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Proton NMR spectroscopy assignment of D-glucose residues in highly acetylated starch

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Abstract

^{1}H NMR spectroscopy assignments have been obtained for starch acetates using COSY and HOHAHA experiments by comparison with the spectra of amylose triacetate and of peracetylated malto-oligosaccharides (maltotriose, maltotetraose, maltoheptaose). These assignments are valuable for the location and evaluation of the substitution pattern in modified starches. The bulk of the ^{1}H NMR spectra of highly acetylated starch strongly resembles the spectrum of amylose triacetate in which all protons are identified and display distinct chemical shifts. The resolving power of the HOHAHA experiment allowed the distinction of minor spin systems. Beside these strong signals pertaining to an average 2,3,6-tri-*O*-acetyl-α-(1 $\rightarrow$ 4) linked D-glucopyranose unit in an infinite chain, the combination of COSY and HOHAHA experiments allowed the identification of these systems to the terminal, n-1, n-2, and to partially acetylated glucopyranosyl units. As an example, two different preparations of starch acetates with degrees of substitution 2.74 and 2.63 were examined. In one case, NMR demonstrates that the defects of acetylation are random on the polymeric chain (with corresponding signals for unacylated secondary hydroxyl positions at δ 3.61 and 3.40) while in another case, these signals are not detectable, probably due to the presence of clusters of non-acetylated residue forming solid-like zones.

Keywords: Peracetylated malto-oligosaccharides; Amylopectin acetate; Amylose triacetate; Starch acetate; ^{1}H NMR; COSY; HOHAHA

1. Introduction

The increasing use of native and modified polysaccharides in thermoplastic polymers [1] has resulted in a growing interest in starch as a renewable and environmentally compatible polymer source. In the course of a program devoted to the chemical modification of starch, the possibility of using NMR spectroscopy for the characterization of modified starches has been investigated.

Starch, the generic name for the D-glucose storage

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polysaccharide in plants, consists of two families of macromolecules, amylose, and amylopectin, which differ in molecular structure, physical, chemical, and biological properties. Amylose is mainly a linear (1 → 4)-α-D-glucopyranan with a degree of polymerization ranging from 6×10^2 to 6×10^3 ($M = 10^5$–10^6 Da) and with a limited degree of α-(1 → 6) branching [2,3]. Amylopectin, more complex, is a (1 → 4)-α-D-glucopyranan with α-(1 → 6) linkages and a higher dp ranging from 6×10^4 to 6×10^5 (10^7–10^8 Da). The α-(1 → 6) branching accounts for 5–6% of all the glycosidic bonds of amylopectin [2]. Although amylopectin is by far the major component of starch granules, representing around 80% in weight of the macromolecules, its content may vary widely depending on its botanical origin and the genetic selection [4]. The two components are separable due to their different physical properties. In the native starch granule, amylose is included in the highly ordered semicrystalline amylopectin complex and can be washed out of the native granule by solubilization in warm water, leaving intact amylopectin. Amylose is precipitated from the solution as insoluble inclusion complexes with small organic molecules [5].

In contrast to other families of complex macromolecules, relatively few results have been published on solution NMR spectroscopy of chemically modified starch. In this paper, NMR spectroscopy is shown to be an extremely powerful tool in the investigation of these complex macromolecules.

2. Results and discussion

Previous results [6] involving proton NMR spectroscopy of amylopectin demonstrated that, when

