Relative reactivities of amylose hydroxyl groups in the reaction with diketene

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^{13}C nuclear magnetic resonance (n.m.r.) spectra at 75.4 MHz of partially modified amylose with β-keto ester groups (degree of substitution (*DS*) ranging from 0.52 to 2.42) were studied in order to evaluate the selectivity of the reaction of amylose with diketene in the homogeneous phase. Analysis of the spectra of ring carbons in the anhydroglucose units shows that the reactivity of individual hydroxyl groups decreases in the order C-6>C-3>C-2 for *DS* values up to ~1.8. For higher *DS* values a negative deviation is observed for the hydroxyl group at C-6 and a positive deviation for the hydroxyl groups at C-2 and C-3. The *DS* values determined by ^{13}C n.m.r. spectra are in good agreement with those found by chemical analysis.

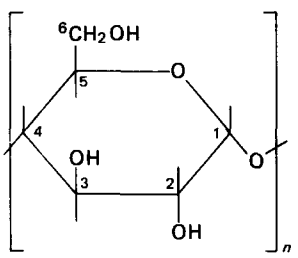
(Keywords: amylose; β-keto ester; relative reactivity; degree of substitution; ^{13}C nuclear magnetic resonance)

INTRODUCTION

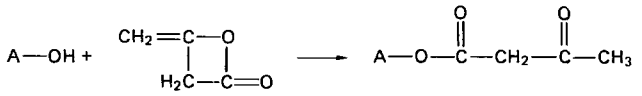
Considerable attention has recently been given to the relative reactivity of hydroxyl groups at the glucopyranosyl units of polysaccharides in view of both theoretical interest and the fact that the distribution of substituents in the anhydroglucose (AHG) units may exert important effects on the behaviour and properties of partially modified polysaccharides.

The difference in reactivity of hydroxyl groups in the AHG residue has been investigated by chemical methods[1-3]. However, at present ^{1}H and ^{13}C n.m.r. have been suggested as reliable methods to evaluate the distribution of substituents in the AHG units[4-8]. The reactivity of hydroxyl groups can be estimated not only from the signals of the ring carbons of AHG but also from the signals of the carbon atoms of the side groups.

As is well known, the AHG unit constituting an amylose molecule is a trihydric alcohol consisting of a primary and two secondary hydroxyl groups at the C-6 and the C-2 and C-3 positions, according to the following scheme:



which offers a variety of possibilities for making useful derivatives. Thus the partial modification of amylose with diketene using tertiary amines as catalyst and dimethylsulphoxide as solvent[9]:



A = amylose residue

EXPERIMENTAL

Preparation of the modified polymers

Partially modified amylose polymers with β-keto ester groups were obtained by reaction of diketene (Fluka) with amylose (Baker Chemical) using dimethylsulphoxide (DMSO) as solvent and pyridine as catalyst[9]. Different precipitants were used to isolate the polymers depending on the degree of substitution. All the samples were purified by reprecipitation, and then they were dried *in vacuo* in the presence of phosphorus pentoxide. The degree of substitution (*DS*) was determined by titration with sodium methoxide in benzene in an *N,N*-dimethylformamide solution of the polymer (1 g/100 ml).A benzene solution of thymol blue (0.3 g/100 ml) was used as indicator[10,11]. Samples of 0.1–0.2 g were taken for titration.

Nuclear magnetic resonance

^{13}C n.m.r. spectra were recorded with a 75.4 MHz Varian XL-300 in the proton-noise decoupled mode using solutions of 10%(w/v) in DMSO-d_6 at 80°C and hexamethyldisilane (HDMS) as internal reference standard. The spectral measurement conditions were similar to those of the structural analysis of cellulose derivatives[7]. The spectra were accumulated for about 16 000 scans with a repetition time of 3 s. The signal areas of the spectra were determined by spectral integration as well as by tracing over the peaks with a planimeter.

