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# Comparative Reactivity of the Three Glucosyl -OH Positions -2, -3 and -6 during esterification and saponification of starch by nmr spectroscopy of partially Deutero-acetylated material

Christophe Bliard, Olivier Durand, G. Massiot

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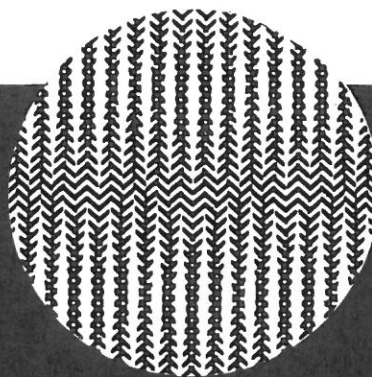
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# Biopolymer Science: Food and Non Food Applications

Montpellier (France)  
September 28-30, 1998

P. COLONNA  
S. GUILBERT

*Editors*



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Montpellier (France), September 28-30, 1998

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## **Comparative reactivity of the three glucosyl -OH positions 2-, 3-, and 6-, during esterification and saponification of starch by NMR spectroscopy of partially deuterio-acetylated material**

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### **ABSTRACT**

The individual reactivity of the three hydroxyle group, (OH-2, OH-3 and OH-6) of the glucosyl monomer residue in starch, under esterification and saponification conditions, were analyzed by  $^1\text{H}$  nmr spectroscopy. A first sample, obtained by direct acetylation, led to an intermediate DS of 1.5, leaving half of hydroxyle positions untouched. These free -OH groups were then esterified with deuterated acetic anhydride. In order to analyze the reactivity under saponification conditions, a second sample was fully acetylated, then, half of the acetate groups were removed by saponification with ethanolic KOH. These regenerated OH groups were then re-esterified with deuterated acetic anhydride as per the previous sample. The proton nmr spectra of each sample was recorded in deuterated chloroform. Integration of the (CH<sub>3</sub>) peaks in the 1 to 2 ppm region allowed the measurement of the relative ( $^1\text{H}$ ) acetate level on each of the three hydroxyle positions.

### **INTRODUCTION**

Faced with new environmental contingencies, polymer industries need to develop new biodegradable products. Starch, the most abundant glucidic reserve polysaccharide in plants, constitutes an interesting renewable source of biopolymers. However, chemical modifications

are required in order to modulate the physico-chemical properties of this highly hydrophilic, soluble biopolymer for use in the plastics field.

In previous works (1) we have shown that high field multidimensional nmr analysis of chemically modified, acetylated starch samples, revealed the precise location of several well-differentiated glucose residues : terminal t, n-1 and n-2 as well as the mean repetitive m unit (fig. 1) and several partly acetylated residues with one of the two secondary positions 2 or 3 free.(fig 2 ) In addition we noticed that two acetylated starch samples, obtained via two different chemical routes, having similar DS, had different  $^1\text{H}$  nmr spectra. These different acetylation patterns on the polymer at a molecular level could result in different behaviors and properties of the material at a macroscopic level.

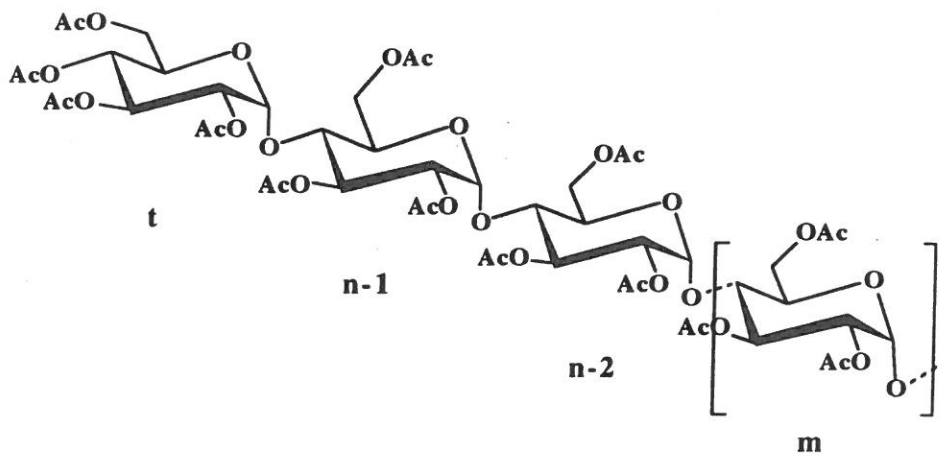
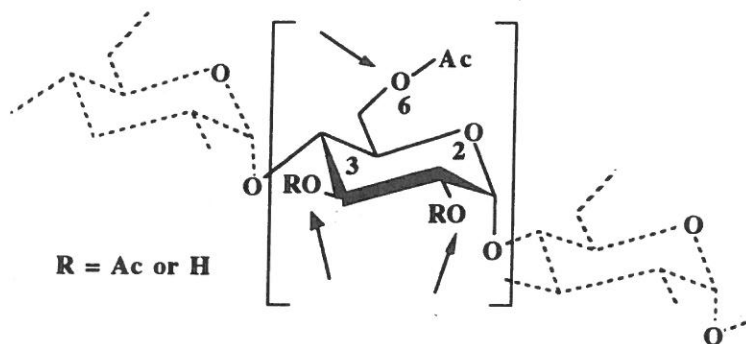


