Homopolysaccharides Interaction with the Dimethyl Sulphoxide–Paraformaldehyde Cellulose Solvent System. Selective Oxidation of Amylose and Cellulose at Secondary Alcohol Groups [1]

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A ^{13}C n.m.r. study of the interaction properties of the cellulose solvent dimethyl sulphoxide–paraformaldehyde with amylose suggests the reversible formation of hemiacetals between formaldehyde or its oligomers and hydroxy-groups at C-6 and C-2 of the polysaccharide. Such selectivity is, however, not found for the model compound methyl 4-*O*-methyl-α-D-glucopyranoside, which shows O-3 substitution as well. In agreement with this result, oxidation of amylose in this solvent system by adding acetic anhydride to the homogeneous solution leads to selective formation of carbonyl groups at C-3 in excellent yield. A similar result is obtained when acetic anhydride is added to a cellulose solution in dimethyl sulphoxide–paraformaldehyde. This suggests that the interaction mechanisms of cellulose and amylose with this solvent system must be similar. As a matter of fact, oxidation of a 6-substituted cellulose, such as 6-*O*-acetylcellulose, mainly takes place at C-2. The 6-*O*-acetylcellulose was prepared by the action of acetic acid–acetic anhydride in pyridine–tetrachloromethane on a pertrimethylsilyl-cellulose followed by hydrolysis of the remaining 2,3-bis(trimethylsilyl) ether. Previous results on the oxidation of 6-*O*-triphenylmethyl-cellulose or -amylose have been confirmed and corroborated. Determination of the site and degree of oxidation with the partly oxidized homopolysaccharides was achieved through their reduction with sodium borodeuteride, hydrolysis, and study by g.l.c.–mass spectrometry of the resulting hexoses in the form of their trimethylsilyl *O*-methyl oximes, or alditol acetate derivatives.

THE dimethyl sulphoxide–paraformaldehyde (DMSO–PF) system introduced by Johnson *et al.*[2] in 1976 is now a reasonably well established solvent for cellulose. Several other polar, aprotic solvents with similar solubilization properties have been proposed [3] more recently in conjunction with formaldehyde. A mechanism involving the reversible formation of hemiacetals between the 6-hydroxymethyl groups of cellulose and formaldehyde has been suggested to account for the solubilization process, and related studies [4,5] indicated that secondary hydroxy-groups may also be involved. The complexity and heterogeneity of the cellulose solubilization reaction has been pointed out [6] and the reversible formation of polyoxymethylol chains of statistical length, depending on the reaction conditions, confirmed [2-5] by n.m.r. spectroscopy. Although problems of recovery of reagents correlated with fibre regeneration remain to be resolved,[7,8] the DMSO–PF system has already been proposed for the production of regenerated fibres, films,[9] and the grafting of cellulose.[10] Another interesting application is the possibility of selective functionalization of cellulose, and some limited selectivity has been reported for the alkylation [11,12] and acylation [13,14] of the polysaccharide at secondary positions. In a program dedicated to the selective modification of polysaccharides of technological interest, the possible use of the DMSO–PF combination as solvent in the selective oxidation of cellulose and such other homopolysaccharides as amylose was considered. In a first approach, the interaction properties of this solvent system with amylose were investigated.

† Associated with the University of Science and Medicine, Grenoble. Results obtained in the framework of the Action Thèmatique Programmée ' Papier ' of the C.N.R.S.

RESULTS AND DISCUSSION

As previously [5] pointed out, ^{1}H n.m.r. spectra of solutions of cellulose in the dimethyl sulphoxide–paraformaldehyde solvent system are poorly resolved and, in spite of several attempts,[2,4] unambiguous assignments are hazardous because of multiple overlapping of signals from large quantities of formaldehyde, formaldehyde oligomers, and water, and due to the usual broad line-shaping of these polymers ascribable to their short relaxation times. ^{13}C N.m.r. spectroscopy is more informative, and previous investigations [5] have shown that 6-*O*-substitution with hydroxymethyl (CH_2OH) and poly(oxymethylene)ol [$(CH_2O)_nH$] functional groups is clearly evident from the ^{13}C chemical shifts of the C-6 signals compared with those of cellulose oligomers in dimethyl sulphoxide. Unfortunately, the lack of resolution for the C-2, C-3, and C-5 resonances, which appear as a broad signal at 73.6—72.1 p.p.m., does not allow a precise assignment of possible secondary substitution sites. Two signals at lower field (75.6 and 76.8 p.p.m.) ‡ however can be attributed to C-2 and C-3 hydroxy-group substitutions [5] but may also result from both hydroxymethyl and poly(oxymethylene)ol substitution on one single secondary hydroxy-site, possibly C-3. This is in agreement with an upfield shift of C-1 in the DMSO–PF solvent system.

