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METHYLATION AS A MEANS OF LOCATION OF ANIONIC GROUPS IN SOME CELLULOSE PHOSPHATES

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ABSTRACT

The distribution of methyl groups after methylation of celluloses containing phosphate groups is quite different from that obtained after methylation of cellulose. The results indicate a probable high level of phosphorylation at positions 2 and 6 of D-glucopyranosyl units, and give supporting evidence for the recognised fibrillar models for cotton.

INTRODUCTION

In order to assist the interpretation of results from ion-exchange studies of some cellulose phosphates of low d.s. (degree of substitution), it was considered desirable to determine the locations of the phosphate groups in the cellulose chains. Esterified celluloses have been little studied as a means of providing structural information relevant to cellulose, because of the relative instability of such polymers. For water-insoluble cellulose derivatives, such studies have been mainly concerned with methoxyl groups[1,2], but other cellulose ethers, *e.g.*, 2-(diethylamino)ethyl[3] or methyl vinyl sulphone[4], have also been utilised. The extent to which HO-2,3,6 enter into heterogeneous chemical reaction under various degrees of swelling, *etc.*, has been utilised[1,2] to interpret the fine structure of cellulose. The distribution of substituents in the industrially important acetates and nitrates of high d.s. has been investigated by using chemical methods[5–7]. More importantly, distributions of methyl groups in methylated, cellulose acetates have been used as a method of determining substituent positions. Thus, methylation of partially deacetylated cellulose triacetate[8,9] has been used to ascertain the position of hydroxyl groups, and methylation of formaldehyde cross-linked cellulose[10] to determine the position of the cross-links.

Generally, for esterification, the rate constants for HO-2,3,6 are in the order $k_6 > k_2 > k_3$, with increasing difference in magnitude as reactions become more homogeneous. This sequence contrasts with that for methylation where HO-2 reacts

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preferentially[1], but is expected, as structural models of cellulose show that HO-6 is sterically the most-accessible hydroxyl group[1]. However, the relative reactivities of HO-2,3,6 are dependent on reaction conditions. The use of these reactivities to obtain data on accessibilities (more meaningful for structural interpretation) is described here.

The relative reactivity of cellulose hydroxyl groups towards phosphorylation has not been reported. However, Petrov *et al.*[11] have claimed that HO-6 is the most reactive site in the formation of cellulose phosphite. The effectiveness of certain phosphorus-containing substituents as flame retardants for cellulose has been explained by phosphorylation of position 6, which inhibits formation of 1,6-anhydro-β-D-glucopyranose and prevents further breakdown to inflammable gases[12] on heating.

We now report on the phosphorylation of cellulose.

EXPERIMENTAL

Materials. — Desized, scoured, and bleached, unmercerised poplin cellulose (weave 125 × 70) and mercerised cellulose (weave 90 × 80) were used. The cellulose phosphates were prepared according to the method of Ford and Hall[13]. Cellulose phosphates *P1* and *P2* were obtained by soaking unmercerised poplin fabric in a mixture of sodium dihydrogen phosphate (10 and 30 g), urea (24 and 35 g), and water (66 and 35 ml) for 5 min. The data for *P1* and *P2* are summarised in Table I. The fabric was then adjusted to a wet "pick-up" of 140%, heated on a pin-frame for 30 sec at 93°, cured (170°, 10 min) in a small oven, and then washed with deionised water (5 min), hot aqueous 1% sodium carbonate (60°, 5 min), and deionised water (5 min).

Samples referred to as "padded" and "cured" were used as controls (see Results section). The former is unmercerised cotton padded with the reagents used for the preparation of *P2* and oven-dried (1 h, 60°), and the latter is cotton padded and cured as for *P1* and *P2*, but with omission of the sodium dihydrogen phosphate.

