525. *The Detection and Determination of "End Groups" in O-Methylcelluloses.*

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Tetra-*O*-methylglucose end groups have been detected and determined in hydrolysates of *O*-methylcelluloses. Degrees of polymerisation have been calculated from physico-chemical data and from end-group contents. To minimise degradation during methylation oxygen must be very rigorously excluded. The presence of end groups has been demonstrated in *O*-methylcelluloses soluble in chloroform–light petroleum (1 : 1) of degree of polymerisation about 1000 and in derivatives insoluble in this solvent and of greater chain length. This evidence is contrary to that upon which Haworth (*Chem. and Ind.*, 1939, **17**, 917) formulated his theory of the behaviour of cellulose during methylation. The results do not exclude the possibility that the original cellulose might be branched to a slight extent. With the rigorous exclusion of oxygen during the methylation a high percentage of the product is insoluble in the chloroform–light petroleum solvent. These insoluble materials, although possessed of end groups, do not, on analysis, yield evidence which would provide a sound basis for conclusions as to the structure of the original molecule.

Naturally occurring celluloses have long been recognised as polysaccharides composed almost entirely of D-glucose residues, but knowledge of the finer structural features of the molecule remains incomplete. The terminal non-reducing residue of a polyanhydroglucose chain will, after methylation and hydrolysis, give rise to tetra-*O*-methylglucose and the presence of this sugar has been demonstrated in hydrolysates of methylated cotton celluloses (Haworth, Hirst, Owen, Peat, and Averill, *J.*, 1939, 1885; Haworth, Montanna, and Peat, *ibid.*, p. 1899; Hess, Grigorescue, Steurer, and Frahm, *Ber.*, 1940, **73**, 669). In the case of an unbranched long-chain molecule determination of the tetra-*O*-methylglucose will give a value for the number average degree of polymerisation, but the method used by Hess *et al.* for this analysis (Neumann and Hess, *Ber.*, 1937, **70**, 721; Leckzyck, *ibid.*, 1938, **71**, 829) has been shown to be unreliable (Averill and Peat, *J.*, 1938, 1244; Hirst and Young, *ibid.*, p. 1247). Haworth and his co-workers found that if the methylation was carried out in the presence of oxygen the cellulose was degraded and end groups were determined in amounts to agree with physico-chemically estimated degrees of polymerisation; on the other hand they reported that if oxygen was excluded during the methylation the cellulose was degraded as before but no end groups could be detected after hydrolysis. On the basis of these results Haworth put forward a theory (*Chem. and Ind.*, 1939, **17**, 917) of the re-combination of reducing and non-reducing end groups to give ring structures in the absence of oxygen, this linking being prevented if oxygen was present and made possible by some unspecified type of bridge which held the cellulose chains in positions favourable for ring closure. This problem has now been re-examined, paper-chromatographic analysis being used for the separation of the end group before its determination.

In order to minimise degradation unscoured cotton was used as the raw material for most of these experiments. The procedure was essentially as described by Haworth *et al.* (*loc. cit.*) except that the methylation liquors were neutralised with sulphuric acid while still under nitrogen. Degrees of polymerisation were calculated from viscosity data and in some cases from osmotic pressures. These determinations on natural cellulose were carried out on the trinitrate prepared under anhydrous conditions and dissolved in butyl acetate, and those on the soluble *O*-methylcelluloses in chloroform. It was found that 90% formic acid hydrolysed *O*-methylcellulose much faster and under less drastic conditions than the methods which had been used by previous workers. When pure 2 : 3 : 6-tri-*O*-methylglucose was subjected to this hydrolytic procedure, no demethylation was observed.

Dewaxed American cotton was methylated in an atmosphere of pyrogallol-washed nitrogen (Expt. 1). The dried *O*-methylcellulose was extracted with chloroform, giving a

very viscous solution containing undissolved material. In order to separate on the centrifuge the soluble from the insoluble *O*-methylcellulose it was necessary to reduce the viscosity of the chloroform extract by the addition of an approximately equal volume of light petroleum. That fraction which then remained in solution was used for the end-group determination. To cause its precipitation it was necessary to add a further four volumes of light petroleum. It is the first solvent, chloroform–light petroleum (1 : 1), which is implied when *O*-methylcelluloses are referred to below as soluble or insoluble. Measurement of the viscosity of this soluble *O*-methylcellulose in chloroform showed that extensive degradation had accompanied the methylation. After hydrolysis and methyl glucoside formation any methyl tetra-*O*-methylglucoside present was concentrated by extraction of an aqueous solution of the glucosides with purified light petroleum (Brown and Jones, *J.*, 1947, 1344). These extracts were hydrolysed and the neutralised hydrolysates examined on a paper chromatogram. Two components were detected, the faster of which travelled with 2 : 3 : 4 : 6-tetra- and the other with 2 : 3 : 6-tri-*O*-methylglucose in the two solvent systems used. The ratio of tetra- to tri-*O*-methylglucose was determined by buffered sodium hypoiodite (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1294) after separation of the two sugars by paper chromatography with ethyl methyl ketone as solvent.

