LXXIII. WOOD CELLULOSE.

II. THE DEPOLYMERISED CELLULOSES OF WOOD AND COTTON.

By DAVID JAMES BELL.

From the Chemistry Research Laboratory of the United College of St Salvator and St Leonard, The University of St Andrews.

(Received April 1st, 1932.)

IN a previous communication [1932] on the behaviour of wood α -celluloses towards methylation and the subsequent cleavage of the methylates, it was demonstrated that these α -celluloses could not be completely etherified with respect to the three hydroxyl groups known to be present in the C_6 unit. Treatment with methyl sulphate and alkali, by a method adequate to accomplish complete methylation of cotton cellulose, and of starch [Bell, 1931] (a notoriously resistant polysaccharide) brought about etherification to the extent of only 36.3–39.1 % of methoxyl. Cleavage of the methylated wood celluloses, under conditions promoting complete fission of trimethylated cotton cellulose, resulted in the dissection of the materials into two parts.

(a) A fully methylated fraction, reacting with the cleavage reagents and manifesting itself as a virtually homogeneous cleavage product consisting of 2:3:6-trimethyl-methylglucoside.

(b) A "resistant portion," methoxyl content 27–30 %, insoluble in all solvents, and completely resistant to cleavage by any known means. By exhaustive methylation the methoxyl content could be raised to 30–32 % but no further.

Since these "resistant portions" appeared to have a hitherto unsuspected existence in all the four wood celluloses examined, an investigation of their properties and, if possible, an elucidation of their inert behaviour, appeared to be of importance. The obvious explanation of the non-reactive behaviour is that of a high polymerisation within these fractions, a condition not obtaining in either cotton or esparto cellulose.

Were high polymerisation the reason, and could depolymerisation of this ultra-complex fraction be effected, then resistance should disappear.

Acetylation by Barnett's method [1921] appeared, from experiments on starch carried out in this laboratory by Irvine and Rutherford [1932], to effect considerable depolymerisation of intact polysaccharides. It was therefore considered that the application of this process to wood celluloses might afford a possible means of determining whether or not the behaviour of the "resistant

portions" was a result of high polymerisation. Subsequent methylation of the acetate, followed by cleavage of the resulting methylate, would provide definite information as to whether or not resistance survived acetylation and depolymerisation. A retention of non-reactivity would be a strong reason for assuming that mere molecular complexity was not the basal cause of the properties of the "resistant portions."

Acetylation.

The action of Barnett's reagents on the wood celluloses, under carefully standardised conditions, resulted in the production of triacetates in almost theoretical yields. The specific rotations of the acetates, in chloroform solution, showed various values, as will be seen from Table I. The alkali-extracted

Table I.

Source of cellulose	Yield of triacetate %	$[\alpha]_D$ in CHCl_3
<i>F. sylvatica</i>	97.3	-19.6°
Thiriena pulp	98.4	-18.4°
<i>Q. sessiliflora</i>	94.5	-11.9°
Sitka spruce	96.7	-10.4°
<i>Q. pedunculata</i>	97.2	0.0°
Controls:		
Standard cotton	97.6	-22.7°
„ (alkali-extracted)	96.8	-23.0°

cotton was prepared by extraction with 12 % NaOH at 90° for 9 hours. The specific rotation of the acetate shows that alkali-extraction does not account for the low values found for the wood acetates.

The wood acetates were all non-reducing, contained no pentose residues, and were soluble in highly halogenated paraffins, but not in alcohol or acetone. Acetyl estimations, in general, gave slightly high values for triacetates, but no significance can be attached to this fact bearing in mind the nature of the processes involved in this determination.

The simultaneous de-acetylation and methylation of wood-cellulose acetate ($[\alpha]_D - 18.4^\circ$).

Employing the procedure of Irvine and Robertson [1926], methylation of the depolymerised Thiriena pulp cellulose was found to take place with greater ease than that of the corresponding intact cellulose. Ten treatments effected the introduction of methyl groups amounting to 39.2 % OMe, and exhaustive treatment resulted in no increase. Yields amounted to 91.7-94.0 % of that theoretically possible. The methylated depolymerised cellulose was a white powder, soluble in chloroform, benzene, pyridine, alcohol and glacial acetic acid. It was insoluble in acetone. In most of these solvents the solutions were clear, easily filterable and of inconsiderable viscosity. In 5 % solution in chloroform the methylate had a specific rotation of +2.3°, a value in close

agreement with that of the chloroform-soluble fraction of methylated intact Thiriena pulp [Bell, 1932].