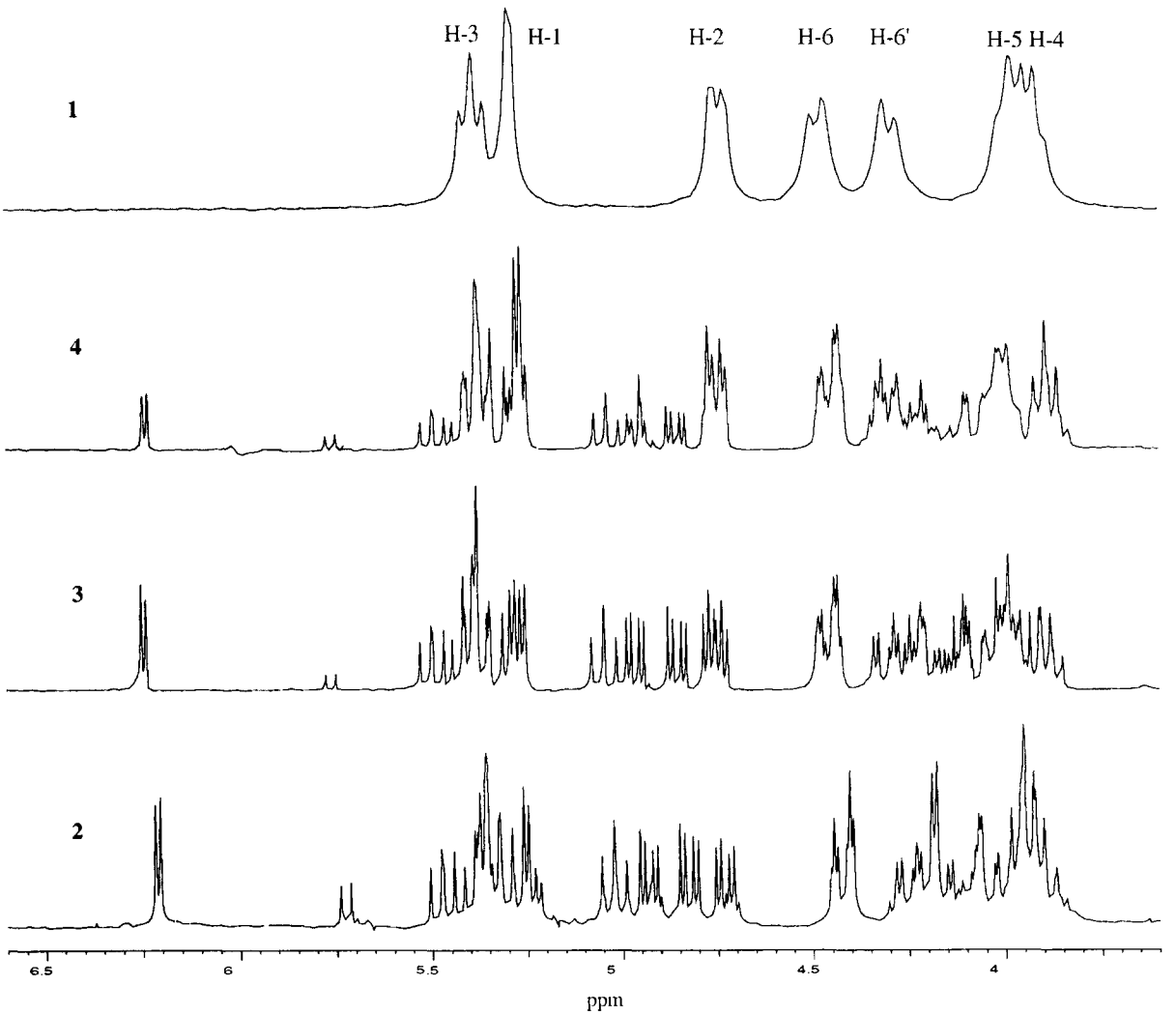


Fig. 1. 300-MHz ^{1}H NMR spectra of amylose triacetate (**1**), maltoheptaose peracetate (**4**), maltotetraose peracetate (**3**), and maltotriose peracetate (**2**), in $CDCl_3$ at 50 °C.

compared with the anomeric protons of unbranched residues, the anomeric proton of the α-(1 → 6) branched D-glucose residue is slightly shielded. We now report the full assignment of the entire spin systems of several D-glucose units in acetylated polymers by means of ^{1}H NMR spectroscopy. Two different starch acetates were investigated with reference to spectra of model molecules: amylose triacetate and acetylated malto-oligosaccharides.

Amylose triacetate.—The spectrum of amylose triacetate **1** has been previously assigned (Fig. 1), despite broadening of lines due to relaxation parameters on one hand and to the superimposition of a large set of nearly equivalent systems on the other. Seven separate proton signals were shown, whose multiplicity and chemical shifts were found to be in good agreement with the spin system expected for a 2,3,6-tri-*O*-acetyl-α-(1 → 4) linked D-glucopyranose unit [7–10] (Table 1). The other two distinct units in amylose, i.e. the upstream terminal 2,3,4,6-tetra-*O*-

Table 1
^{1}H NMR data [a] for amylose triacetate **1** and acetylated malto-oligosaccharides **2**, **3** and **4**