In the second experiment bleached wood pulp was methylated in nitrogen and the soluble portion of the product subjected to an end-group analysis as described before. Degradation had again occurred during methylation. The details of these two experiments are summarised in Table 1.

TABLE 1.

		Natural cellulose: D.P. calc. from				*O*-Methylcellulose: D.P. calc. from		
Expt. no.	Source	viscosity	osmotic press.	Sol. in chloroform–petroleum (%)	OMe (%)	viscosity	osmotic press.	end group
1	American Cotton	3900	2200	75	43·7	256	—	245
2	Bleached Wood Pulp	800	—	84	43·1	260	200	244

(D.P. = degree of polymerisation.)

The sugars isolated from the *O*-methylcellulose hydrolysates were proved to be 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methylglucose by comparison of the infra-red spectrum of an end-group concentrate from a further sample of *O*-methylcellulose with the spectra of known specimens. The reliability of the concentration and analytical method for the determination of the end group was tested by the analysis of a synthetic 1 : 1500 mixture of tetra- and tri-*O*-methylglucose; four paper-chromatographic determinations gave an average value within 6% of the theoretical.

It is considered that these two experiments simulated those reported by Haworth *et al.* (*loc. cit*) for the methylation of cellulose under " oxygen-free " conditions. They confirm the degradation under those conditions. No difficulty was experienced, however, in detection and determination of the tetra-*O*-methylglucose, and the values they afford for the degrees of polymerisation agree with those values obtained physico-chemically. Thus there is no evidence in these experiments for linking of end groups in the absence of oxygen.

In order to reduce the degradation, dewaxed Egyptian cotton was methylated under two sets of conditions (Expts. 3—4 and 5—6), the second set being more efficient than the first in exclusion of oxygen. Experiment No. 6 was the most carefully conducted of the series. Parallel with this exclusion of oxygen the percentage of the resulting *O*-methylcelluloses which was soluble in chloroform–light petroleum (1 : 1) diminished. These soluble materials were hydrolysed and the end groups which were detected in the hydrolysates were determined, giving values for the degrees of polymerisation. The results obtained in experiments 3—5 are shown in Table 2.

The discrepancy between the values for the number average degrees of polymerisation obtained by osmotic and end-group measurements may be expained by assuming a slightly branched structure for these soluble *O*-methyl celluloses. The maximum permissible number of branches (about one per cellulose molecule) is, however, sufficiently small to

require further evidence for substantiation. It is considered that the errors inherent in the classical methylation technique render it incapable of providing a final decision on this point.

The soluble *O*-methylcelluloses prepared under increasingly oxygen-free conditions are seen to be less and less degraded, in accord with the well-established degradation of cellulose in the presence of alkali and oxygen. From a consideration of the degrees of polymerisation

TABLE 2. *Natural cellulose*: *Egyptian cotton, D.P.* (*viscosity*) 4600, *D.P.* (*osmotic*) 2300.

		O-Methylcellulose			
	Product. sol. in		Degree of polymerisation calc. from		
Expt. no.	chloroform–petroleum (%)	OMe (%)	viscosity	osmotic press.	end groups
3	50	44·0	890	610	495
4	64	42·5	850	720	540
5	47	42·7	1250	1050	880

of the *O*-methylcelluloses in the range 1000—200, as determined by the physico-chemical and end-group methods, it may be concluded that each break in the cellulose chain gives rise to one non-reducing end group. These may be formed either by direct action on a β-glucosidic linkage or by the oxidation of one or more glucose residues producing in the molecule linkages which are labile to alkaline hydrolysis, the oxidised residues themselves then being completely broken down.

A high proportion of the *O*-methylcelluloses prepared in experiments 3—6 is insoluble, increasingly as the precautions to exclude oxygen are increased. Indeed in experiment 6 the *O*-methylcellulose contained 39·5% of methoxyl, and 75% of the material was insoluble. These insoluble fractions were hydrolysed and the presence of end groups in the hydrolysates demonstrated. Determination of the end group is the only method of assessing the degree of polymerisation in this instance, but the values will tend to be high owing to the under-methylation. The degrees of polymerisation are of the same order as that of the starting material (Table 3).