RESULTS AND DISCUSSION

In general, the ^{13}C n.m.r. spectra of amylose-modified polymers with β-keto ester groups show three spectral yields polymers with β-keto ester groups. The groups can either react with various organic compounds or be employed in the formation of chelates with transition-metal ions.

In the present work, a ^{13}C n.m.r. study is performed for the quantitative assay of the distribution of β-keto ester groups of some partially modified amylose polymers.

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regions, 29–50, 60–101 and 160–202 ppm, which arise from the methylene and methyl carbon atoms in the β-keto ester groups, from the ring carbons of AHG and from the carbonyl carbon atoms of ketonic and ester groups from the grafted β-keto ester groups, respectively. However, the analysis of these three spectral regions enables us to conclude that only the range 60–101 ppm gives adequate analytical information.

The ^{13}C n.m.r. spectra of the ring carbon region of amylose and several samples of partially modified amylose with β-keto ester groups (*DS* ranging from 0.52 to 2.42) are shown in *Figure 1*. According to Gagnaire *et al.*[12] we have assigned the corresponding signals in the spectrum of amylose to carbons of the AHG units as quoted in *Table 1*.

The spectral analysis of different samples (*Figure 1*) shows a decrease in the intensity of the signals assigned to C-1 to C-6 carbons relative to the amylose spectrum, together with the appearance of several new peaks at 95.5, 76.0, 74.5, 73.0, 70.6, 69.6, 69.0 and 63.4 ppm. In this respect, it is well known that the esterification of a hydroxyl group of glucopyranosic compounds causes an upfield shift of the resonance of the adjacent carbons 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[5,7,8]. On this basis, *Table 1* shows the spectral assignments for monosubstituted amylose polymers taking into account, as a qualitative guide, the chemical shift data reported for oligosaccharides, cellulose and dextran. *Table 1* also shows, for comparison, the chemical shifts of the ring carbon atoms of tri-*O*-acetyl amylose[12]. For monosubstituted derivatives the chemical shifts calculated for resonances of carbons C-1, C-4, C-5 and C-6 appear to be in good agreement with those obtained from the spectral analysis of tri-*O*-acetyl amylose. This result can be attributed to the fact that each of the above-mentioned carbons have a similar environment to that of the corresponding carbons of tri-*O*-acetyl amylose. For resonances of carbons C-2 and C-3 it was not possible to make a similar comparison because each of these monosubstituted derivatives has an unsubstituted hydroxyl group in the adjacent carbon.

In the case of the sample with the highest modification level (*DS* = 2.42) it is difficult to distinguish the resonance signals that appear in the interval 69.6–71.0 ppm because resonance signals of both C-2 and C-3 bearing substituted hydroxyl groups can be expected.