Residue	Protons	Amylose triacetate	Acetylated malto-oligosaccharides		
		1	**2**	**3**	**4**
α-Glc^I	H-1 ($J_{1,2}$)	–	6.22 d (3.7)	6.25 d (3.7)	6.25 d (3.7)
	H-2 ($J_{2,3}$)	–	4.94 dd (10.1)	4.97 dd (9.9)	4.96 dd (9.6)
	H-3 ($J_{3,4}$)	–	5.48 t (9.0)	5.51 t (8.8)	5.50 t (9.7)
	H-4 ($J_{4,5}$)	–	3.96 t (9.3)	4.00 t (9.1)	4.00 t (8.9)
	H-5 ($J_{5,6'}$)	–	3.87 m (3.6)	4.13 m (3.8)	4.10 m (5.3)
	H-6 ($J_{5,6}$)	–	4.43 dd (2.8)	4.47 dd (2.6)	4.47 dd (2.7)
	H-6′ ($J_{6,6'}$)	–	4.26 dd (12.3)	4.32 dd (12.4)	4.30 dd (12.3)
Glc^{II}	H-1 ($J_{1,2}$)	–	–	5.30 d (4.0)	5.31 d (4.4)
	H-2 ($J_{2,3}$)	–	–	4.76 dd (10.1)	4.75 dd (10.5)
	H-3 ($J_{3,4}$)	–	–	5.42 t (8.6)	5.42 t (9.6)
	H-4 ($J_{5,6}$)	–	–	3.89 t (9.2)	3.90 t (9.0)
	H-5 ($J_{5,6'}$)	–	–	4.03 m (4.1)	4.02 m (3.8)
	H-6 ($J_{5,6}$)	–	–	4.46 dd (2.4)	4.46 dd (2.8)
	H-6′ ($J_{6,6'}$)	–	–	4.26 dd (12.4)	4.32 dd (12.4)
Glc$_m$ [b]	H-1 ($J_{1,2}$)	5.30 d (3.7)	–	–	5.28 d (4.0)
	H-2 ($J_{2,3}$)	4.75 dd (10.1)	–	–	4.75 dd (10.1)
	H-3 ($J_{3,4}$)	5.40 t (9.3)	–	–	5.39 t (9.4)
	H-4 ($J_{4,5}$)	3.93 t (9.0)	–	–	3.90 t (9.0)
	H-5 ($J_{5,6'}$)	3.99 m (–)	–	–	4.00 m (3.8)
	H-6 ($J_{5,6}$)	4.49 m (–)	–	–	4.46 dd (2.8)
	H-6′ ($J_{6,6'}$)	4.30 m (–)	–	–	4.32 dd (12.4)
Glc$_{n-1}$	H-1 ($J_{1,2}$)	–	5.26 d (3.9)	5.27 d (4.0)	5.27 d (4.1)
	H-2 ($J_{2,3}$)	–	4.74 dd (10.2)	4.75 dd (10.2)	4.75 dd (10.1)
	H-3 ($J_{3,4}$)	–	5.40 t (8.3)	5.39 t (8.6)	5.38 t (9.5)
	H-4 ($J_{4,5}$)	–	3.91 t (9.0)	3.92 t (9.0)	3.89 t (9.0)
	H-5 ($J_{5,6'}$)	–	3.98 m (3.7)	3.99 m (3.5)	3.96 m (4.2)
	H-6 ($J_{5,6}$)	–	4.44 dd (2.3)	4.48 dd (2.2)	4.47 dd (2.8)
	H-6′ ($J_{6,6'}$)	–	4.17 dd (12.3)	4.19 dd (12.5)	4.21 dd (12.1)
Glc$_n$ [c]	H-1 ($J_{1,2}$)	–	5.38 d (4.3)	5.40 d (4.1)	5.36 d (4.0)
	H-2 ($J_{2,3}$)	–	4.83 dd (10.5)	4.86 dd (10.5)	4.86 dd (10.5)
	H-3 ($J_{3,4}$)	–	5.30 t (10.1)	5.36 t (10.3)	5.35 t (11.1)
	H-4 ($J_{4,5}$)	–	5.03 t (9.7)	5.06 t (9.8)	5.05 t (9.8)
	H-5 ($J_{5,6'}$)	–	3.93 m (2.2)	3.97 m (2.4)	3.96 m (2.8)
	H-6 ($J_{5,6}$)	–	4.21 dd (3.7)	4.24 dd (3.8)	4.23 dd (3.9)
	H-6′ ($J_{6,6'}$)	–	4.05 dd (12.4)	4.08 dd (12.6)	4.09 dd (12.5)

[a] $CDCl_3$, at 50 °C, 300 MHz. Chemical shifts in ppm, J in Hz.
[b] m = Mean residue.
[c] n = Non-reducing terminal residue.

acetyl-α-(1 → 4)-linked D-glucopyranosyl and the downstream 'reducing' 1,2,3,6-tetra-*O*-acetyl-α-(1 → 4)-D-glucopyranose were not observed in the ^{1}H NMR spectrum. This is due to the degree of polymerization of amylose, which varies between 6×10^2–6×10^3. The terminal units represent 0.16–0.016% of the

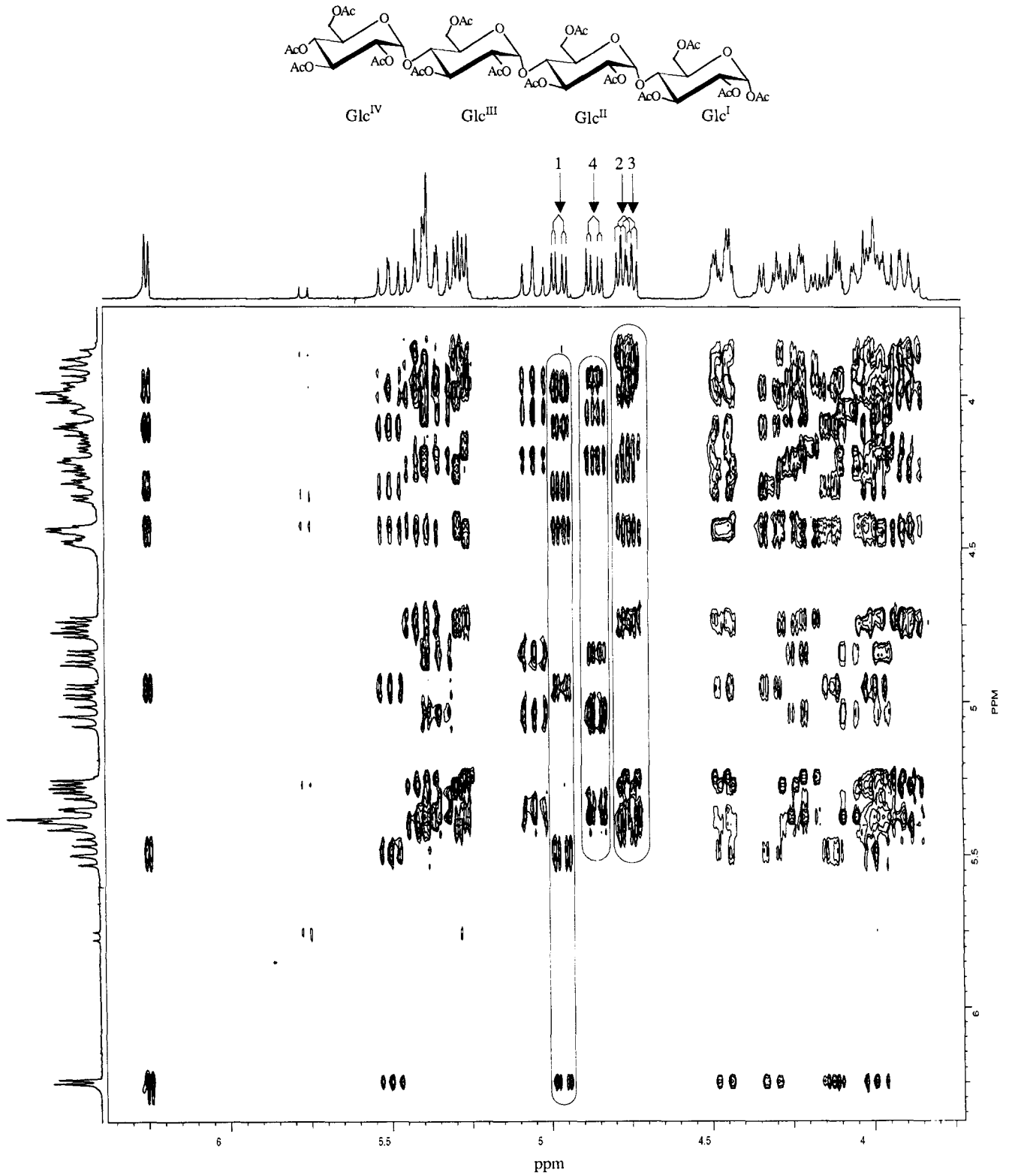
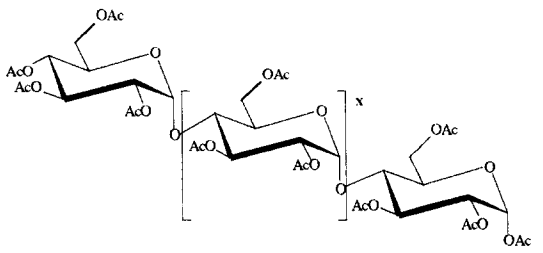