From this last observation, if the doubtful criterion of optical data be adopted, the possibility of depolymerisation of the insoluble fraction of the cellulose to the same level of complexity as the chloroform-soluble one must not be overlooked.

Comparative experiments on depolymerised cotton cellulose.

Cotton cellulose triacetate, $[\alpha]_D - 22.7^\circ$, when treated in the same fashion as the Thiriena pulp acetate, attained a methoxyl content of 43.7 % after 14 methylations. No increase in the methoxyl content could be effected, either by the use of methyl sulphate and alkali on a pyridine solution of the methylate, or by the action of Purdie's reagents on the material dissolved in dry chloroform. It is noteworthy that Purdie's reagents effected no apparent change in the methylated depolymerised cotton, while considerable alteration in the condition of methylated starch takes place under similar circumstances, as has been shown by Irvine and Macdonald [1926] in this laboratory.

Methylated depolymerised cotton was a white powder, possessing the same solubilities as methylated depolymerised wood cellulose. In chloroform solution it showed $[\alpha]_D - 1.7^\circ$; after several treatments with Purdie's reagents the value was found to be virtually the same, namely -1.2° . In this case, it will be observed, there is no agreement between the rotations of the depolymerised methylate and the soluble fraction of intact trimethylated cotton [Bell, 1932].

Eight methylations of the cotton acetate introduced 38.6 % of methoxyl; specimens of this material were employed in comparative cleavage experiments with the methylated derivative of Thiriena pulp.

Acetylation of the "fully-methylated" depolymerised cotton (OMe, 43.7 %) showed that free hydroxyl groups were still present, although they could not be etherified. For the acetyl derivative, an acetyl content of 2.90 % was found; theory 2.63 %.

The investigation of the celluloses of wood and cotton, both intact and depolymerised, is summarised in Table II.

Table II.

Cellulose	Cotton		Wood	
	Intact	Depolym.	Intact	Depolym.
$[\alpha]_D$ of triacetate	-22.7°	—	-18.4°	—
Treatments required for complete methylation	36	14	18	10
OMe of methylate	44.3 %	43.7 %	39.0 %	39.2 %
OMe of methylate (soluble fraction of intact cellulose)		43.9 %		39.1 %
$[\alpha]_D$ of methylate (soluble material)	-10.5°	-1.2°	$+2.5^\circ$	$+2.3^\circ$

The properties of the trimethylated depolymerised cotton are in almost exact agreement with the values given by Hess *et al.* [1923] for his trimethylcellulose "A" in the crude state. In no way did the material resemble the "trimethylcellulose" which Haworth, Hirst and Thomas [1931] claim to have obtained with exceptional facility from an acetone-soluble cellulose acetate. (Attempts to repeat the experiments of these workers, it may be stated, were not successful.) The three substances under consideration are compared in Table III.

Table III.

	Hess	Bell ¹	Haworth
$[\alpha]_D$ of initial acetate	-16.7°	-22.7°	—
Trimethylcellulose, OMe	42 %	43.7 %	45.6 %
„ $[\alpha]_D$ in CHCl_3	0.0°	-1.2°	-10°
„ M.P.	230-245°	233-240°	215-216°*
* Material of OMe 41-42 % had m.p. 242-243°.			

Subsequent to their original experiments, Hess and Schultze purified the cellulose acetate "A," whereby the rotation in chloroform increased to -19.5° [1927]; a genuine "trimethylcellulose A" (OMe, 45 %) was also obtained from the crude product [Hess and Weltzien, 1925].

Relation between the specific rotation of the wood cellulose acetates and the proportion of the "resistant portion" in the material.