Methylation analysis. — Methylation was effected by the method of Roberts

TABLE I

ANALYSES OF CELLULOSE SAMPLES USED IN METHYLATION

Sample	*D.s. of acid groups*	*Phosphorus (%)*	*Cross-linked (%)*[a]
Unmercerised poplin	0.002[b]		
Mercerised poplin	0.002[b]		
Cured poplin	0.003[b]		
Padded poplin	0.005	0.06	
P1	0.07	1.12	12
P2	0.19	2.64	28

[a]Refers to the percentage of phosphate groups in the monobasic form, *i.e.*, $(-CO)_2PO_2^-$; the other phosphate groups are dibasic $-COPO_3^{2-}$; values were determined from the phosphorus contents and the molarity of the ionising group[11]. [b]Estimated as carboxyl groups.

and co-workers[1]. The various celluloses (100 × 10 cm, ~20 g) were soaked in 2M NaOH (45 min), and immersed in methyl sulphate (25 ml)–methyl sulphoxide (50 ml) for a further 34 min. The samples were then rinsed in warm 1% acetic acid, water (60°, 1 h), aqueous 10% sodium carbonate (60°, 5 min), and water (60°, 10 min), and air-dried. Uniformity of treatment was checked by staining[1] with Serisol Fast Red NGGL, and methoxyl contents were determined by a modified Zeisel method[15].

Methylated celluloses were hydrolysed with 72% sulphuric acid, according to the method of Croon and Manley[16]. The resulting syrups were stored in a desiccator over phosphorus pentaoxide.

Samples (10 mg) of the syrups were trimethylsilylated (according to the method of Sweeley *et al.*[17]) with pyridine (1 ml), hexamethyldisilazane (0.2 ml), and chlorotrimethylsilane (0.1 ml), and subjected to g.l.c. (OV1-coated capillary column, 50 m × 0.25 mm). A specially designed sample injector was used[18], so that peaks eluted early could be more accurately determined. The sample was applied to a glass tip, and the solvent was evaporated before application of the sample to the column head. This procedure prevented solvent trail. The peak areas were determined by an "excision and weighing" method.

Samples of methylated D-glucoses (2-, 3-, 6-, 2,3-, 3,6-, and 2,3,6-) were obtained from Dr. J. G. Roberts (Shirley Institute, Manchester). The relative retention time of each was measured, and the relative response of each to an internal standard (D-mannitol) was constant for the flame-ionisation detector up to 10^{-2} mmol.ml^{-1}. Thus, the use of an internal standard was unnecessary, as the peak area of a component in a mixture relative to the whole peak area was a measure of the relative molar amount of that component in that mixture.

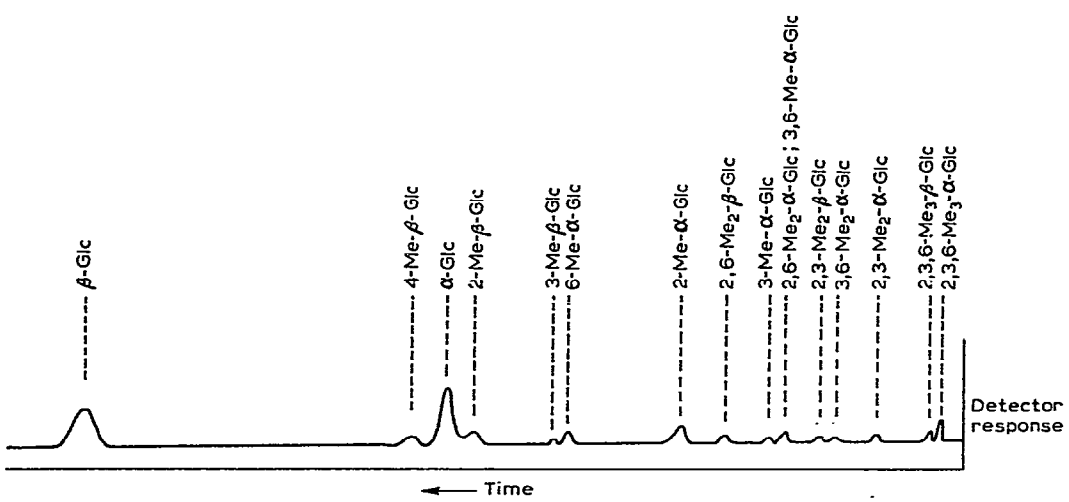

Fig. 1. Gas chromatogram of the trimethylsilylated D-glucose derivatives formed after hydrolysis of thrice-methylated poplin cellulose; obtained at 115° with an OVI-coated glass-spiral capillary column (50 m × 0.25 mm). Key: β-Glc = β-D-glucose; 4-Me-β-Glc = 4-*O*-methyl-β-D-glucose, *etc.*