Fig. 2. 300-MHz HOHAHA spectrum of **3** in $CDCl_3$ at 50 °C. 1, 2, 3, and 4 correspond to H-2 of the Glc^{I}, Glc^{II}, Glc^{III}, and Glc^{IV} units of **3**. Boxes 1, 2, 3, and 4 correspond to Glc^{I}, Glc^{II}, Glc^{III} and Glc^{IV} spin systems of **3** read along H-2 columns.

signal, which is much below NMR spectroscopy detection capabilities.



	x
(1)	600-6000
(2)	1
(3)	2
(4)	5

Peracetylated malto-oligosaccharides.—Representative oligosaccharides, chosen as references, are the (1 → 4)-α-D-gluco-oligosaccharides maltotriose, maltotetraose, and maltoheptaose, which were acetylated in pyridine. For each oligosaccharide, the α-anomers were obtained predominantly by anomerization with zinc chloride in acetic anhydride. The ^{1}H NMR spectra were assigned with the help of COSY [11], HOHAHA [12] and ROESY experiments [13] at 300 MHz.

The NMR subspectra of the three units of maltotriose peracetate **2** were ascertained by means of a COSY experiment. Analysis started with the well-separated anomeric doublets, from which H-2 protons were assigned and hence, H-3, H-4, H-5, and H-6. The 'reducing' 1,2,3,6-tetra-*O*-acetyl-(1 → 4)-α-D-glucopyranosyl unit was differentiated by the chemical deplacement of H-1 which was found to be deshielded as a result of acetylation. As a consequence of the presence of the acetate in position C-4 of the terminal unit, proton H-4 of the terminal unit was also found to be deshielded, whereas H-6 and H-6′ of the terminal unit were found to be shielded with regard to the corresponding protons in the inner sugar unit (Table 1).

The HOHAHA experiment run on maltotetraose peracetate **3** allowed assignment of each individual spin system (Fig. 2). The inter-residual H-4 to H-1 correlations in the ROESY experiment were used to distinguish the spin systems of the two central units