Amylose, the anomer of cellulose, was thought to be a good model compound for comparison. Although different patterns in molecular shape and solubility are

‡ The 0.7 p.p.m. difference between the chemical displacements listed in the present work and the published data is ascribable to difference in the calculated chemical shift for dimethyl sulphoxide (39.6 p.p.m. in the present work).

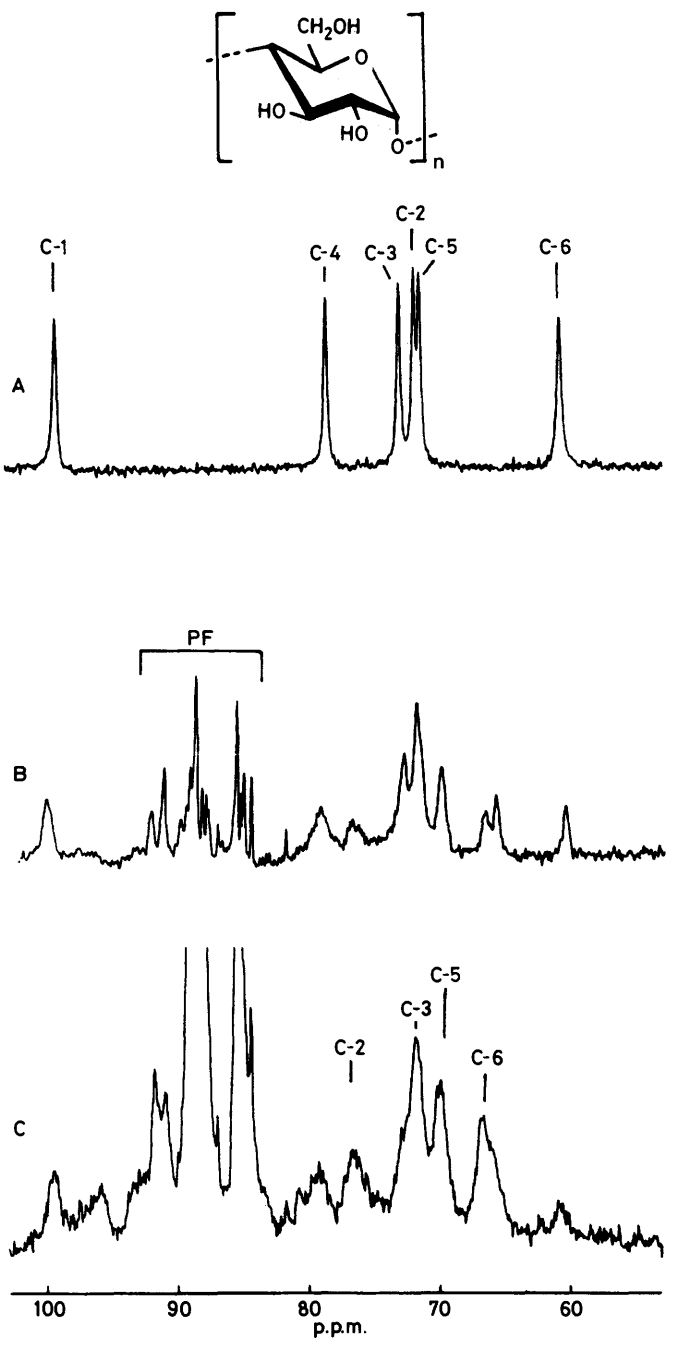


FIGURE 1 ^{13}C N.m.r. spectra in [$^{2}H_6$]Me_2SO of amylose (A) and of the products formed by reaction of amylose with smaller (B) and larger amounts (C) of formaldehyde