TABLE II

DISTRIBUTION OF METHOXYL GROUPS IN METHYLATED CELLULOSES

Cellulose sample	*Number of methylations*	*O-Methyl-D-glucoses in hydrolysate (mol %)*[a]								*D.s. at each position*			*Overall d.s.*
		2,3,6	*2,3*	*2,6*	*3,6*	*2*	*3*	*6*	*Glc*	*2*	*3*	*6*	
Unmercerised (poplin)	3	4.3	3.4	5.6	1.0	10.1	2.6	9.9	63.2	23.4	11.3	20.8	0.56
Mercerised	3	3.7	2.9	3.7	0.9	9.0	3.9	6.2	69.7	17.3	11.4	14.5	0.43
Cured poplin	3	4.4	3.0	4.5	1.0	9.3	2.4	7.0	67.9	21.2	10.8	16.9	0.48
Padded poplin	3	5.7	4.5	6.2	1.2	10.7	3.3	6.7	61.6	27.1	14.7	19.8	0.62
P1	1	0.6	1.4	0.9	0.3	5.1	2.9	4.9	84.2	8.0	5.2	6.5	0.20
	2	1.8	2.3	2.7	0.6	7.2	3.6	7.6	74.1	14.0	7.3	12.7	0.34
	3	3.4	3.0	2.9	1.4	8.2	4.2	6.6	69.7	17.5	12.0	14.3	0.44
P2	1	0.5	1.0	1.2	0.4	4.2	4.2	5.5	83.1	6.9	6.1	7.6	0.21
	2	1.3	2.0	2.1	0.7	7.6	3.1	6.5	77.0	13.0	7.1	10.6	0.31
	3	3.1	2.2	3.0	0.8	5.6	3.7	6.1	76.2	13.9	9.8	13.0	0.37

[a]2, 3, and 6 refer to carbon positions of methyl groups in sugar ethers.

RESULTS AND DISCUSSION

Fig. 1 shows a gas chromatogram of trimethylsilylated methylated glucoses resulting from the hydrolysis of thrice-methylated, unmercerised, poplin cellulose. From the series of chromatograms obtained for each methylated cellulose subjected to methylation treatment (Table I), the relative proportions of the methyl ethers were obtained, and consequently the d.s. at each position was determined (Table II).

The phosphates were of d.s. comparable to that for the methylcellulose, so that comparisons could be used to determine the location of anionic groups. The extent of degradation of the cotton was not appreciable (shown by retention of tensile strength), and the effect of the conditions on polyanion structure and the accessibility of the hydroxyl groups was determined by methylation of the "padded" and "cured" celluloses. Comparison of the data for these samples with that for the poplin sample showed that the reaction conditions do not have a large effect on the position of methyl substitution. The somewhat lower reactivity of "cured" cellulose is probably due to high-temperature collapsing of chains, resulting in a structure of "higher order". Mercerised cellulose, of higher order than unmercerised[3], shows a similar rate of methyl substitution. Since 4M sodium hydroxide is necessary to mercerise cellulose[19], the methylation preswelling treatment of using 2M base will not transform the poplin cellulose I into cellulose II, although the presence of substituents may be responsible for some structural swelling (see below).