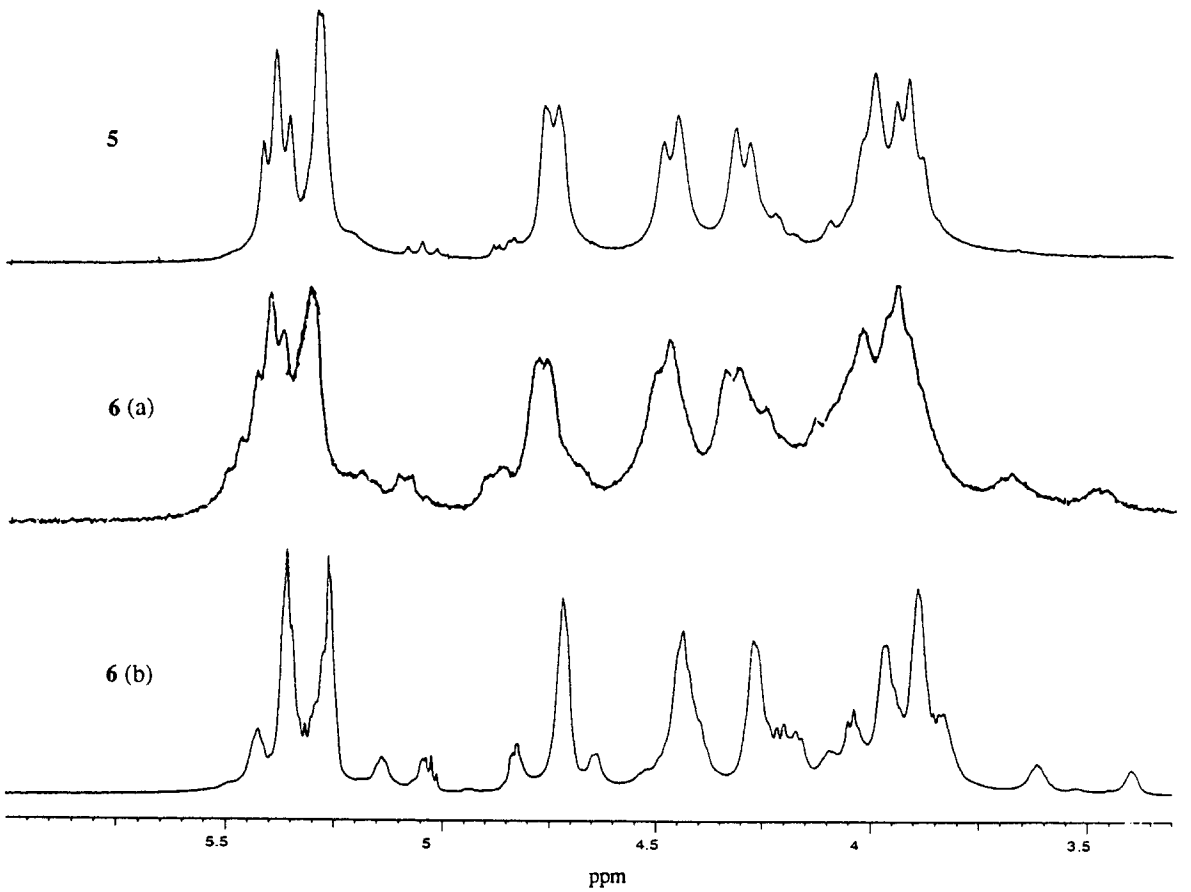


Fig. 3. ^{1}H NMR spectra in $CDCl_3$ at 50 °C of starch acetates **5** at 300, **6**(a) at 300, and **6**(b) at 800 MHz.

(Table 1). The other signals were assigned using the same approach as for the trisaccharide.

The major α-anomer of maltoheptaose peracetate **4** displayed signals for seven glucosidic units, some of which were superimposed at the 300-MHz observation field. The signals of stronger intensity corresponded to the mean unit 2,3,6-tri-*O*-acetyl-(1 → 4)-α-D-glucopyranose. The HOHAHA experiment allowed the assignment of the spin systems of the terminal and the 'reducing' units, with characteristic chemical shifts for H-2 and H-4 in the fully acetylated upstream terminal residue and for H-1 in the 'reducing' 1,2,3,6-tetra-*O*-acetyl-(1-4)-α-D-glucopyranosyl unit (Table 1). Two other spin systems were also observed which differed from the mean unit by the chemical shifts of H-6 and H-3. By comparison with the spectral data of **2** and **3**, it was concluded that they corresponded to the penultimate units of **4**.

Spectra of samples **1–4**, drawn at the same scale, are shown in Fig. 1. The less complex spectrum of maltotriose peracetate displays three sets of seven spin systems for the predominant α-anomer plus a detectable seven spin system for the upstream 'reducing' end of the minor β isomer. A maximum complexity is reached with the spectrum of maltoheptaose peracetate **4**, with eight different seven spin systems. When the sugar chain is long enough, the reducing unit was no longer observable and the spectrum of an average 2,3,6-tri-*O*-acetyl-α-(1 → 4)-α-D-glucopyranan in an infinite chain was obtained. The protons which seemed to be less sensitive to their environment were H-1, H-2, H-3, and one H-6. Protons H-4, H-5, and the other H-6 showed a broader distribution of chemical shifts.

Starch acetate of ds 2.74 (Method A).—Starch acetate **5** of ds 2.74 was prepared by reaction of destructured wheat starch in pyridine with acetic anhydride at 120 °C for 5 h. In the 3–6 ppm area, the most intense signals had multiplicities and chemical shifts corresponding to the mean 2,3,6-tri-*O*-acetyl-(1 → 4)-α-D-glucopyranosyl unit (Fig. 3). Beside these main signals, well resolved signals of weak intensity at δ 4.85 (dd) and δ 5.04 (t) were also observed (Table 2). The COSY spectrum (300 MHz) allowed the assignment of the protons of the associated spin system, namely seven protons with chemical shifts corresponding to a terminal 2,3,4,6-tetra-*O*-acetyl-(1 → 4)-α-D-glucopyranose unit. On the 1D ^{1}H spectrum at 300 MHz, only protons H-2, H-4, H-6, and H-6′ were directly observed. The other protons of the system were found to be superimposed with the stronger signals belonging to the mean unit.