known for these polysaccharides, one could expect similar reactivity with the DMSO–PF solvent system, provided that such an interaction would not involve any secondary conformational arrangement. Amylose is, in fact, soluble in dimethyl sulphoxide and a ^{13}C n.m.r. spectrum shows all carbon resonances [C(1)–C(6) carbon atoms] at 99.4, 71, 72.2, 77.9, 70.6, and 59.6 p.p.m., respectively (Figure 1A).[15] Treatment of the dimethyl sulphoxide solution with paraformaldehyde for 30 min results in the appearance of three major signals at 65.1, 66, and 69.2 p.p.m. together with a decrease in the intensity of the C-6 and C-5 signals, in agreement with substitution at C-6 of amylose (Figure 1B). A smaller signal at 76.2 p.p.m. and the appearance of a small, broad signal near 96 p.p.m. may indicate an incipient reaction involving secondary hydroxy-groups. A larger amount of formaldehyde, as seen from the spectrum in Figure 1C, emphasizes these features. Thus, for C-6, the signal at 66 p.p.m. increases relative to that at 65.1. This may reflect the fact that an increasing number of CH_2O groups become attached to O-6, resulting in a downfield shift of C-6.[5] An increase of the 76.2 p.p.m. signal is also noted, in agreement with an increase of the substitution level at the secondary hydroxy-sites. As substitution of either the C-2 or the C-3 hydroxy-groups would lead to a concomitant upfield shift of *ca.* 0—2 p.p.m. of the β-carbon atoms together with a downfield shift of 8—11 p.p.m. of the substituted one,[16,17] the region of 67—70 p.p.m. is of special interest for such a characterization. Since the total area of the signals at 65.1—66 p.p.m. (C-6 substituted hydroxy-groups) is nearly equivalent to that of the 69.2 p.p.m. signal (β-shift of C-5) and since no other spectral perturbation is apparent, it becomes clear that the substitution at secondary hydroxy-sites must involve largely C-2. The notably increased resonance at 71 p.p.m. is consequently assigned to an overlapping of the residual C(2)–C(5) signals associated with the upfield shift for the C-3 neighbouring substitution. In agreement with this conclusion, the C-4 signal at 78.3 p.p.m. does not appear to be seriously affected during the formaldehyde treatment, apart from the expected general resonance broadening. Still unexplained, however, is the upfield shift found for C-1. This is also observed in the spectrum obtained from the model compound methyl 4-*O*-methyl-α-D-glucopyranoside under similar reaction conditions (Figure 2). In addition to the aforementioned considerations which appear to be valid for this monosaccharide derivative, there may be noted a lowered selectivity for C(2)–C(3) secondary hydroxy-substitution, which can be deduced from a decrease of both the signals at 72.4—73.00 p.p.m. together with an increase of the expected resonances for the corresponding substituted carbon atom around 78 p.p.m. The appearance of two additional signals for C-1 clearly shows that substitution takes place to a considerable extent at both C-2 and C-3. Of further interest is the completeness of the O-6 substitution, as evidenced by the disappearance of the original C-6 signal in Figure 2B. Heating of either amylose or methyl 4-*O*-methyl-α-D-glucopyranoside–paraformaldehyde–dimethyl sulphoxide solutions for 20 h at 95 °C in an open vessel results in complete regeneration of the pure starting materials (Figure 1A and Figure 2A), in agreement with the expected reversible hemiacetal formation. The reversibility of the reactions excludes the possibility of intra- or inter-molecular methylene acetals,[18] as such derivatives are known to be stable.