Assuming a direct comparison to be possible, a methylated polyanion of methyl d.s. x and acid d.s. y will have the same total substituent distribution as a methylated cellulose (containing no acid groups) of methyl group d.s. $(x + y)$. However, the contents of 3-*O*-methyl-D-glucose in the samples from methylated *P1* and *P2* are more

than can be accounted for by mere substitution of positions 2 and 6 by anionic groups. This discrepancy can be considered in view of the assumptions underlying the above reasoning. (*a*) The phosphorus content, which is the same after alkali treatment and methylation (Table I), is 1.12 and 2.64% for *P1* and *P2*, respectively, whereas the corresponding losses in exchange capacity are 28.7% and 28.4%, respectively, suggesting that methylation of phosphoric ester groups is mainly responsible for capacity loss. (*b*) Hydrolysis of phosphate only regenerates hydroxyl groups. No phosphorus could be found in the hydrolysates, and studies involving cellulose acetates have shown virtually complete acetate–hydroxyl conversion[9]. (*c*) There are no other substituents. Potentiometric titrations have shown the phosphorus contents to be in agreement with measured $=PO_2^-$ and $-PO_3^{2-}$ contents[14]. Nitrogen in the phosphates (0.4% in *P1*, 0.5% in *P2*) is mainly removed after alkali treatment and is probably[20] due to carbamate groups, $-O(CO)NH_2$. (*d*) There is no migration of anionic group during alkali treatment.* Alkali-catalysed phosphate migration in D-glucose 2-phosphate is known[21]. However, the lack of availability of neighbouring hydroxyl groups, or inaccessibility within the polymer structure, would disfavour this possibility. (*e*) There is no decomposition on acid treatment. Methoxyl contents of the cloths are in good agreement with those obtained by g.l.c. of the hydrolysates. (*f*) In an unmercerised cellulose, HO-2,3,6 have the same relative initial reactivities to phosphorylation as to methylation. This assumption can be considered in terms of a relationship[1]:

$$\frac{\text{cellulose-HO-2}}{\text{cellulose-HO-6}} = \frac{k_6}{k_2} \times \frac{dP_6}{dP_2}. \qquad (1)$$

The ratio of the accessibilities of HO-2 and HO-6 in a partly crystalline cellulose is a function of the rate constants and the distribution of isomers, dP_2 and dP_6 at positions 2 and 6, respectively[3]. Presumably, the hydroxyl groups will show different relative rates (k_2 and k_6) for methylation as opposed to phosphorylation; thus, a different ratio of substituents will be obtained, *i.e.*,

$$dP_2/dP_6 \text{ (methylation)} \neq dP_2/dP_6 \text{ (phosphorylation)}. \qquad (2)$$

Ethylation of cellulose gives a range of substituents quite different to that formed on methylation[19]. The difference was assigned to a "propping open" of the water-swellable areas of cellulose by the ethyl groups, giving an initial increase in moisture regain, followed by a subsequent decrease when the ethyl d.s. is increased, because the greater bulk of the ethyl groups hinders unsubstituted hydroxyl groups in addition to those that had been substituted. Similarly, although reagent-treated celluloses did not greatly affect the distribution of the methyl isomers (Table II), it seems likely that

*A referee has pointed out that no migration occurs for inositol phosphates under alkaline conditions [S. J. Angyal and M. E. Tate, *J. Chem. Soc.*, (1961) 4122–4128]. Although phosphodiesters migrate under these conditions, such cleavage could only occur for cross-linked groups, which do not preponderate here.

