensities of C-1, C-2 and C-3 peaks decrease as the value of the total *DS* increases, together with the appearance of several new peaks at 95.2, 75.6, 73.0, 72.3, 71.3 and 67.8–67.4 ppm. The presence of these new peaks is consistent with the fact that the esterification of a hydroxyl group of glucopyranosyl compounds not only causes an upfield shift (−1.8 to −3.3 ppm) of the resonance of the adjacent carbons, but also the resonance of the carbon directly linked to a modified hydroxyl group is shifted (0.9 to 1.9 ppm) downfield with respect to the chemical shift of the carbon bearing an unsubstituted hydroxyl group[3].

The assignments of these new signals were made on the basis of comparison with the substituent-induced shifts observed for the 2-, 3- and 4-mono-*O*-myristoyl-α-D-glucopyranose[3] and assuming the additivity of the substituent effects[3,14,15]. These assignments were also correlated with the systematic variation of the relative intensity of each individual peak with the total *DS*.

The peak at 95.2 ppm may be ascribed to C-1 carbons adjacent to C-2 carbons bearing a substituted hydroxyl group. The peak at 75.6 ppm may be attributed to C-3 carbons bearing a substituted hydroxyl group in monosubstituted AHG units.

The C-2 carbons bearing a substituted hydroxyl group in monosubstituted AHG units are assumed to give a peak at 73.3 ppm, which appears at the same position as that of the C-3 carbons of the original dextran. This assignment was made taking into account the chemical shifts of C-2 in 2-mono-*O*-myristoyl-α-D-glucopyranose[3] and the variation of the relative intensity of this peak with the total *DS*.

The peak at 72.3 ppm was attributed to C-2 carbons bearing a substituted hydroxyl group in 2,4-disubstituted anhydroglucose units. This assignment may be justified considering the downfield shift of C-2 carbons in penta-*O*-acetyl-β-D-glucopyranose[16] by desacetylation at the C-3 position. The order of magnitude of this downfield shift is similar to that found when C-2 carbons in triacetyldextran and 2,4-diacetyldextran are compared. This assumption was also confirmed by considering the evolution of the relative intensity of the peak at 72.3 ppm with the total *DS*.

Table 2 Variation of molar fraction of mono-, di- and trisubstituted AHG units with the total *DS* in modified dextran polymers prepared by reaction with acetyl chloride

O-acetyl substitution	*DS*				
	0.41	0.97	1.38	1.73	2.50
2	0.18	0.38	0.40	0.29	0
3	0.12	0.14	0.09	0.05	0
4	0.04	0.06	0.03	0.03	0
2,3	0	0.06	0.12	0.17	0.12
2,4	0.04	0.12	0.23	0.36	0.41
3,4	0	0	0	0	0
2,3,4	0	0	0.04	0.09	0.47

Table 3 Variation of molar fraction of mono-, di- and trisubstituted AHG units with the total *DS* in modified dextran polymers prepared by reaction with acetic anhydride

O-acetyl substitution	*DS*				
	0.45	0.88	1.37	1.82	2.18
2	0.14	0.17	0.11	0.09	0.04
3	0.12	0.23	0.22	0.19	0.13
4	0.06	0.10	0.07	0.02	0
2,3	0	0.05	0.12	0.16	0.14
2,4	0.05	0.10	0.15	0.18	0.19
3,4	0	0.02	0.08	0.09	0.11
2,3,4	0	0	0.09	0.23	0.39

The weak peak at 71.3 ppm may be assigned to C-4 carbons bearing a substituted hydroxyl group in monosubstituted AHG units. The peaks at 68.8–67.4 ppm are considered to belong to C-5 carbons adjacent to C-4 carbons bearing a substituted hydroxyl group as well as to C-4 carbons in 3-monosubstituted, 2,3-disubstituted and trisubstituted AHG units.

The peak at 69.2 ppm is assumed to result from the C-4 carbons in 3,4-disubstituted glucopyranosyl units.

It is necessary to indicate an anomaly observed with respect to the general additivity of α and β effects. The C-3 resonance in disubstituted rings at positions C-2 and C-4 is not shifted to the 67–69 ppm region as would be expected. We assume that the C-3 resonance remains in the group of signals in the 69–71 ppm region. A similar behaviour was observed in dextran modified with ethyl carbonate groups[6].

The substituent-induced shifts observed for the monosubstituted AHG units are similar in direction and amount to those reported for monoesterification of α-D-glucopyranose[3] and α-D-xylopyranose[15].

