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Note

A ^{1}H- and ^{13}C-n.m.r. study of bromine-oxidised potato starch

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Starch can be oxidised to obtain a high-solid, low-viscous dispersion with minimum retrogradation, properties that are of technical importance especially in the paper industry. Alkaline hypochlorite, the most common commercial oxidant, introduces carbonyl and carboxylic functions, and also causes depolymerisation[1]. Oxidation with bromine at neutral pH selectively introduces keto groups into carbohydrates[2–4] and eventually causes[4–6] ring cleavage and the formation of carboxylic groups at C-2 and C-3.

Because of the instability of the products of oxidation, determination of the quantity and of the type of functional groups is difficult. We now report the application of n.m.r. spectroscopy to monitor the oxidation of potato starch with bromine.

Potato starch was oxidised with bromine at several different concentrations, and the products were reduced with borohydride, then subjected to sugar analysis[7] in order to determine the position of the keto groups. The detection of mannose and allose indicated that oxidation had occurred at positions 2 or 3 of the glucose residues.

The positions of the keto or hydrated keto groups and other functional groups were obtained by 1D and 2D ^{1}H- and ^{13}C-n.m.r. spectroscopy. In a 2D-COSY experiment, the H-1 signals were assigned by correlation with H-2. The lack of correlation with H-2 indicated oxidation at position 2 or ring cleavage between C-2 and C-3. The relay COSY experiment revealed oxidation at position 3. The ^{13}C signals were assigned by 2D-heteronuclear correlations and the multiplicities by the APT technique. The assignments were compared with data in the literature[4,8–14] and are presented in Tables I and II.

There were several signals in the region for H-1. The signals from (1→4)- and (1→6)-linked α-D-glucopyranose residues appeared at 5.38 and 5.02 p.p.m., respectively. The signals at 4.78 and 5.15 p.p.m. had no correlations in the COSY analyses and were assigned to H-1 of hydrated forms of 2-keto residues in different environments.

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TABLE I

^{1}H-N.m.r. data for oxidised potato starch (δ in p.p.m.)

Residue	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
(1→4)-Glc	5.38	3.59	3.92	3.61	3.80	3.80
(1→6)-Glc	5.02	3.58	3.97			
(1→4)-2-Keto	5.15					
	4.78					
(1→4)-3-Keto	5.76	4.63				
	5.60	4.65				
	5.41	4.61				
(1→4)-DicarboxylicA[a]	4.95					
(1→4)-GlcA	5.42	3.59	3.81	4.25	4.58	
α-Glc (terminal)	5.20	3.59				
β-Glc (terminal)	4.61	3.30				

[a] Residue ring cleaved between C-2 and C-3 with the formation of carboxylic acid groups at these positions.

TABLE II

^{13}C-N.m.r. data for oxidised potato starch (δ in p.p.m.)

Residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
(1→4)-Glc	100.4	72.4	74.2	77.5	72.0	61.2
(1→6)-Glc	98.4					
(1→4)-2-Keto	98.6	95.4				
	98.2	94.4				
(1→4)-3-Keto	103.0	75.8	206.4[b]			
	102.4	74.8	206.4[b]			
		76.2	206.4[b]			
(1→4)-DicarboxylicA[a]	100.7	174.0[b]	174.0[b]			
(1→4)-GlcA	99.0					174.0[b]
α-Glc (terminal)	92.9					
β-Glc (terminal)	96.5					

[a] Residue ring cleaved between C-2 and C-3 with the formation of carboxylic acid groups at these positions. [b] Cluster of signals.

The signals at 5.76, 5.60, and 5.41 p.p.m. had no correlation peak between H-1 and H-3 in a relay COSY experiment. The low chemical shifts of these signals indicated 3-keto units in different environments. The signal at 4.95 p.p.m. had no correlation in the COSY analysis and originated from ring-cleaved residues. This signal increased in intensity when oxidation was prolonged. A signal from H-1 of the glucuronic acid residues was present at 5.42 p.p.m. The signals at 4.61 and 5.20 p.p.m. were assigned to the reducing end groups of β- and α-D-glucopyranose, respectively.

Some of the signals from H-2/6 were assigned readily by the n.m.r. techniques used, and are given in Table I. The H-2 signals of the three different 3-keto residues were found around 4.63 p.p.m. and almost overlapped the signal for H-1 of β-D-glucopyranose. The H-2 signal of the glucuronic acid residue was at 3.59 p.p.m., in accord with that in the literature[14], and the resonances of H-3,4,5 were also identified.

The C-1 signal of (1→4)- and (1→6)-linked α-D-glucopyranose residues appeared at 100.4 and 98.4 p.p.m., respectively (Table II). The shifts at 98.6 and 98.2 p.p.m. corresponded to C-1, and those at 95.4 and 94.4 p.p.m. to C-2 of the two hydrated 2-keto residues. The last two signals were verified by the APT experiment (a keto group could not be excluded by the APT analysis, but the resonance of a carbon in a keto group appears elsewhere[4]). The C-1 signals of two of the three 3-keto units appeared at 103.0 and 102.4 p.p.m. (the third anomer could not be verified), whereas the C-2 signals of the 3-keto residues were found at 75.8, 74.8, and 76.2 p.p.m. The C-1 signal of the ring-cleaved dicarboxylic residues appeared at 100.7 p.p.m., whereas the C-1 signal of the glucuronic acid residue was found at 99.9 p.p.m. The C-1 signals of the α- and β-D-glucopyranose residues at the reducing end appeared at 92.9 and 96.5 p.p.m. respectively.