TABLE 3.

Sample no.	No. of methylations	Product insol. in chloroform–petroleum (%)	OMe (%)	D.P. (end group)
6*a*	6	77	38·1	2340
6*b*	7	74	37·9	2040

The conditions under which these two samples of *O*-methylcellulose were prepared are considered to be essentially non-degradative and the detection of end groups in the hydrolysates indicates that the original cellulose molecule had an open-chain and possibly an unbranched structure.

As degradation during the methylation is minimised by the exclusion of oxygen the amount of insoluble fraction increases. No valid conclusions as to the structure of the original cellulose molecule can be drawn if this very significant fraction is neglected. These insoluble materials however, by virtue of their under-methylation, are unsuitable for use in the determination of the fine details of molecular stucture. These results are included therefore to show the limitations in the method.

Paper chromatography and ionophoresis (Foster, *Chem. and Ind.*, 1952, 828, 1050; Foster and Stacey, *J. Appl. Chem.*, 1952, **3**, 19) of the constituents of these insoluble *O*-methylcellulose hydrolysates has shown the virtual absence of 3-*O*-methyl- and 3 : 6-di-*O*-methyl-glucose and the presence of all the other possible isomers. The possibility thus arises that in a proportion of the glucose residues in the cellulose positions 3 and 6 may be prevented from reacting under these conditions of methylation, for reasons which are being studied further.

EXPERIMENTAL

The cotton, in the form of card sliver, was de-waxed by extraction (Soxhlet) with ethanol for 17 hr., followed by ether for 12 hr., and was dried at 50°. It was then milled in a Raymond Laboratory Mill to a staple length of 1—2 mm. The ethanol extracts, after filtration and evapor-

ation, were shown by paper chromatography to contain mannose, glucose, and galactose, together with cellobiose and higher oligosaccharides (see also Guthrie and Reeves, *Textile Res. J.*, 1950, **20**, 859). Two solvent systems were used for the paper chromatography: Ethyl methyl ketone, 10% undersaturated with water; and the organic layer of a butanol–ethanol–water (4 : 1 : 5) (approx. 10% undersaturated with water). Undersaturation was to prevent streaking of the chromatograms by the separation of water from the organic solvent should the temperature fall overnight. *p*-Anisidine hydrochloride (Hough, Jones, and Wadman, *J.*, 1950, 1702) was used as spray. Evaporations were carried out at 40°/15 mm., unless otherwise stated. The nitrogen used was British Oxygen Co. "oxygen free," said to contain less than 10 p.p.m. oxygen.

Molecular-weight Measurements.—The osmometer was of a modified Fuoss–Mead type, with a vertical membrane, and kept at 25·0° ± 0·002°. Membranes were prepared from never-dried Cellophane (British Cellophane Ltd., 300 gel film). The viscometer was of the capillary type, kept at 25·0° ± 0·05°, and so designed that the corrections for non-laminar flow were negligible.

Natural Cellulose.—The trinitrate was prepared under anhydrous conditions (Sharples, unpublished work). Osmotic pressure determinations were made on its solution in acetone and corrected for the density effect (Higginbotham, *J. Text. Inst.*, 1951, **42**, T235). The error at this degree of polymerisation was *ca.* 10%. The viscosity determinations were carried out on butyl acetate solutions and the results were corrected for rate of shear and converted into degrees of polymerisation by D.P. = 92·5η (Harland, unpublished results).

O-*Methylcellulose in Chloroform Solution.*—Osmotic results were corrected for the density effect. The viscosity results were not corrected for rate of shear. At D.P. (visc.) = 2000 the values could be 5% low; this error at D.P. (visc.) = 1000 would be negligible. Intrinsic viscosities were converted to degrees of polymerisation by D.P. = 91η (Staudinger and Reinecke, *Annalen*, 1938, **535**, 47).

Experiment No. 1.—Milled, de-waxed American cotton (20 g.) was suspended in sodium hydroxide solution (1500 ml.; 30%) with rapid stirring, under nitrogen previously washed with alkaline pyrogallol solution. After 2 hr., methyl sulphate (275 ml.) in dioxan (275 ml.) was added dropwise, at approx. 40°; the current of nitrogen and agitation were continued overnight. The temperature was lowered to 0° and the alkali neutralised by sulphuric acid (50%), the nitrogen atmosphere and the stirring being maintained meanwhile. The insoluble partially methylated cotton was separated from the neutral liquors by filtration on sintered glass, and washed with hot water to remove the majority of the inorganic material.