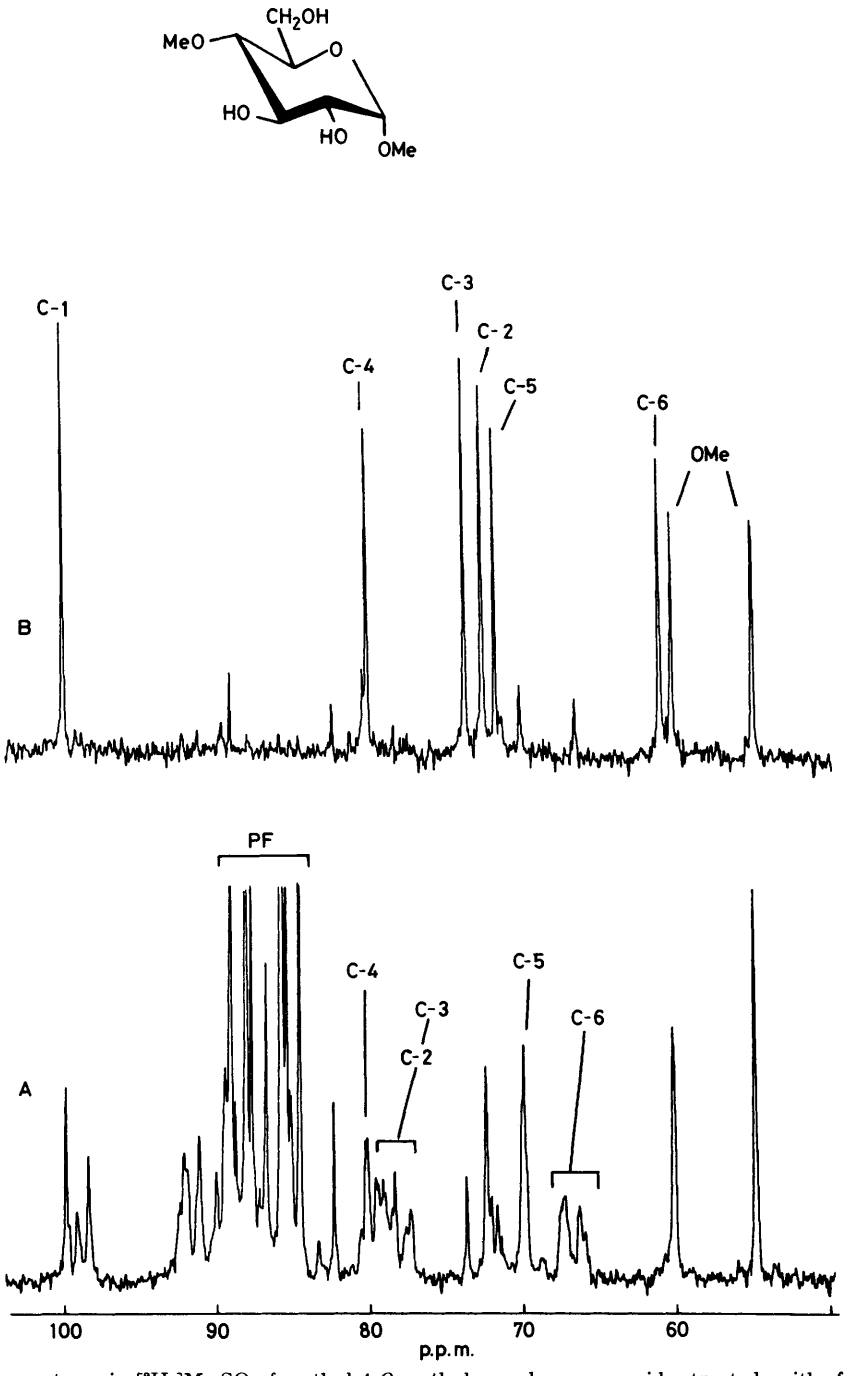


FIGURE 2 A, ^{13}C N.m.r. spectrum in $[^2H_6]Me_2SO$ of methyl 4-*O*-methyl-α-D-glucopyranoside treated with formaldehyde. B, After evaporation of formaldehyde

As previously mentioned, little selectivity has been previously observed in the functionalization of cellulose in the DMSO–PF solvent, and this might be due either to interference with the polar reagents used (which may shift the equilibrium or preferentially interact with the newly formed hemiacetal hydroxy-group) or to incomplete substitution conditions. Dimethyl sulphoxide, in conjunction with an electrophilic activating species like an acid anhydride, is known to oxidize hydroxy-groups smoothly.[19] This reagent, was used for the oxidation of cellulose [20] as well as cellulose [21] and amylose [22] substituted at O-6 with a triphenylmethyl group. One could expect that the addition of an acid anhydride to the DMSO–PF solution of cellulose or amylose would lead to oxidation of the unprotected hydroxy-groups without giving rise to side effects, and could thus allow precise assignment of the hydroxy-groups not involved in the interaction with the solvent, leading eventually to a