The above assignments for the different carbon atoms of the substituted AHG units permit us to estimate the relative reactivities of hydroxyl groups in the reaction of dextran with acetyl chloride and acetic anhydride. In this sense, according to several authors[4,6,17–19], it may be considered that, under the experimental conditions used in the present work, the spin–lattice relaxation times and the nuclear Overhauser effect factors of all six carbons of the AHG units must be very similar. Therefore, the peak area measurements can be used for evaluation of the relative *DS* values for individual hydroxyl groups attached to C-2, C-3 and C-4 carbons.

In the present study, the mole fractions (A) of monosubstituted AHG units at C-2 (A_2), C-3 (A_3) and C-4 (A_4), of disubstituted AHG units at C-2,C-3 ($A_{2,3}$), C-2,C-4 ($A_{2,4}$) and C-3,C-4 ($A_{3,4}$), and of trisubstituted AHG units ($A_{2,3,4}$) were calculated (*Tables 2* and *3*) from the expanded forms of the spectral ranges at 71–100 and 67–69 ppm previously mentioned, using in each case an adequate combination of the relative intensities of the signals at 98.4, 95.2, 75.6, 73.3, 73.0, 72.3, 71.8 and 67.8–67.4 ppm.

The relative *DS* values (DS_i) of individual hydroxyl groups attached to the C-2, C-3 and C-4 carbons have been estimated from the following equations:

$$DS_2 = A_2 + A_{2,3} + A_{2,4} + A_{2,3,4}$$

$$DS_3 = A_3 + A_{2,3} + A_{3,4} + A_{2,3,4}$$

$$DS_4 = A_4 + A_{2,4} + A_{3,4} + A_{2,3,4}$$

The average degree of substitution of each polymer sample was calculated from:

$$DS = DS_2 + DS_3 + DS_4$$

The values obtained from the above equations are summarized in *Tables 4* and *5*. It may be noticed that in all cases the magnitude of the total *DS* determined by chemical analysis and that obtained from the sum of the partial *DS* values of individual hydroxyl groups attached at C-2, C-3 and C-4 carbons are in good agreement.

Figures 3 and *4* show the variation of the relative *DS* of individual hydroxyl groups in the reaction of dextran with acetyl chloride and acetic anhydride, respectively, as a function of the total *DS* value.

The analysis of data from the reaction between dextran and acetyl chloride (*Figure 3*) clearly indicates that the relative reactivity of hydroxyl groups at C-2 is much higher than at C-3 and C-4. However, the reactivities of hydroxyl groups at C-3 and C-4 positions are similar for *DS* values up to ~1.0. At higher *DS* values, the relative reactivity of the hydroxyl at C-4 with respect to that of the hydroxyl at C-3 is enhanced in the course of the modification of dextran chains. This behaviour relative to

Table 4 Quantitative structural analysis of substitution at individual hydroxyl groups in the esterification of dextran with acetyl chloride

DS determined by chemical analysis	*DS* at position			Total *DS*
	C-2	C-3	C-4	
0.41	0.21	0.12	0.08	0.41
0.97	0.56	0.20	0.18	0.94
1.38	0.79	0.25	0.30	1.34
1.73	0.91	0.31	0.48	1.70
2.50	1.00	0.59	0.88	2.47

Table 5 Quantitative structural analysis of substitution at individual hydroxyl groups in the esterification of dextran with acetic anhydride

DS determined by chemical analysis	*DS* at position			Total *DS*
	C-2	C-3	C-4	
0.45	0.19	0.12	0.11	0.42
0.88	0.32	0.30	0.22	0.84
1.37	0.47	0.51	0.39	1.37
1.82	0.66	0.67	0.52	1.85
2.18	0.76	0.77	0.69	2.22

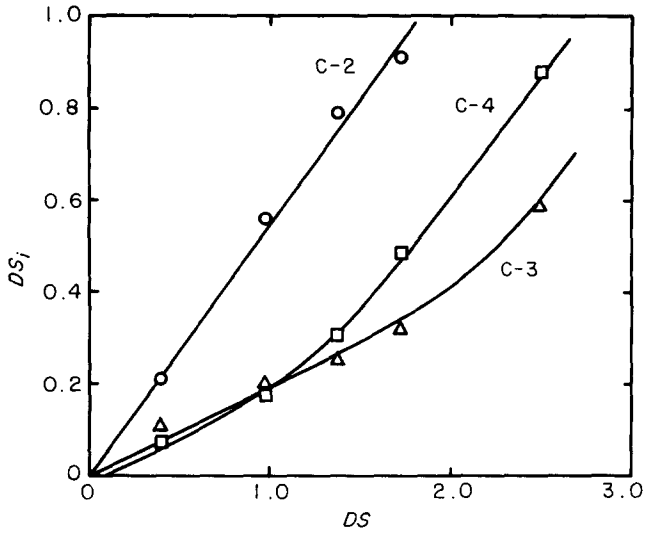


Figure 3 Variation of the degree of substitution at individual hydroxyl groups (DS_i) with the total degree of substitution in modified dextrans prepared by reaction with acetyl chloride

a reactivity change of a hydroxyl group in a carbohydrate during the course of the modification reaction has also been observed by other authors[6,20].