Figure 1



R = Ac or H

Figure 2

This last result prompted us to further investigate the details of the chemical reaction on the polymer more closely by the use of quantitative  $^{13}\text{C}$  (2), and  $^1\text{H}$  nmr spectroscopy. Analysis of the preferential location of acetate groups was possible by quantitative  $^{13}\text{C}$  nmr on highly esterified samples, but due to differences in relaxation times of carbon atoms in various chemical environment and low signal/noise level, precise analysis was difficult to ascertain.

The aim of the present work is to show the respective reactivity of the three -OH positions -2, -3 and -6 of the glucosyl monomer residues ( fig. 2 ) in starch under esterification or saponification conditions by means of  $^1\text{H}$  nmr spectroscopy.

## MATERIAL AND METHODS

On the proton nmr spectrum of fully acetylated starch (fig. 2 R = Ac), the  $\text{CH}_3$  resonance at 1.89, 1.94 and 2.11 ppm (fig 3) are attributed to the three acetate methyl groups in position 3-, 2- and 6- respectively. In order to analyze preferential reactivity on the anhydroglucose monomer, an intermediate DS (close to 1.5) was chosen for the study.

Intermediate DS modified starch samples are not soluble in deuterated chloroform, an nmr solvent allowing a good separation of the acetate's methyl resonance frequencies. Therefore acylation of these samples was completed with deuterated acetic anhydride leading to homogeneous peracylated starch ( acetate/ deutoacetate ) chloroform soluble samples.

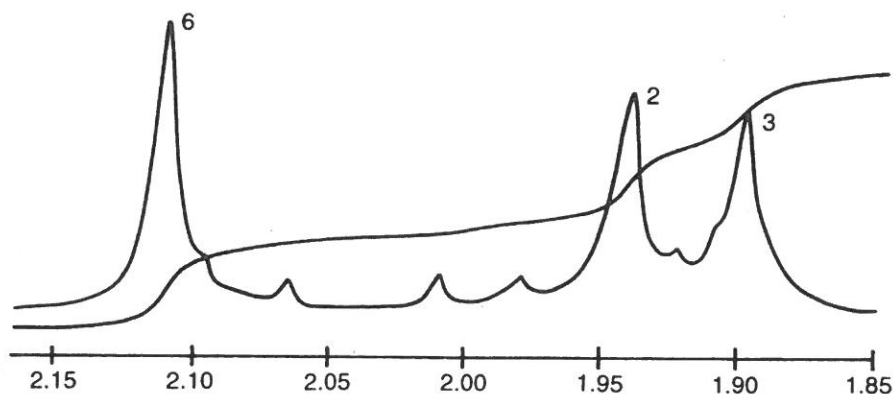


Figure 3

The first sample, A was obtained by direct acetylation of a starch in non-solvent (acetic anhydride/sodium hydroxyde) conditions (3). Using the kinetics of the reaction, the acetylation was stopped at an intermediate DS of 1.5, leaving half of hydroxyle positions untouched. These free -OH groups were then esterified with deuterated acetic anhydride.



The second sample : **B**, used to analyze the reactivity under saponification conditions, was first acetylated in solvent conditions using pyridine and DMAP catalyst up to a DS close to 3 (2.85). Half of the acetate groups were then removed by saponification with ethanolic KOH leading to a DS 1.3. These regenerated OH groups were finally re-esterified with deuterated acetic anhydride as for the previous sample.

Using this procedure, both DS 1.5 and DS 1.3 samples obtained respectively through direct acetylation and partial saponification could be compared. Both samples had identical spectra in the 3 to 6 ppm region (fig. 3) but in the 1-3 ppm region, each peak had different surfaces corresponding to the acetylation level on each of the three positions.