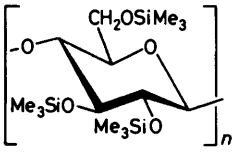
new method of selective polysaccharide oxidation. Following this idea, solutions of amylose or cellulose in the DMSO–PF solvent, at the optimal paraformaldehyde concentration (^{13}C n.m.r.) for maximum substitution, were treated at room temperature with acetic anhydride for 17 h. The oxidized polysaccharides were recovered by precipitation with methanol–water and the carbonyl content (degree of oxidation: $\overline{D.O.}$) estimated, *via* the hydroxylamine method,[23] to be 0.6—0.7 for both amylose and cellulose. Reduction of the oxidized samples with sodium borohydride followed by hydrolysis and conversion into alditol acetate derivatives led, for both polysaccharides, to the exclusive characterization of allitol and glucitol (see Table), implying that the oxidation occurred mainly at the C-3 hydroxy-group of both polysaccharides. Indeed, as it is known from previous work on the anomeric methyl D-*arabino*-hexopyranos-2-ulosides,[24–26] one could expect that borohydride reduction of C-2 oxidized β-linked polysaccharides would have led to appreciable amounts of mannosyl components. Although the yield of the allitol derivative from the oxidized cellulose sample (59%) was in good agreement with the number of carbonyl groups, as estimated by the hydroxylamine technique,[23] such an excellent correlation was not found for amylose (allitol yield, 40%). This may reflect the fact that some oxidation may also have occurred at C-2 of this polysaccharide. This point is further confirmed through reduction of the oxyamylose and oxycellulose samples with sodium borodeuteride, hydrolysis, and conversion of the resulting monosaccharides into *O*-methyl oxime *O*-trimethylsilyl ethers.[27] These derivatives were selected for the high intensity of the C(2)–C(3) fragmentation signals in electron-impact mass spectrometry, which allows accurate measurement of deuterium incorporation at both sites. A study by combined gas-liquid chromatography–mass spectrometry of these derivatives using a capillary column (see Table) led to the conclusion that deuterium incorporation indeed involved exclusively the C-3 hydroxy-group of cellulose, whereas some 10% incorporation occurred at C-2 of amylose.

The selectivity of the oxidation process for amylose and cellulose using the DMSO–PF reaction medium contrasts sharply with the known behaviour of 6-*O*-(triphenylmethyl)-cellulose or -amylose with the dimethyl sulphoxide–acetic anhydride reagent which is known to lead mainly to oxidation at C-2.[21,22] Since such specificity may be ascribable in this case to the presence of the bulky triphenylmethyl protective group at C-6, which would hinder the approach at C-3 of the oxidant, a comparative sequence was devised for 6-*O*-acetylcellulose (3). A convenient route to this compound is the selective *O*-acetylation of the primary positions developed for trimethylsilylated monosaccharides or polyols by Lehmann *et al.*[28] and recently extended to amylose.[29] Cellulose, regenerated from its commercial tri-*O*-acetyl derivative, was trimethylsilylated in pyridine at 70 °C. After 8 days, the originally heterogeneous suspension became homogeneous and the fully protected derivative (1), which did not show any hydroxy-band in its i.r. spectrum, was treated with pyridine–acetic anhydride–acetic acid in tetrachloromethane to yield after 6 days at 70 °C a mono-*O*-acetyl-di-*O*-trimethylsilylcellulose (2). Removal of the remaining *O*-trimethylsilyl groups with acetone–methanol–acetic acid yielded 6-*O*-acetylcellulose (3), the structure of which was established after acetylation with [$^2H_6$]acetic anhydride to give compound (4). Comparison of the ^1H n.m.r. spectrum of this compound (4) with that of a tri-*O*-

Estimation of the site and degree of oxidation in oxy-amylose and oxy-cellulose *via* conversion into the corresponding alditol acetates (A) or deuteriated *O*-methyl oxime *O*-trimethylsilyl ethers (B) *

Oxidized samples from the compound shown	A Relative proportions (%) of alditol acetates			B† Relative proportions (%) of deuterium incorporated at C-2 and C-3 of *O*-methyl oxime *O*-trimethylsilyl derivatives					
				C-2			C3		
	allo	*gluco*	*manno*	*allo*	*gluco*	*manno*	*allo*	*gluco*	*manno*
Amylose (DMSO–PF)	40	53	-	-	10	-	70	10	-
Cellulose (DMSO–PF)	59	40	-	-	-	-	85	-	-
6-*O*-Acetylcellulose (3)	-	56	30	-	20	95	-	20	25
6-*O*-(Triphenylmethyl)-cellulose	-	57	36	-	25	95	-	25	30

* A hyphen means that no detectable compound (A) or deuterium incorporation (B) was noted according to the respective experimental parameters described under the heading ' General methods.' † The values for deuterium incorporation are given within a 5% approximation.