After analysis and substraction of correlations per-

Table 2
^{1}H NMR data [a] for starch acetates **5** and **6**

Starch acetate	Protons	Residue					
		fully acetylated residue				partially acetylated residue	
		Glc_m [b]	Glc_{n-2}	Glc_{n-1}	Glc_n [c]	2-OH	3-OH
5 [d]	H-1	5.27	5.26	5.26	5.38	–	–
	H-2	4.73	4.73	4.73	4.85	–	–
	H-3	5.37	5.37	5.37	5.33	–	–
	H-4	3.90	3.90	3.90	5.04	–	–
	H-5	3.97	3.97	3.95	3.95	–	–
	H-6	4.45	4.45	4.45	4.22	–	–
	H-6′	4.27	4.23	4.17	4.04	–	–
6 [e]	H-1	5.26	5.26	5.25	5.36	5.05	5.29
	H-2	4.72	4.72	4.72	4.84	3.40	4.64
	H-3	5.36	5.36	5.36	5.31	5.14	4.04
	H-4	3.89	3.85	3.89	5.02	3.82	3.61
	H-5	3.97	3.97	3.95	3.94	3.94	3.83
	H-6	4.42	4.42	4.42	4.21	4.42	4.43
	H-6′	4.27	4.23	4.16	4.04	4.24	4.27

[a] $CDCl_3$, at 50 °C. Chemical shifts in ppm.
[b] m = Mean residue.
[c] n = Non-reducing terminal residue.
[d] 600 MHz.
[e] 800 MHz.

taining to the two preceding spin systems, several other correlations in the COSY map appeared unassigned. One of these corresponded to a correlation between protons at δ 4.17 and 3.95, which were found to be included into a third spin system, differing from the average unit by the chemical shift of one of the H-6 protons at δ 4.17 (HOHAHA experiment at 300 MHz). By increasing the magnetic field to 600 MHz, a fourth full spin system was observed, which also differed from the average unit by the chemical shift of one of the H-6 at δ 4.23. These two spin systems correlate with 2,3,6-tri-*O*-acetyl-α-(1 → 4)-D-glucopyranosyl units in different chemical environments with regard to the average unit. Comparison with the observations made on the peracetylated malto-oligosaccharides **2**, **3**, and **4**, suggested that they corresponded to the n-1 and n-2 units (n = non-reducing terminal unit).

Starch acetate of ds 2.63 (Method B).—Starch acetate **6** of ds 2.63 was prepared from native wheat starch, in the presence of acetic anhydride and sodium hydroxide, at 120 °C, for 5 h [14]. Despite the similar degree of substitution, samples **5** and **6** were surprisingly different as seen on their ^{1}H NMR spectra (Fig. 3). The COSY and HOHAHA spectra at 300 MHz allowed a partial assignment of the spin systems. A HOHAHA experiment at 800 MHz was recorded to gain further insight into the spin systems. The map, displayed in Fig. 4, shows the four spin systems present in **5** with good resolution: a mean 2,3,6-tri-*O*-acetyl-(1 → 4)-α-D-glucopyranosyl unit, a terminal 2,3,4,6-tetra-*O*-acetyl-(1 → 4)-α-D-glucopyranosyl unit, n-1 and n-2 2,3,6-tri-O-acetyl-(1-4)-α-D-glucopyranosyl units. Two broad peaks at δ 3.61 and 3.40 were observable in the high field part of the spectrum of **6**, which were not present in the spectrum of **5**. The HOHAHA experiment allowed access to all the protons of these spin systems (Fig. 4) and their sequence was determinated by the COSY map recorded at 300 MHz. Those spin systems were