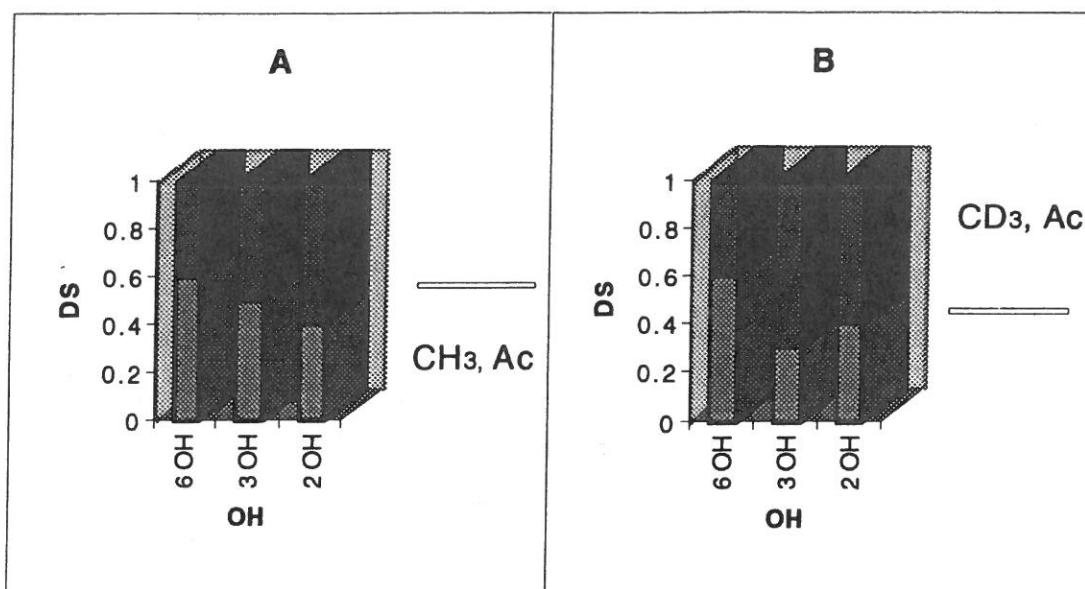
## RESULTS AND DISCUSSION

Results are shown in table 1. In Sample **A**, obtained under kinetics controlled esterification, the order of reactivity is  $6 > 3 > 2$ . In sample **B**, obtained by saponification, the order is  $6 > 2 > 3$ .

Table 1

	DS CH <sub>3</sub> Ac	DS CD <sub>3</sub> Ac		DS CH <sub>3</sub> Ac	DS CD <sub>3</sub> Ac
6A	0.6	0.4	6B	0.6	0.4
3A	0.5	0.5	3B	0.3	0.7
2A	0.4	0.6	2B	0.4	0.6

These results (graph. 1) can be interpreted as follows : In direct partial acetylation, although the order of reactivity is the same as previously found by quantitative <sup>13</sup>C nmr analysis (2), the magnitude is different. Previous analysis showed a similar reactivity for the primary 6-hydroxyle group and the secondary 3-hydroxyle, and a lower reactivity for the secondary 2-hydroxyle group. Here, the difference in reactivity seems more regular, but still, no clear gap can be seen between primary or secondary OH groups. This result seems to indicate a difference in the reactivity of hydroxyle groups of similar positions on the anhydroglucose monomer but in a different location within the macromolecule (i.e. the amorphous versus crystalline zone).



Graph 1

In the sample obtained by saponification, the position 3-OH appears to be the most reactive during deacetylation and unexpectedly, the more reactive, less hindered, primary hydroxyle 6-OH position seems to be much less reactive in these deacetylation conditions, a result which does not seem to be consistent with the others.

As a tentative explanation, an intra- or inter-molecular transesterification of the regenerated reactive primary 6-OH might be shifting an acetate group from a neighboring secondary 2- or 3- position during the saponification process. Although intramolecular transesterification from position 4- to position 6- is a well described (4) reaction in carbohydrate chemistry and widely used in monosaccharide derivative synthesis, to our knowledge, no examples of inter-glycidic transesterification have been described in the oligo or polysaccharide field for acetylated material.

## CONCLUSION

The deuteroacetylation of partially acetylated starch material allowed the quantification by <sup>1</sup>H nmr of the acetate groups on each position of the glucosyl monomer, and therefore the comparative reactivity of each hydroxyle function during acetylation or saponification procedures. Differences in reactivities during esterification and saponification were observed for each of the three hydroxyle positions of the polysaccharide molecules.

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