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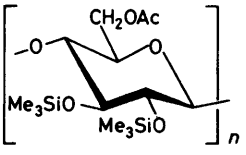

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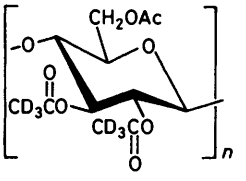

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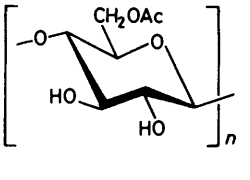

(3)

acetylcellulose in [^{2}H]chloroform showed a 3 H singlet at δ 2.21 and only traces of signals from the acetyl groups at O-2 and O-3. Oxidation of 6-*O*-acetylcellulose (3) with the dimethyl sulphoxide–acetic anhydride reagent, using previous reaction conditions, gave an oxidized derivative with a $\overline{D.O.}$ of 0.7 according to the hydroxylamine estimation technique.[23] Reduction of this sample with sodium borohydride and subsequent conversion into alditol acetates led to the identification of mannitol and glucitol in an approximate ratio of 3 : 5.6. This is in good agreement with the result obtained from similar oxidation of 6-*O*-triphenylmethyl derivative of cellulose,[21] which means that the selectivity in this oxidation at C-2 of a 6-protected cellulose is not related to the bulkiness of the substituent.* Of interest is the occurrence of some double labelling at C-2 and C-3 as detected after reduction with sodium borodeuteride of 6-*O*-trityl- as well as 6-*O*-acetyl-cellulose and conversion into the corresponding *O*-methyl oxime *O*-trimethylsilyl ethers of D-mannose and D-glucose (see Table). Although most of the labelling is found as expected [24–26] at C-2 of the resulting *manno*-derivative, some 25—30% of deuterium incorporation at C-3 has still to be considered.† This result contrasts with the absence of double-deuterium incorporation with the oxycellulose and its very low extent with the oxy-amylose resulting from the oxidation step in the DMSO–PF solvent system. In the latter case, there may be some residual protection of the C-2 hydroxy-group with formaldehyde hemiacetals during the reduction step.

Conclusion.—The close similarity in the oxidation of amylose and cellulose in the DMSO–PF solvent system, which leads to selective oxidation at C-3 in both cases, provides good confirmation that the interaction of these homopolymers with the solvent involves a reversible, covalent reaction with the C-2 and C-6 hydroxy-groups, as similar oxidation of 6-protected amylose and cellulose leads to carbonyl-group formation at C-2. This difference in selectivity gives strong support to the results of ^{13}C n.m.r. spectroscopy, which suggest the reversible formation of hydroxymethyl and poly(oxymethylene)ol groups at O-2 and O-6 when a solution of amylose in dimethyl sulphoxide is kept in contact with paraformaldehyde. Although a similar ^{13}C n.m.r. spectroscopic investigation does not allow such ready conclusions with cellulose, because of the complexity of the spectrum, one can extrapolate with some confidence to cellulose from the results with amylose which, furthermore, are in good agreement with the known reactivity parameters for alkyl D-glucopyranosides in the $^4C_1$ conformation.[31]

Apart from these theoretical considerations, these results open a ready access to C-3 oxidized derivatives of amylose and cellulose and lead to the expectation that a wider use of the interaction properties of cellulose solvents may be of interest for the specific derivatization of these polyfunctional, natural polymers.

EXPERIMENTAL

General Methods.—Solutions were evaporated *in vacuo* at temperatures below 45 °C or freeze-dried. ^{1}H N.m.r. spectra were recorded at 250 MHz with a Cameca 250 instrument (Thomson-CSF, Paris) using tetramethylsilane as internal reference. ^{13}C N.m.r. spectra of methyl 4-*O*-methyl-α-D-glucopyranoside,[32] cellulose, and amylose were measured at 25.18 MHz on a Bruker WP-100 spectrometer. Chemical shifts are in δ values relative to the central peak of [$^2H_6$]dimethyl sulphoxide at 39.6 p.p.m. For ^{13}C n.m.r. spectra at 25.18 MHz, recorded in non-deuteriated solvents unless otherwise specified, a few drops of [$^2H_6$]dimethyl sulphoxide were added to provide a lock signal. ^{13}C N.m.r. spectra of amylose were recorded at 60 or 80 °C using a 75° pulse angle and a spectral width of 5 681.1 Hz; spectra of methyl 4-*O*-methyl-α-D-glucopyranoside were recorded at 30 °C. Gas-liquid chromatography was performed with a Girdel 3000 instrument (Paris) fitted with a flame-ionization detector and *A*, a SP-2340 glass SCOT capillary column (20 m × 0.22 mm) kept isothermally at 180 °C for 20 min and then programmed at 4 °C min^{-1} to 220 °C for alditol acetates; and *B*, an OV-17 glass WCOT capillary column [33] with temperature programming of 2 °C min^{-1} from 100 to 170 °C for *O*-methyl oxime *O*-methylsilyl ethers.