What interpretation, if any, can be placed on the magnitude of rotation of a cellulose acetate? A host of reducing and non-reducing derivatives of cellulose of widely different degrees of apparent complexity display closely similar optical activity. Ost and Kayama [1912] from alkali-treated cellulose have obtained acetone-soluble triacetates showing $[\alpha]_D$ -17° to -22°. The "hexacetyl anhydrobiose" of Hess [1921], $[\alpha]_D$ -17.8°, his "triacetyl cellulose 'A,'" and the wood cellulose triacetates described above are examples which appear only to confuse attempted explanation.

A consideration of the wood cellulose acetates reveals that an almost quantitative relationship exists between their specific rotations and the proportion of resistant material obtained in the cleavage of the corresponding methylates [Bell, 1932] (see Table IV). While the relationship is not uniformly

Table IV.

Source of α -cellulose	$[\alpha]_D$ of acetate in CHCl_3	% "resistant portion"
<i>F. sylvatica</i>	-19.6°	24
Thiriena pulp	-18.4°	23
Sitka spruce	-10.4°	30
<i>Q. pedunculata</i>	0.0°	40

constant, it is apparent that an increase of 1 % in the content of "resistant portion" results in an increase in dextro-rotation of approximately 1°. While

¹ Dr N. K. Adam has informed the author, after this paper had been sent to the printer, that this depolymerised product gives surface-film results identical with those given by the trimethylcellulose of Hess.

this observation, together with a consideration of the results of the methylation experiments, suggests profound differences between the nature of the "resistant portions" and that of the remainder of the wood cellulose, the investigation of the "resistant portions" has by no means been confined to such empirical observations. This side of the problem will be dealt with in a forthcoming communication.

Cleavage of the methylated depolymerised cellulose.

The derivative of Thiriena pulp was subjected to the action of methyl alcohol containing hydrogen chloride; at the same time fully methylated (OMe, 43.7 %) and partially methylated (OMe, 38.6 %) depolymerised cotton celluloses were treated under the same conditions. Employing the technique described previously [1932] for the cleavage of the methylated intact celluloses, the following results were obtained.

Table V.

Methylated cellulose (depolymerised)	Thiriena pulp (39.2 % OMe)	Cotton (43.7 % OMe)	Cotton (38.6 % OMe)
Duration of treatment	400 hrs.	400 hrs.	400 hrs.
Cleavage products isolated	81.9 %	93.0 %	95.0 %
2:3:6-Trimethyl-methylglucoside	92.3 %	83.5 %	—
Dimethyl-methylglucoside*	7.2 % (a)	13.8 % (b)	—
Resistant material found	14.0 %	Nil	Nil
Methoxyl content of resistant material	29.9 %	—	—

* These materials had OMe (a) 36.5 %; (b) 38.4 % values indicating contamination with lower homologues.

Both the cotton derivatives were completely broken down; on the other hand the wood methylate, initially soluble in the cleavage reagent, had become split into soluble cleavage products and an insoluble resistant residue. The proportion of the latter was decreased compared with the amount of resistant material obtained from the methylated intact Thiriena pulp, 14 % in place of 22.5 %, but the amount of dimethyl-methylglucoside among the cleavage products was relatively increased.

A comparison of the cleavage experiments on the methylates of intact and depolymerised Thiriena pulp is of interest (see Table VI).

Table VI.

Methylate of Thiriena pulp	Intact %	Depolymerised %
Methoxyl content	39.0	39.2
"Resistant portion"	22.5	14.0
Methoxyl content of "resistant portion"	28.0	29.9
Cleavage products	71.4	81.9
Trimethyl-methylglucoside	94.9	92.3
Dimethyl-methylglucoside	4.1	7.2
Ratio $\frac{\text{Trimethyl-methylglucoside}}{\text{Dimethyl-methylglucoside}}$	23.1	12.8

From the above, and preceding results, it will be seen that the inert properties of the "resistant portion" have been proved to survive acetylation,

disintegration of the cellulose fibre and extensive depolymerisation of the polysaccharide to a much less complex state of molecular aggregation. It must be noted, however, that the "resistant portion," although still incompletely etherifiable, had become so depolymerised as to be no longer completely resistant to cleavage. The evidence in favour of this resistance being due to conditions more fundamental than mere high polymerisation seems definitely convincing.

The presence of tetramethylglucose among the cleavage products of methylated celluloses.