Repetition of this process 4 times yielded crude *O*-methylcellulose (22 g.) (Found: OMe, 41·3%). The cotton was not allowed to dry between the methylations. The methoxyl content could not be increased by further treatment with the methylating reagents.

The crude material was dried (50°) and shaken with chloroform (1 l.). The resulting viscous suspension was diluted with light petroleum (b. p. 40—60°; 1 l.), and the highly swollen solid (fraction 1) separated centrifugally from the clear viscous solution. It was then triturated with light petroleum until free from chloroform and dried (50°) (5·4 g.) (Found: OMe, 39·6%). Fraction 2 was obtained from the centrifugate by complete precipitation with light petroleum. It was triturated and dried as above (15·7 g.), viscosity average D.P. 256 (Found: OMe, 43·7%).

Hydrolysis of methylated cellulose. Fraction 2 (8·0 g.) was treated with formic acid (170 ml.; 90%) on a boiling-water bath until the rotation was constant (5 hr.). The acid was removed by evaporation, and water (3 × *ca.* 100 ml.) was added, with evaporation between additions. The product was dried by distillation of its solution in ethanol–benzene, to leave a syrup (8·65 g.) which readily crystallised. These free sugars were refluxed with methanolic hydrogen chloride (200 ml.; 2—3%) for 16 hr. and after working up in the usual way the glucosides were obtained as a clear syrup (9·1 g.).

Partial fractionation of the methyl glucosides by solvent extraction. The glucosides were dissolved in water (80 ml.) and divided into two portions, each of which was extracted for 2 periods of 3 hr. with light petroleum (b. p. 38—41°) in a liquid–liquid extraction apparatus (Brown and Jones, *loc. cit.*). The corresponding extracts (fractions 1 and 2) from each portion were combined and the solvent evaporated with very slight reduction in pressure, giving from fraction 1 0·5148 g., and from fraction 2 0·4955 g.

Hydrolysis of petroleum extracts and paper chromatography. Hydrolysis of the petroleum-soluble glucosides was effected by treatment with sulphuric acid (50 ml.; 2N) on a boiling-water bath for 16 hr. Neutralisation with barium carbonate, filtration, washing of the solids, and evaporation gave the free sugars (fractions 1*a* and 2*a*) which readily crystallised *in vacuo* over phosphoric oxide (fraction 1*a* 0·4751 g.; fraction 2*a* 0·4504 g.). These materials were treated

in water (5 ml.) with a little "Deminrolite" ion-exchange resin (Permutit Co. Ltd.) before chromatography. Each fraction contained only two components: the faster travelled with 2 : 3 : 4 : 6-tetra- and the other with 2 : 3 : 6-tri-*O*-methylglucose in both the solvent systems used. R_G values for the ethyl methyl ketone system, the solvent front having travelled 90—100 cm. in 12 hr. at 20°, were: 2 : 3 : 4 : 6-tetra-*O*-methylglucose 1·00; 2 : 3 : 4 : 6-tetra-*O*-methylmannose 0·97; 2 : 3 : 6-tri-*O*-methylglucose 0·75; 2 : 3 : 4 : 6-tetra-*O*-methylgalactose 0·85.

The ratio of tetra- to tri-*O*-methylglucose in the above fractions was determined by hypoiodite oxidation (Chanda, Hirst, Jones, and Percival, *loc. cit.*). The sugars were washed from the relevant portions of the chromatogram with cold distilled water (5 ml.) dropping slowly from burettes through the paper into B.24 boiling-tubes. 0·1N-Iodine (1 ml.) was added from a 1-ml. pipette, with a standardised technique, being followed by buffer solution (3 ml.; pH 11·4). The stoppers were sealed with potassium iodide solution (10%), and the tubes kept in the dark for 3 hr. After acidification with sulphuric acid (3 ml.; 2N) the liberated iodine was titrated against *ca.* 0·01N-sodium thiosulphate. Paper blank determinations were run concurrently. The differences between these two titrations gave the required ratio. The value of this ratio for each fraction was derived from at least four paper-chromatographic estimations. Ratios of tetra- to tri-*O*-methyl sugars were 1 : 16·0 in fraction 1*a* and 1 : 51·7 in fraction 2*a*. Light petroleum thus extracted 38·4 mg. of tetra-*O*-methylglucose (0·163 millimole of tetra-*O*-methylanhydroglucose); 8·0 g. of tri-*O*-methylcellulose is equiv. to 39·21 millimoles of tri-*O*-methylanhydroglucose, whence D.P. by end-group estimation = 245.