The g.l.c.–m.s. analysis was conducted with a AEI MS-30 double-beam mass spectrometer, directly coupled to the capillary columns, using electron-impact ionisation with a source temperature of 150 °C, trap current 100 μA, ionisation potential 70 eV, acceleration energy 3 kV for *O*-methyl oxime *O*-trimethylsilyl ethers and 4 kV for alditol acetates. Retention times (min) for acetates: 28.2 (allitol), 29.6 (D-altritol), 30.0 (D-mannitol), 32.6 (D-glucitol), and for *O*-methyl oxime *O*-trimethylsilyl ethers (*E*–*Z* isomers), 24.8—25.6 (D-allose), 25.6 (D-mannose), 26.8—27.2 (D-glucose).

The extent of deuterium incorporation at C-2 and C-3 in reduced oxy-amyloses and -celluloses (see Table) were calculated for the corresponding hexose *O*-methyl oxime *O*-trimethylsilyl derivatives from the intensity ratios of the ions *m*/*z* 161/160 and 320/319, following the known [27] fragmentation pathway of these derivatives under electron impact [C(2)–C(3) fragmentation].

Preparation of the Dimethyl Sulphoxide–Paraformaldehyde Solutions for N.m.r. Studies.—Amylose and cellulose. The sample (potato amylose, pract. grade, Sigma, or cellulose, regenerated from cellulose acetate,[34] 10 g) in dimethyl sulphoxide (100 ml) was treated at 95 °C with a stream of gaseous formaldehyde generated by heating solid paraformaldehyde (Merck) in an external vessel, at a flow rate of 0.33 g min^{-1}. At given times, aliquots (2 ml) were taken in 10-mm n.m.r. sample tubes for the spectral recordings.

Methyl 4-O-*methyl*-α-D-*glucopyranoside.* The sample (2.5 g) in [$^2H_6$]dimethyl sulphoxide (5 ml) was treated at 95 °C with formaldehyde as described above.

2,3,6-*Tri*-O-(*trimethylsilyl*)*cellulose* (1).—A suspension of cellulose (5 g), regenerated [34] from commercial cellulose triacetate (Prolabo, Paris), in pyridine (250 ml) was stirred for 24 h at 100 °C and then cooled to room temperature; a mix-

* According to D. Horton and T. Usui (unpublished results), oxidation of 6-*O*-acetylamylose using the dimethyl sulphoxide–acetic anhydride reagent leads also to oxidation at C-2 as previously found for 6-*O*-(triphenylmethyl)amylose.[22]

† The double deuteriation may arise either from a double oxidation at both secondary sites of a glucopyranosyl moiety (although this type of compound is supposed to be unstable [30]) or, more probably, from sequential conventional deuterium incorporation at an enolate anion followed by the deuterium reduction step of the ketone group. Such a possibility is not unexpected in view of the slowness of this reduction step, which occurs mostly under heterogeneous conditions.

ture of hexamethyldisilazane (50 ml) and chlorotrimethylsilane (25 ml) was added with vigorous stirring (glass balls). This stirring was continued for 8 days at 60—70 °C, after which the cold mixture was poured into methanol (1 l) and kept for 1 h with stirring. Water (250 ml) was then added and the resulting suspension was left for 0.5 h. The precipitate was filtered off, washed several times with water, and methanol, then freeze-dried (9.8 g, 84%). The i.r. spectrum showed no hydroxy-absorption [Found: C, 47.4; H, 9.0. Calc. for $(C_{15}H_{34}O_5Si_3)_n$: C, 47.58; H, 9.05%].

6-O-*Acetyl*-2,3-*di*-O-(*trimethylsilyl*)*cellulose* (2).—A similar sequence as used for the corresponding amylose derivative [29] was followed, except that solubilization of the per(trimethylsilylated)cellulose in pyridine–acetic acid required heating to 70 °C for 6 days. Starting from compound (1) (5 g), the yield of compound (2) after freeze-drying was 3.8 g (81%); $\nu_{max.}$ (KBr) 1 752 cm^{-1} (C=O ester), no OH band [Found: C, 48.1; H, 7.95. Calc. for $(C_{14}H_{28}O_6Si_2)_n$: C, 48.24; H, 8.10%].