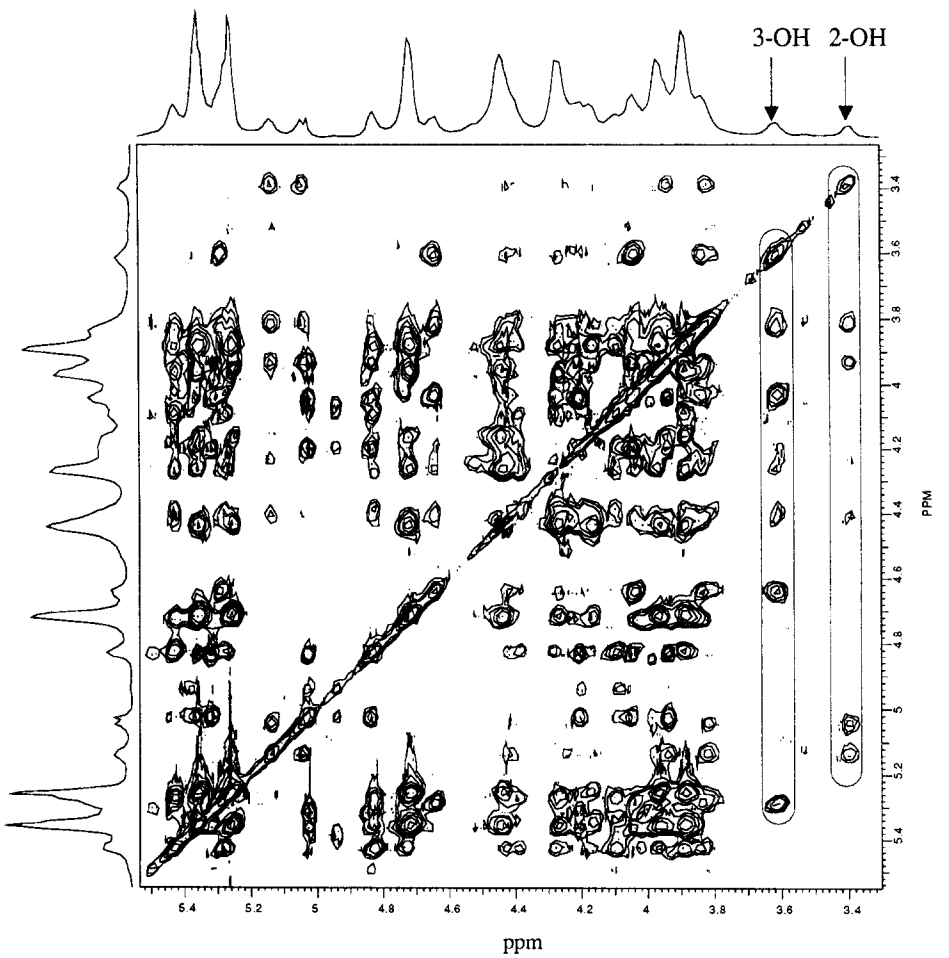


Fig. 4. 800-MHz HOHAHA spectrum of **6** in $CDCl_3$ at 50 °C. Spin systems indicated by arrows are those of unacetylated glucose units at C-2 and C-3.

assigned to partially acetylated units: 2,6-di-*O*-acetyl-(1 → 4)-α-D-glucopyranose and 3,6-di-*O*-acetyl-(1 → 4)-α-D-glucopyranose (Table 2). The 2,6-di-*O*-acetyl-(1 → 4)-α-D-glucopyranosyl unit differs from the average unit by the chemical shift of H-3 (δ 4.04) and H-4 (δ 3.61). In the 3,6-di-*O*-acetyl-(1 → 4)-α-D-glucopyranose system H-2 (δ 3.40) and H-1 (δ 5.05) are shielded to a noteworthy extent.

Respectively, 8.7% and 12.3% of hydroxyl functionalities of the starting material in sample **5** and **6** remained untouched by the acetylation. In a random acetylation mechanism, the resonances of non-acetylated positions would be expected in the 3–3.8 ppm area with all intermediates: 2, 3, and 6 mono-, and di-acetylated glucose unit combinations. Resonances at δ 3.61 and 3.40 corresponding to the C*H*OH of two di-acetylated glucose units were only present in sample **6**. The reason why, in sample **5**, the residual free hydroxyl groups seen on the IR spectrum, remained undetected by ^{1}H NMR could be linked to the formation of clusters of non-acetylated glucose residues, unsolvated in deuterochloroform, and behaving like solid lumps in the remainder of the solvated polymer and thus appearing as extremely broad and undetectable signals.

The origin of the **5** versus **6** difference is not clear and may arise from reaction conditions or starting materials. Native starch was used under Schotten–Baumann conditions and it may be expected that, in this sample, the sugar chain is more entangled and thus less accessible to the reagents. When the starch is amorphous (destructured), all the free hydroxyl functionalities are equally accessible and hence the sample is randomly acetylated. From another point of view, this observed difference appears to resemble the incomplete acetylation of maltose [15], whose 3-OH group is left intact as a consequence of the difficulty of breaking down the inter-residual hydrogen bond between 3-O*H* and 2′-O*H* of the neighboring glucose unit [16], without a dissociating solvent.

3. Conclusion

High field NMR studies of starch derivatives are potentially useful in the fine characterization of samples from both chemical and structural standpoints. First, they enable us to distinguish between samples **5** and **6** and to identify precisely the position of the defect of acetylation. The second area for which NMR may become important is the measurement of branching ratios. The number of terminal units was found to be measurable by integration of the signal for isolated H-4, corresponding to the number of ramifications plus one. In these large molecules, one may consider these numbers to be the same. The figure obtained for sample **5** is 3% and must be corrected for amylopectin content (73%) and acetylation ratio (ds 2.74), assuming that imperfect acetylation is random. The direct quantification of signals belonging to sugars engaged in ramifications would provide an interesting confirmation of these values, but even at 800 MHz, it was not possible to assign these signals with any certainty. A description of a model for amylopectin branching published in the literature [17] suggests that, in our spectra, H-6 resonances occur at δ 3.8 and 4.1, precisely in areas of strong signals overlap.

4. Experimental

Materials.—Maltotriose, [α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose] hydrate, maltotetraose [α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 4)-D-glucopyranosyl-(1 → 4)-D-glucopyranose] hydrate, maltoheptaose hydrate, amylose [(1 → 4)-α-D-glucopyranan] were obtained from Aldrich. Amylopectin from corn was obtained from Sigma. Native wheat starch, containing 13% water (w/w), was obtained from Agro-industrie Recherches et Developpements (Route de Bazancourt, F-51110 Pomacle, France).