For the reaction of dextran with acetic anhydride, the reactivities of the hydroxyl groups towards acetylating agent decrease in the order C-2≃C-3>C-4.

The different substitution pattern we have obtained in the reaction of dextran with acetyl chloride and acetic anhydride is consistent with previous studies on the substitution reaction of monosaccharides[1]. In this sense, Williams and Richardson[21] have studied the reaction of methyl-α-D-glucopyranoside with benzoyl chloride in pyridine. They found that the relative reactivities of the secondary hydroxyl groups were in the order C-2>C-3>C-4. The greater reactivity of hydroxyl groups at the C-2 position has been correlated in terms of hydrogen bonding with the oxygen in the axial methoxyl group. Sivakumaran *et al.*[22], in a study of the reaction of benzyl-α-D-xylopyranoside with benzoyl chloride, have reported that the order of reactivity was C-2>C-4>C-3. This result was explained by considering that the hydroxyl group at the C-2 position is *cis* to the 1-alkoxy substituent, whereas the hydroxyl group at C-3 suffers *gauche* interactions with a benzyloxy group at position C-2. Jeanloz *et al.*[23] have studied the relative acylation of methyl-4,6-*O*-benzylidene-α-D-glucopyranoside with carboxylic anhydrides and acid chlorides in pyridine. The reaction patterns show a marked dependence on the reagent employed. Acid chlorides yield mainly ester groups at the C-2 position, whereas carboxylic anhydride gave mainly ester groups at C-3. Horton *et al.*[24] have studied the reaction of methyl-α-D-glucopyranoside with acetic anhydride in pyridine, and the reactivity of three secondary hydroxyl groups, as revealed by ^1H n.m.r., was approximately identical. However, in the reaction of methyl-4,6-*O*-benzylidene-α-D-glucopyranoside with acetic anhydride, the substitution molar ratio at C-2 and C-3 positions was 0.30:0.37.

On the other hand, de Belder *et al.*[8] reported that the substitution reaction of dextran with acetic anhydride depends upon the reaction medium, since, whereas all the three secondary hydroxyl groups show almost similar reactivities for the acetylation reaction in aqueous alkali, the reactivity of a hydroxyl group at position C-2 is noticeably enhanced when the acetylation takes place in the presence of pyridine.

Recently, the selectivity of the reaction of dextran with ethyl chloroformate using pyridine as catalyst has been studied[6]. The results obtained show that the hydroxyl group at C-2 is selectively substituted and that the reactivity of the hydroxyl groups at C-3 and C-4 positions are in the order C-4>C-3. These results were explained by considering the formation of intramolecular hydrogen bonding between the hydroxyl group at C-2 and the *cis* anomeric oxygen as well as between the hydroxyl group at C-4 and the carbonyl of the ethyl carbonate at C-2 on the adjacent AHG unit.

The relative reactivities of hydroxyl groups at C-2, C-3 and C-4 positions that we have obtained in the reaction between dextran and acetyl chloride follow the same order reported for the reaction of dextran with ethyl chloroformate. Therefore, the highest reactivity of the hydroxyl group at the C-2 position may be correlated with the *cis* character of the anomeric oxygen and the hydroxyl at C-2, which gives a very favourable 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 in the course of the modification reaction can also be explained in view of molecular models, as follows. It is considered that, once a particular anhydroglucose unit has been modified at position C-2, the hydroxyl group at position C-4 of the adjacent AHG unit presents a spatial orientation with respect to the acetyl group at C-2 mentioned above, which favours 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 hardly form intramolecular hydrogen bonds with the carbonyl group of the substituent at the C-2 or C-4 positions of the same AHG unit.

The order of reactivity of the secondary hydroxyl groups during the acetylation of dextran with acetic anhydride using pyridine as catalyst is presumably a

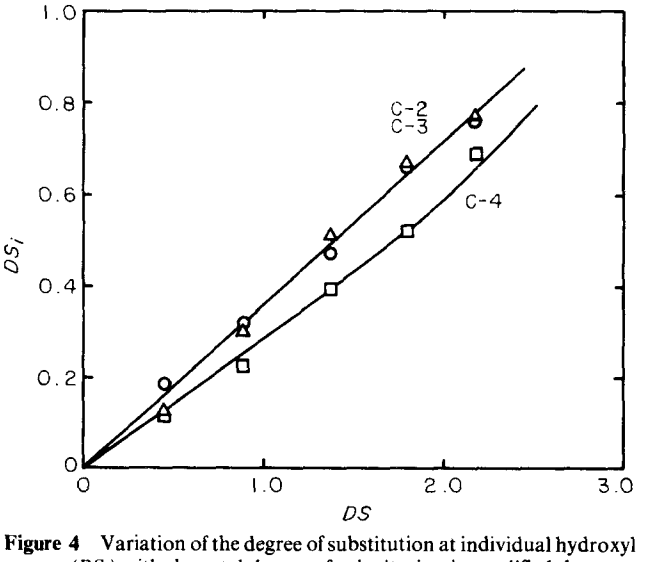
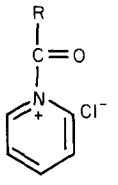
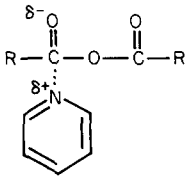