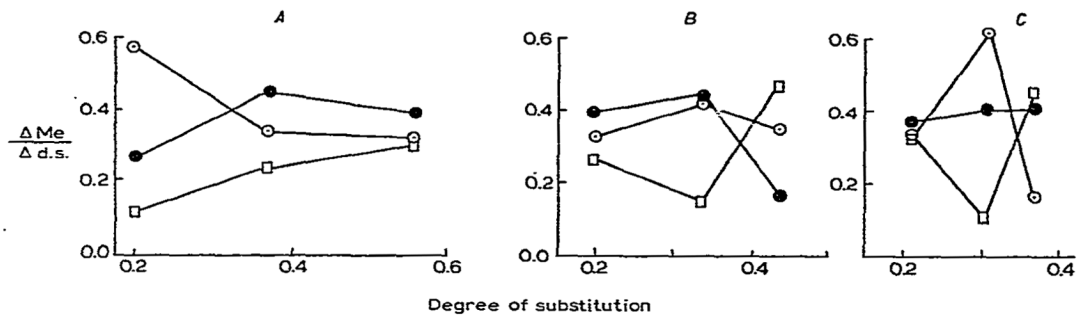

Fig. 2. Increase in methylation with d.s. at HO-2 (—○—), HO-3 (—□—), and HO-6 (—●—), as a function of d.s. for poplin (*A*), cellulose phosphate *P1* (*B*), and *P2* (*C*).

interspersal of the bulky phosphate group in the cellulose molecule will have a large effect on the positions of subsequent methylation. Thus, assumption (*f*) may be invalid, and it may not be possible to compare directly methylated celluloses that are unsubstituted and substituted with phosphate groups, in order to assign quantitatively the location of the acid groups. However, the methyl distributions in the polyanions can be used to give structural information.

Fig. 2 compares the incremental increase in methylation with d.s. (ΔMe/Δd.s.) of the poplin cellulose and the two phosphates as a function of d.s. The initial high reactivity of HO-2 in cellulose to methylation is not observed and could be attributed to extensive substitution of HO-2 with acid groups, whereas HO-6 is initially of similar reactivity for all three celluloses, with some decrease at higher d.s., markedly so with *P1*. The initial methylation of *P1* and *P2* is the same as for poplin (~0.2 after one methylation), but further methylation takes place less readily. The phosphate group appears to behave as a large alkyl group, initially "propping up" the chains and, as the d.s. increases, causing a "blocking effect", *i.e.*, the accessibility of the hydroxyl groups in the fibril surface is limited. These assumptions accord with measured moisture-regain values of 8.0, 5.8, and 4.9%, respectively, for *P2*, poplin, and thrice-methylated *P2*. The high reactivity of HO-3 in *P1* and *P2* is probably due to disruption of the structure, principally (*i*) separation of chains by interspersed bulky phosphate groups, and (*ii*) disruption of O-5...H-3 intermolecular hydrogen-bonding. Activation of HO-3 by other substituents in the D-glucopyranosyl units could also be a contributory factor[22].

Although the methylated polyanions show less selectivity towards methylation than a poplin of the same d.s., the relatively high proportions of D-glucose and di- and tri-*O*-methyl-D-glucoses (of the same order to those obtained for methylated poplin of the same d.s.) suggest that methylation takes place at the surface of the fibrils.

We have applied methylation analysis to a cellulose phosphate prepared under anhydrous conditions (anionic d.s., 0.05), a cellulose sulphate (d.s. 0.05), and a carboxymethylcellulose (d.s. 0.04). There was decomposition and loss of anionic

groups, and the results could not be satisfactorily interpreted. However, some similar trends were recognised, namely, the formation of esters having high methyl content at position 3 and a decrease in the rate of methylation on subsequent methylations. The highly swelled carboxymethylcellulose structure showed high reactivity to methylation, with the hydroxyl accessibilities resembling those of amorphous cellulose.

Despite evidence of structural disruption, the high content of D-glucose in the hydrolysates of all the methylated cellulosic materials is indicative of the preservation of crystalline regions. The observed methyl distributions are attributed to bulky phosphate groups separating the cellulose chains at the surfaces of the fibrils, thereby disrupting intermolecular hydrogen-bonds (*e.g.*, O-5...HO-3) and leading to a more even distribution of methyl groups. This explanation seems to be more likely than ascribing the reactive sites to amorphous regions, and supports the ideas of Roberts and co-workers[1], and of Rowland and Roberts[23], in that cotton of the cellulose I structure is highly crystalline and that, under heterogeneous conditions, reaction takes place primarily at surfaces.

The results also demonstrate a higher phosphate substitution at positions 2 and 6 in the D-glucopyranosyl units, which is in keeping with the proposed fibrillar models for cotton cellulose I.

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