The assignments made by using the chemical shifts

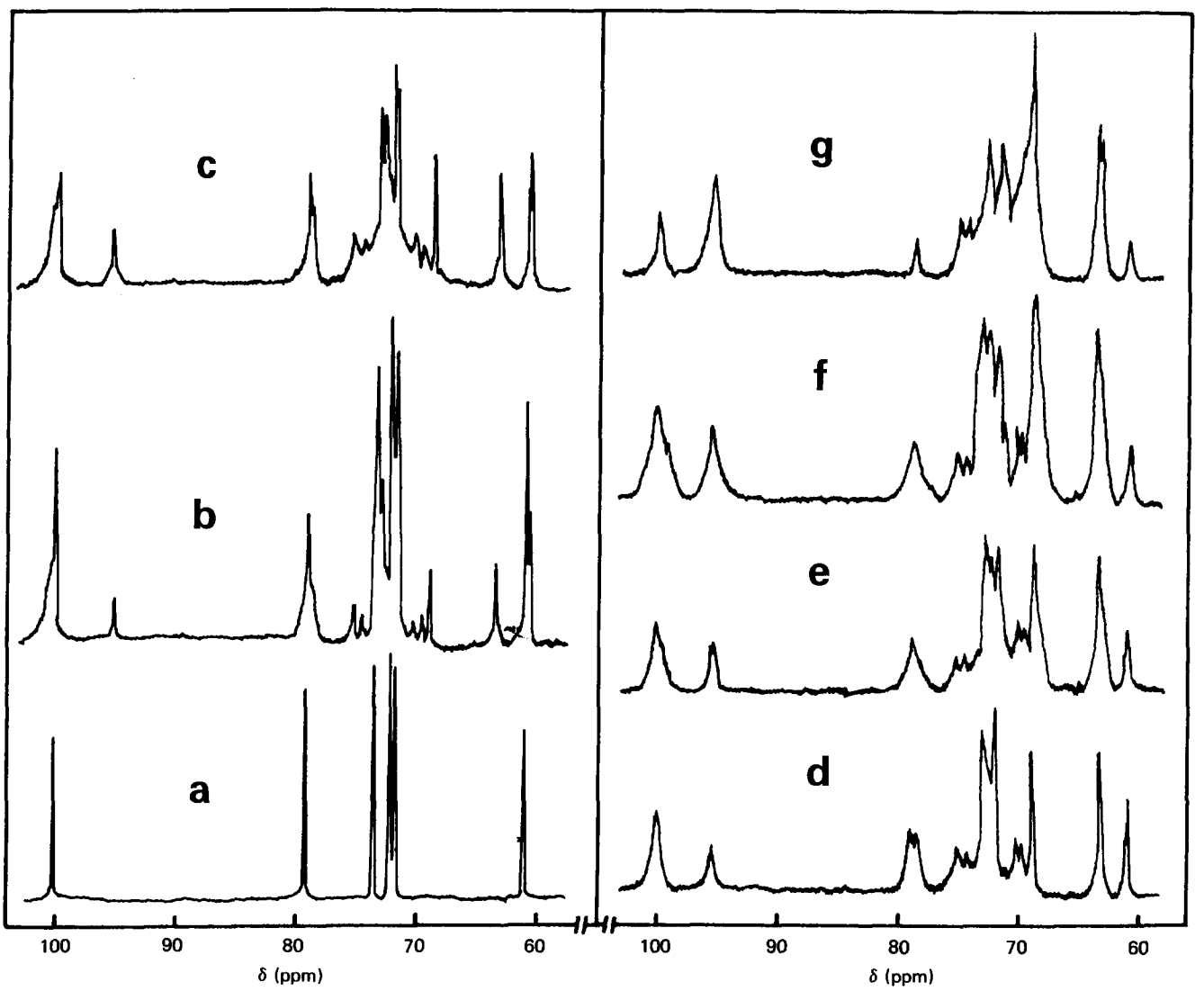
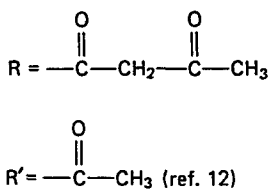


Figure 1 ^{13}C n.m.r. spectra of the ring carbon region of some partially modified amyloses with β-keto ester groups: (a) *DS* = 0; (b) *DS* = 0.52; (c) *DS* = 0.87; (d) *DS* = 1.08; (e) *DS* = 1.40; (f) *DS* = 1.76; (g) *DS* = 2.42

Table 1 Chemical shifts and assignments of ^{13}C n.m.r. signals of partially modified amylose with β-keto ester groups

Substitutents at positions			Chemical shift (ppm)					
2	3	6	C-1	C-2	C-3	C-4	C-5	C-6
OH	OH	OH	100.2	72.3	73.5	79.2	71.9	60.9
OR	OH	OH	95.5	74.5	70.6	79.2	71.9	60.9
OH	OR	OH	100.2	69.6	76.0	73.0	71.9	60.9
OH	OH	OR	100.2	72.3	73.5	79.2	69.0	63.4
OR′	OR′	OR′	95.8	70.6	71.9	73.7	69.2	62.8