Experiment No. 2.—A sample (20 g.) of a bleached wood pulp (viscosity average D.P. 800) was treated as described above. The results are shown in Table 1.

Determination of Tetra-O-methylglucose in a Known Mixture by the Above Method.—Pure distilled methyl 2 : 3 : 4 : 6-tetra- (3·524 mg.) and distilled methyl 2 : 3 : 6-tri-*O*-methyl-α-D-glucoside (5·2861 g.) in water (50 ml.) were analysed as above, 3·74 mg. (106%) of tetra-*O*-methylglucose being recovered.

Determination of the Extent of Demethylation during Hydrolysis.—Chromatographically pure 2 : 3 : 6-tri-*O*-methylglucose (1·0 g.) was hydrolysed as was the *O*-methylcellulose. The crystalline material recovered (0·955 g.) was analysed on the paper chromatogram and found to contain only 2 : 3 : 6-tri-*O*-methylglucose.

Infra-red Spectra.—These were taken on a Sir Howard Grubb, Parsons S3 sepctrometer fitted with a rock-salt prism. The spectrum of 2 : 3 : 6-tri-*O*-methylglucose was recorded for its solution in dry chloroform; the 2 : 3 : 4 : 6-tetra-*O*-methylglucose was dissolved in dry carbon tetrachloride, as was the end-group concentrate. This concentrate was obtained by ether-extraction of an aqueous solution of the free sugars produced on hydrolysis of a further sample of *O*-methylcellulose prepared under the conditions used in Experiments 1 and 2 and after removal of any formyl esters by hydrolysis by dilute sulphuric acid.

Methylations under more rigorously Controlled Conditions.—Egyptian cotton (Sudan Sakel, type G.3.L.) was used in these experiments. The reactions were carried out in the dark with the quantities of reagents used in experiment 1. The results are given in Tables 2 and 3.

Experiment No. 3.—The sodium hydroxide solution was saturated whilst hot with nitrogen, previously washed by passage through a train of bottles fitted with sintered-glass bubblers containing an alkaline solution of sodium anthraquinone-2-sulphonate (Whelan and Peat, *J. Soc. Dyers and Col.* 1949, **65**, 756). The dioxan and methyl sulphate were distilled off under nitrogen, the latter under reduced pressure, and subsequently stored under nitrogen. The nitrogen entering the reaction flask was also washed by the above solution. Milled de-waxed cotton (20 g.) after four methylations yielded a crude granular methyl derivative (22·4 g.) (Found: OMe, 41·6%). A portion (10 g.) of the soluble fraction (11·2 g.) (Found: OMe, 44·0%) was hydrolysed and the end groups which were detected in the hydrolysate were determined, giving a value for the number average degree of polymerisation of this methylcellulose.

Experiment No. 4.—The same precautions were observed for the exclusion of oxygen as in experiment 3. Cotton (20 g.) after four methylations gave a granular methyl derivative (22·3 g.) (Found: OMe, 41·7%). This was fractionated as before and a portion (11·5 g.) of the soluble material (13·5 g.) (Found: OMe, 42·5%) analysed for end groups.

Experiment No. 5.—In addition to the above precautions to exclude oxygen, the flask containing the milled cotton was evacuated and filled with washed nitrogen four times before each methylation. Cotton (20 g.) after five methylations gave a granular methyl derivative (23·5 g.) (Found: OMe, 41·5%) which was fractionated as above. The soluble methylcellulose (9·2 g.) (Found: OMe, 42·7%) was hydrolysed and analysed for end groups.

Experiment No. 6.—The above precautions for the exclusion of oxygen during the methylation were applied as rigorously as possible. Cotton (20 g.) was subjected to six methylations, and the still fibrous material (21 g.) (Found : OMe, 39·5%) was divided into two portions (samples 6*a* and 6*b*) one of which (6*b*; 10 g.) was remethylated. It remained fibrous and of the same methoxyl content. Both samples were fractionated as before. End groups were present in the hydrolysates of the soluble and the insoluble fractions, but the weights of the soluble materials (from 6*a* : 2·3 g.; 23%; OMe, 43·0%. From 6*b* : 2·4 g.; 26%; OMe, 42·9%) were too small to permit accurate determination of the end group. The insoluble materials (from 6*a*; 7·7 g.; OMe, 38·1%. From 6*b* : 6·9 g.; OMe, 37·9%) were subjected to end-group assay.

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