Figure 4 Variation of the degree of substitution at individual hydroxyl groups (DS_i) with the total degree of substitution in modified dextrans prepared by reaction with acetic anhydride

result of the reaction mechanism. Thus, by admitting that the reactive intermediate species in the esterification reactions with acid chlorides in the presence of pyridine is an acyl pyridinium ion:



and that with an acid anhydride it is an ion pair[1]:



the lack of cationic character for the ion pair permits us to consider that the usual steric considerations could dominate the reactivity of hydroxyl groups in the reaction of dextran with acetic anhydride. In this sense molecular models revealed that the hydroxyl group at C-3 is less sterically hindered than the hydroxyl groups at C-2 and C-4 positions. Thus, the hydroxyl group at C-3 is acetylated faster than that at C-4 and is similar to that at the C-2 position in spite of the more favourable orientation of this hydroxyl group for the formation of hydrogen bonding with the *cis* anomeric oxygen.

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REFERENCES

1 Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* 1976, **33**, 11
2 Miyamoto, T., Sato, Y., Shibata, T., Tanahashi, M. and Inagaki, H. *J. Polym. Sci., Polym. Chem. Edn.* 1985, **23**, 1373
3 Yoshimoto, K., Itatani, Y. and Tsuda, Y. *Chem. Pharm. Bull.* 1980, **28**, 2065
4 Miyamoto, T., Sato, Y., Shibata, T., Inagaki, H. and Tanahashi, M. *J. Polym. Sci., Polym. Chem. Edn.* 1984, **22**, 2363
5 Takahashi, S., Fujimoto, T., Barua, B. M., Miyamoto, T. and Inagaki, H. *J. Polym. Sci., Polym. Chem. Edn.* 1986, **24**, 2981
6 Arranz, F., San Román, J. and Sánchez-Chaves, M. *Macromolecules* 1987, **20**, 801
7 Miyaji, H. and Misaki, A. *J. Biochem. (Tokyo)* 1973, **74**, 1131
8 de Belder, A. N. and Norrman, B. *Carbohydr. Res.* 1968, **8**, 1
9 Seymour, F. R., Knapp, R. D., Bishop, S. H. and Jeanes, A. *Carbohydr. Res.* 1979, **68**, 123
10 Vogel, A. I. 'Textbook of Practical Organic Chemistry', 4th Edn., Longman, New York, 1978, p. 277
11 Riddick, J. A. and Bunger, W. B. Techniques of Chemistry. Vol. II, 'Organic Solvents', 3rd Edn, Wiley Interscience, New York, 1970, p. 836
12 Friebolin, H., Keilich, G., Frank, N., Dabrowski, U. and Siefert, E. *Org. Magn. Reson.* 1979, **12**, 216
13 Gagnaire, D. and Vignon, M. *Makromol. Chem.* 1977, **178**, 2321
14 Reuben, J. and Conner, H. T. *Carbohydr. Res.* 1983, **115**, 1
15 McEwan, T., McInnes, A. G. and Smith, D. G. *Carbohydr. Res.* 1982, **104**, 161
16 Komura, H., Matsuno, A., Ishido, Y., Kushida, K. and Aoki, K. *Carbohydr. Res.* 1978, **65**, 271
17 Wu, T. K. *Macromolecules* 1980, **13**, 74
18 Kowsaka, K., Okajima, K. and Kamide, K. *Polym. J.* 1986, **18**, 843
19 Sei, T., Ishitani, K., Suzuki, R. and Ikematsu, K. *Polym. J.* 1985, **17**, 1065
20 Lehrfeld, J. *Carbohydr. Res.* 1975, **39**, 364
21 Williams, J. M. and Richardson, A. C. *Tetrahedron* 1967, **23**, 1369
22 Sivakumaran, T. and Jones, J. K. *Can. J. Chem.* 1967, **45**, 2493
23 Jeanloz, R. W. and Jeanloz, D. A. *J. Am. Chem. Soc.* 1957, **79**, 2579
24 Horton, D. and Lauterbach, J. H. *J. Org. Chem.* 1969, **34**, 86