NMR spectra.—^{1}H NMR spectroscopy measurements were performed with Bruker AC-300, AMX-600, and DRX-800 spectrometers at 300, 600, or 800 MHz in 5 mm tubes at 50 °C in $CDCl_3$ at concentrations of 20–40 mg per mL. Chemicals shifts were referenced from the residual proton signal of $CDCl_3$ (δ 7.27 for ^1H). Magnitude mode 2D H–H COSY maps were obtained by acquisition of 1024 × 256 data matrices, with 88 scans per experiment, relaxation delay of 2 s between scans, and a spectral width of 1923 Hz in both dimensions. The spectrum was processed by using a sine bell filtering function in both dimensions before zero filling in F_1. The 2D FT and magnitude calculation yielded a 512 × 512 real data matrix. HOHAHA measurements were performed on a Bruker AMX-600 or Bruker DRX-800 spectrometer with 2048 × 256 or 2048 × 512 data matrix, 96 or 16 scans per increment, a spectral width of 3521 Hz or 4807 Hz in both dimensions, a relaxation delay of 2 s and a mixing time of 200 ms. The spectrum was processed by using a cosine bell filter-

ing function in both dimensions before zero filling in F_1. The size of the RR quadrant of the hypercomplex 2D FT data matrix was 1024 × 256 or 1024 × 512. ROESY measurement was carried out on a Bruker AC-300 with 2048 × 256 data matrix and a mixing time of 200 ms.

General procedure.—IR spectra were recorded on Bomem MB-Series spectrophotometer, with 30 scans and a resolution of 4 cm^{-1}. Optical rotations were measured on a Perkin–Elmer 241 automatic polarimeter in $CHCl_3$.

Determination of the degree of substitution (ds).—A dried sample of powdered starch acetate (1 g) was placed in a 250 mL flask and 75% EtOH (50 mL) were added. The soln was stirred at 50 °C for 30 min, and cooled to room temperature, 0.5 N KOH (40 mL) was added with swirling. The flask was stoppered, then allowed to stand 72 h with occasional swirling. The excess of alkali was back titrated with 0.5 N hydrochloric acid using phenolphthalein as an indicator. The soln was allowed to stand for 2 h, then any additional alkali which might leach from the sample was titrated. A blank was titrated in parallel.

$$\%\text{acetyl} = \frac{(V_a - V_b) \times N_{HCl} \times M_{acetyl}}{m_s}$$

where V_a is the volume of HCl for the blank in L, V_b is the volume of HCl for the sample in L, $N_{HCl} = 0.5$ N is the normality of HCl, $M_{acetyl} = 43$ g/mol, and m_s is the weight of sample (dry) in g.

$$\text{ds} = \frac{162 \times \%\text{acetyl}}{(M_{acetyl} \times 100) - (M_{acetyl} - 1) \times \%\text{acetyl}}$$

General procedure for oligosaccharide acetylation.—To a solution of the oligosaccharide in dry pyridine was added a twofold excess of Ac_2O. The mixture was stirred and heated at 50 °C for 24 h under inert atmosphere. The mixture was cooled at room temperature, poured into ice-water, and stirred for an additional hour. The precipitate was filtered off, washed several times with water, and dissolved in CH_2Cl_2. The filtrate was extracted three times with CH_2Cl_2. The organic phases were combined, washed with 1 M aq HCl, then with satd aq $NaHCO_3$, water, dried ($NaSO_4$), and evaporated to dryness. In the case of amylose triacetate, an extra step of purification was used at this stage. The solid was stirred in abs EtOH, filtered off, washed twice with of abs EtOH (50 mL), and dried in vacuo at 100 °C for 24 h.

Starch acetate (**5**)*; method A.*—Native wheat starch (100 g) was suspended in 400 mL of distilled water in a three necked round bottom flask equipped with reflux condenser and mechanical stirrer. The mixture was stirred for 1 h at 100 °C. The resulting starch gel was rapidly cooled and freeze-dried. An aliquote of the freeze-dried starch (5 g) was added to 75 mL of dry pyridine, and the mixture was refluxed for 1 h, with vigorous stirring. Acetic anhydride (17 mL) was then added to the soln and the mixture was stirred for 5 h at 120 °C. The soln was poured into 500 mL of ice-water and stirred vigorously for 1 h. The precipitated solid was filtered off and washed twice with water (50 mL). The solid was suspended in 100 mL of water and neutralized. The solid was filtered again and washed twice with water (50 mL). The white solid was then stirred in abs EtOH (100 mL), filtered off, washed twice with abs EtOH (50 mL), and dried under reduced pressure at 100 °C for 24 h resulting in 6.58 g of product with ds 2.74. $[\alpha]_D = +156.9°$ (*c* 0.2, $CHCl_3$); IR (KBr): ν 3600–3400, 2962, 1749, 1371, 1229, and 1034 cm^{-1}.

Starch acetate (**6**)*; Method B.*—Native wheat starch (57 g, 50 g dry weight) was placed in a 500 mL three-necked round bottom flask equipped with a mechanical stirrer, a reflux condenser, and an immersion thermometer. Acetic anhydride (184.8 mL) was added with stirring, followed by 11 g of 50% aq NaOH soln. The mixture was stirred and refluxed for 5 h, then allowed to cool. Ethanol (300 mL) was poured into the soln with vigorous agitation to precipitate a white solid. The product was neutralized with an aq soln of $NaHCO_3$, filtered, and washed twice with water. The product was stirred in abs EtOH (200 mL), filtered, and washed twice on the filter with 100 mL portions of abs EtOH. The product was dried under reduced pressure at 100 °C for 24 h resulting in 84.5 g of product with ds 2.63; $[\alpha]_D = +175.8°$ (*c* 0.2, $CHCl_3$); IR (KBr): ν 3600–3400, 2960, 1746, 1371, 1233, and 1036 cm^{-1}.

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