6-O-*Acetylcellulose* (3).—The technique described for the amylose [29] derivative was followed. Starting from compound (2) (2 g), the yield of the title compound was 1.4 g (97%). This derivative was soluble in pyridine and in tetrachloromethane [Found: C, 46.9; H, 6.0. Calc. for $(C_8H_{12}O_6)_n$: C, 47.06; H, 5.92%].

6-O-*Acetyl*-2,3-*di*-O-(*trideuterioacetyl*)*cellulose* (4).—Compound (3) (100 mg) in a mixture of pyridine (2 ml) and [$^2H_6$]acetic anhydride (0.5 ml) was kept for 7 days at room temperature and then diluted with pyridine (2 ml) and poured into methanol with stirring. The resulting precipitate was filtered off and freeze-dried (yield 80 mg). The 100 MHz, ^1H n.m.r. spectrum ([^2H]chloroform) was identical with that of a sample of tri-*O*-acetylcellulose, except that it showed essentially a 3-H singlet in the *O*-acetyl region at δ 2.21 (6 OAc, lit.,[35] δ 2.09) and the integrated intensity in the region 1.94—1.99 (2,3-OAc) was negligible (< 1 H).

Oxidation of 6-O-*Acetylcellulose* (3) *and* 6-O-(*Triphenylmethyl*)*cellulose*.—6-*O*-Acetylcellulose (3) or 6-*O*-(triphenylmethyl)cellulose [34] (1 g) were dissolved in dimethyl sulphoxide (10 ml) with stirring at room temperature. Acetic anhydride (5 ml) was then added and the stirring was continued for 17 h. The oxidized product was precipitated by pouring the solution into methanol–water (1 : 1, v/v, 100 ml), and the suspension was filtered off. The precipitate was washed exhaustively with water and then methanol, and finally dried *in vacuo*. The yield was 0.8 g for both derivatives and the degree of oxidation 0.57—0.60 as estimated by the hydroxylamine method.[23]

Oxidation of Amylose and Cellulose in the Dimethyl Sulphoxide–Paraformaldehyde Solvent System.—Cellulose (5 g) regenerated [34] from commercial tri-*O*-acetylcellulose (Prolabo, Paris) was suspended in dimethyl sulphoxide (100 ml) and heated at 95 °C with stirring. A stream of gaseous formaldehyde generated by heating paraformaldehyde (Merck, 5 g) in a separate container was then bubbled into the suspension during 15 min and the solution was kept in a closed vessel for 3 h at room temperature; the treatment with formaldehyde was then repeated. Acetic anhydride (50 ml) was then added to the stirred solution at room temperature. After stirring for 17 h, the mixture was poured into methanol–water (1 : 1, v/v) and the resulting precipitate was filtered off, washed exhaustively with methanol, and dried *in vacuo* (yield 6.5 g).

A similar procedure was used for amylose (potato, Sigma, practical grade), except that a homogeneous solution was immediately obtained in dimethyl sulphoxide. Yields were identical with those obtained from cellulose. The degrees of oxidation [23] for both cellulose and amylose derivatives were 0.60—0.70.

Borohydride or Borodeuteride Reduction of the Oxidized Amylose and Cellulose Derivatives and Conversion into Alditol Acetates or O-*Trimethylsilyl* O-*Methyl Oxime Derivatives*.—To a suspension of the oxidized sample (200 mg) in methanol–water (1 : 1, v/v) sodium borohydride (or sodium borodeuteride for deuterium labelling and further conversion into *O*-trimethylsilyl *O*-methyl oxime derivatives, 200 mg) was added with stirring at room temperature. After the mixture had been stirred for 48 h, the excess of reducing agent was destroyed by the addition of a few drops of aqueous acetic acid. The solution was evaporated under diminished pressure and the solid residue successively washed with water (2 × 20 ml), methanol (2 × 20 ml), and toluene (2 × 20 ml).

The resulting, reduced polysaccharide sample in aqueous sulphuric acid (72%, w/w, 2 ml) was stirred for 1 h at room temperature. Water (56 ml) was then added and the solution was heated at 100 °C for 14 h. It was then made neutral with Amberlite IR 45 (OH^-) and the resulting monosaccharide solution further processed to give the alditol acetates [36] or *O*-methyl oxime *O*-trimethylsilyl ethers [27] according to the usual procedures.

[1/1699 *Received*, 2*nd November*, 1981]

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