reported in *Table 1* for the different carbons of the AHG units permit us to estimate the relative reactivities of hydroxyl groups in the reaction of amylose with diketene. According to several authors[7,8,13] it has been considered that in 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 must be very similar. Therefore, the peak area measurements can be used for the evaluation of the relative *DS* values of individual hydroxyl groups attached to C-2, C-3 and C-6 carbons. Accordingly, the relative intensities of peaks at 95.5 and 100.2 ppm can be taken as a measure of the relative *DS* of hydroxyl groups at the C-2 position. The relative intensities of signals at 63.4 and 60.9 ppm can give the relative *DS* of hydroxyl groups at C-6. The peak at 73.0 ppm overlaps the signals of C-2 and C-3 carbons and it is difficult to estimate its intensity in order to calculate the relative *DS* of hydroxyl groups at the C-3 position. However, it is possible to determine that value by comparing the relative intensity of the peak at 79.2 ppm, belonging to C-4 carbons adjacent to C-3 carbons bearing an unsubstituted hydroxyl group, with the sum of the whole band intensities in the range of 60–101 ppm.

The values so obtained are summarized in *Table 2*. It may be noticed that in all cases the magnitude of the total *DS* determined by chemical analysis and those obtained from the sum of the partial *DS* values of individual hydroxyl groups attached to C-2, C-3 and C-6 carbons are in good agreement.

Figure 2 shows the variation of the relative *DS* of individual hydroxyl groups at C-2, C-3 and C-6 positions as a function of the overall *DS* value. A linear dependence is observed in the initial stages of the esterification reaction ($DS < 1.8$). At higher *DS* values a negative deviation is observed for the hydroxyl group at C-6 and a positive deviation for the hydroxyl groups at C-2 and C-3.

The analysis of data shown in *Figure 2* clearly indicates that the relative reactivities of the three hydroxyl groups of AHG units in the reaction of amylose with diketene using pyridine as catalyst decreased in the following order: C-6 > C-3 > C-2 for *DS* values up to ~1.8. For samples with higher *DS* values the order is C-3 ≃ C-6 > C-2.

The highest reactivity of the primary hydroxyl groups at the C-6 position in the initial stages of the reaction of amylose with diketene is consistent with previous studies on the esterification of amylose[14] and cellulose[7]. According to the mechanism proposed for the reaction of amylose with diketene[9] this behaviour may be explained in terms of a larger steric effect of secondary hydroxyl groups upon further substitution in comparison with primary alcohol groups. A similar correlation between steric effects and reactivity of hydroxyl groups was also proposed in order to justify the results obtained in the reaction of ketenes with low molecular weight alcohols[15].

On the other hand, it is difficult to give a definitive interpretation of the higher reactivity of the hydroxyl groups attached to C-3 carbons with respect to the hydroxyl groups at the C-2 position in the reaction of amylose with diketene. A similar phenomenon was observed by Takahashi *et al.*[16] in the xanthation of cellulose. This behaviour is contrary to the accepted generalization that hydroxyl groups at C-2 are more reactive than secondary hydroxyl groups in α-D-glucopyranoside compounds[17,18]. The remarkable low reactivity of the hydroxyl group at C-3 in acetylation of maltose[19] was attributed to the presence of a strong intramolecular hydrogen bond between the hydroxyl group at C-2 in the first glucopyranoside unit and the hydroxyl group at C-3 in the second glucopyranoside unit, as has been observed in the crystal structures of

Table 2 Comparison of the *DS* at individual hydroxyl groups by ^{13}C n.m.r. in the esterification of amylose with diketene

	DS at position			
DS[a]	C-2	C-3	C-6	Total
0.52	0.11	0.15	0.25	0.51
0.87	0.17	0.24	0.48	0.89
1.08	0.23	0.28	0.56	1.07
1.40	0.33	0.43	0.73	1.49
1.76	0.39	0.53	0.84	1.76
2.42	0.66	0.91	0.89	2.46