The contents of functional groups introduced into the starch by different concentrations of bromine are given in Table III. The proportions of keto and carboxylic functions increased when oxidation was prolonged. The proportion of keto groups was largest at positions 2. At the highest concentration of bromine used, no 3-keto functions were detected probably because of rapid degradation. Fairly good quantification of the keto groups and their corresponding hydrates was obtained from the ^{1}H-n.m.r. spectra by excision and weighing. The content of the carboxylic groups was the sum of the dicarboxylic and uronic acid groups measured by the integral in the ^{13}C-n.m.r. spectra. On prolonged oxidation, the proportion of the dicarboxylic acid increased in comparison with that of glucuronic acid, according to the decrease of the integral of the C-6 signal but also indicated by the proportions of the corresponding H-1 and C-1 signals.

The 2-keto residues appeared only as their hydrated form, whereas the 3-keto residues were not hydrated as found with model compounds[4]. Also, the integral of the keto carbon signal at 206.4 p.p.m. accorded with the calculated content of 3-keto units. The H-2 signal from hydrated 3-keto residues should appear at higher field than δ 4.63 (*cf.* Anteunis *et al.*[15]). The appearance of different signals for each keto unit was

TABLE III

Proportions[a] of modified glucose residues in oxidised potato starch

Br_2:"Anhydroglucose" (molar ratio)	*2-Keto*	*3-Keto*	*Carboxylic acid*[b]
1:40	—	—	—
1:20	1	1	1
1:10	2	1	1
1:5	3	2	2
1:1	9	5	14
3:1	13	8	29
5:1	27	—	48

[a] Determined from the n.m.r. spectra by excision and weighing, or by integration. [b] Includes both dicarboxylic acid and glucuronic acid.

probably due to different environments, as found for cellulose and xylan oxidised with sodium nitrate in orthophosphoric acid[14].

Aldehydic functions and aldonic acids may be introduced by the oxidation of starch[1]. However, in the present study, no such functions were found.

Prolonged oxidation depolymerised the starch, which was indicated by stronger signals from the terminal α- and β-D-glucopyranose residues and in agreement with the decreased molecular weight determined[16] by gel-permeation chromatography.

Studies of glucopyranosides and of dextran[2-4] showed that bulky axial substituents hinder oxidation at the *syn*-diaxial positions. Thus, the glucose residues in starch should be protected from oxidation at position 3. However, in the present study, although oxidation occurred mainly at position 2, some oxidation occurred also at position 3 (Table III) possibly as a result of changes in conformation.

EXPERIMENTAL

Material. — Potato starch (Lyckeby Stärkelseförädling AB, Kristianstad) was oxidised with aqueous bromine at room temperature and pH 7, and the products were reduced conventionally with sodium borohydride[16]. The bromine/carbohydrate molar ratios used were 1:40, 1:20, 1:10, 1:5, 1:1, 3:1, and 5:1.

G.l.c. analysis. — Each oxidised/reduced starch was hydrolysed with M trifluoroacetic acid for 1 h at 125°, and the products were converted conventionally into the alditol acetates and analysed[7] by g.l.c. A Packard 427 instrument fitted with a flame-ionisation detector and a glass capillary OV-225 column (15 m × 0.22 mm i.d.) was used, with a helium flow of 65 cm/s at 210°.

N.m.r. spectroscopy. — The spectra were recorded with a Varian VXR 400 instrument: ^1H (400 MHz) on solutions (6 mg/mL) in D_2O at 85° (spectral width, 4000 Hz; 30K data points, 45° r.f. pulse, 3.75-s repetition time); ^{13}C (101 MHz) for solutions (50 mg/mL) in D_2O, at 20° using an inverse-gated decoupling technique (spectral width, 22 000 Hz; 66K data points, 45° r.f. pulse, 4.5-s pulse delay, 6.0-s repetition time) in order to suppress n.O.e. Chemical shifts are expressed in p.p.m. downfield from the signal for internal sodium 3-trimethylsilylpropionate-d_4. The attached proton test (APT)[17] was included in the 1D-n.m.r. experiment in order to determine the multiplicity of the ^{13}C signals (delay time, 7 ms). 2D-N.m.r. spectroscopy was performed with standard COSY[18], relayed COSY[19,20], and heteronuclear correlated (HETCOR)[21] C/H pulse sequences. The COSY experiments were performed with $90°-t_1-90°-t_2$; 320 transients with a 128 × 512 data matrix zero-filled to 512 × 512 were obtained in both COSY and relay COSY experiments. A delay time of 20 ms was used in relay COSY. In HETCOR, 3000 transients and 32 increments were obtained and zero-filled to a 1024 × 128 data matrix. The spectra widths were ~5000 Hz for ^{13}C and 1000 Hz for ^1H. The percentages of carbonyl and carboxyl groups were calculated from the ^1H- and ^{13}C-n.m.r. spectra.

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