[a] Chemical analysis by titration

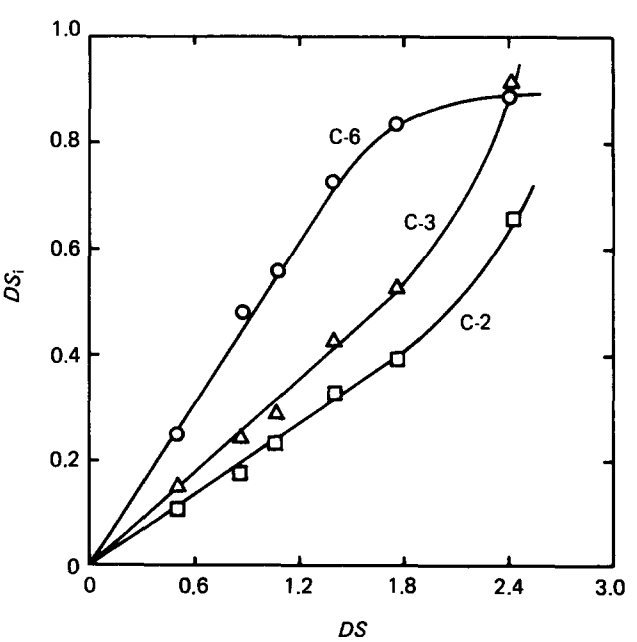


Figure 2 Variation of the degree of substitution at individual hydroxyl groups (DS_i) with the total degree of substitution (*DS*)

maltose monohydrate[20] and has been postulated for maltose in DMSO solution from i.r. and n.m.r. spectral evidence[21]. However, it has been proposed in other cases that the lack of reactivity may be more profound since the intramolecular hydrogen bonding enhances the rate of acylation by acid chlorides[22]. In agreement with this, a similar lack of reactivity of the hydroxyl group at C-3 was found for the β-1,4-linked disaccharide, lactose, which has been shown to adopt preferentially a conformation in which hydrogen bonding between hydroxyl groups at C-2 and C-3 is not possible[23].

From the ^{1}H n.m.r. spectrum of amylose in DMSO, the presence of an intramolecular hydrogen bond between hydroxyl groups at C-2 and C-3 in adjacent AHG units has been postulated[24], as shown in *Figure 3*, which implies substantial right-handed helical character in DMSO. In the reaction of amylose with diketene, there is no clear reason that might justify the link between the preferential reaction of the reactant with hydroxyl groups at C-3 and the formation of an intramolecular hydrogen bond. The difference in the relative reactivities of the two secondary hydroxyl groups in amylose may be due to possibly higher steric hindrance of the hydroxyl group at C-2 with respect to the hydroxyl group at C-3 in the more energetically favourable conformation of amylose.

The low relative reactivity of hydroxyl groups at C-6 observed in the last stages of the reaction between amylose and diketene (*Figure 2*) may be attributed to steric effects of the β-keto ester groups at the C-6 position in the two adjacent AHG units with respect to the unsubstituted hydroxyl group at C-6, as we have verified with molecular models. This may be the reason for the fact that we have found it impossible to prepare completely modified amylose polymers.

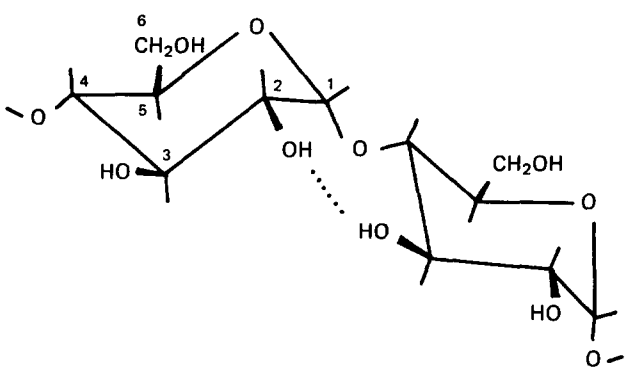


Figure 3 Schematic representation of intramolecular hydrogen bond in adjacent AHG units in amylose

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