Haworth [1932] reports the detection of tetramethylglucose from the cleavage products of a "trimethylcellulose" obtained by methylating acetone-soluble cellulose acetates. This is claimed to be a proof that polysaccharides are constructed on the basis of long, open chains of glucose residues, and it is further proposed that his method affords a clue to the length of the said chains. How far this method may be applied to starch is dealt with by Irvine in a reply to Haworth's suggestions [1932]. Haworth's views do not lend themselves to acceptance without criticism. The extreme ease with which he introduces methyl groups into celluloses, after subjecting them to preliminary treatment, suggests at once that extensive depolymerisation, possibly not unaccompanied by degradation also, has occurred in his materials. Moreover, the production of an acetone-soluble acetate of cellulose requires an intensified treatment, and it is not unreasonable to suspect the possibility of degradation of a closed system occurring, as indicated in the following scheme. (A ring-structure of three anhydroglucose units is employed for convenience only, no suggestion is thereby made of the number of C_6 chains composing the cellulose unit.) The linkage between chains I and II has been acetolysed with the introduction

of acetyl groups in position 4 of chain II and position 1 of chain I. If the same conditions for destruction of reducing groups by alkali hold for polysaccharides as for simple sugars, we should expect, during methylation, that destruction of the major part of chain I would occur, with the result that, after methylation, the following condition would obtain:

On cleavage of the methylate, we should obtain, for every complex unit of n C_6 chains which has undergone degradation at one linkage,

$$\begin{array}{l}
 (n - 2) \text{ molecules of 2:3:6-trimethylglucose,} \\
 1 \quad \text{molecule of tetramethylglucose,} \\
 \text{i.e.} \quad (n - 1) \text{ C}_6 \text{ units recovered.}
 \end{array}$$

The occurrence of tetramethylglucose as a cleavage product from an initially closed ring, is therefore rendered possible, and it is not necessary to postulate a chain structure, such as the following, to account for its presence:

Careful examination of the cleavage products of fully-methylated intact cotton cellulose failed to detect any trace of tetramethylglucose. From 20 g. of crude trimethyl-methylglucoside, by the method described in the Experimental Section, complete absence of even 0.0005 g. (0.0025 %) of tetramethylglucose was proved.

By similar treatment, methylated intact wood cellulose (Thiriena pulp) was shown to yield no tetramethylglucose. After depolymerisation, however, this wood cellulose was found to yield about 0.5 % of the weight of the methylate, as pure, crystalline 2:3:4:6-tetramethylglucose. This is strong evidence of degradation having taken place during acetylation.

Examination of the cleavage products of depolymerised cotton, methylated to the extent of (a) 43.7 % OMe, *i.e.* fully-methylated; and (b) 38.6 % OMe, *i.e.* approximating to the OMe content of a fully-methylated wood cellulose, yielded a result at present inexplicable. No trace of tetramethylglucose was found in the case of (a), but cleavage of (b) gave rise to 0.64 % of 2:3:4:6-tetramethylglucose. In the extremely empirical state of our present knowledge of polysaccharide chemistry, it seems impossible to proffer any feasible explanation of this phenomenon.

EXPERIMENTAL.

Acetylation.

One part of cellulose was suspended in glacial acetic acid (10 parts) and chlorine bubbled through the mixture (for 10 secs. per 10 cc.). Acetic anhydride (10 parts) was then added and sulphur dioxide passed through the whole for twice as long as the time taken for the chlorine. The reaction mixture was heated to 75° on the water-bath and stirred until the solid matter dissolved, forming an almost clear, viscous solution. This was poured into a large excess of water containing sodium acetate, and the bulky solid which separated was collected, washed acid-free and dried at 100°. Purification was effected by dissolving the crude product in chloroform, drying the solution over anhydrous sodium sulphate, filtering through a sintered glass funnel and evaporating under diminished pressure. The triacetates were deposited as tough colourless films, soluble in chloroform, but insoluble in alcohol and acetone. The chloroform solutions, although clear, were too viscous to filter through paper. Observations of optical activity were made on 1 % solutions. Stronger solutions possessed too great a power of light-dispersion to permit of accurate measurement.

The experimental results are given in Table VII.

Table VII.

Source of cellulose	Weight of starting material g.	Yield	Acetyl content %	Specific rotation in CHCl ₃
Standard cotton	—	(97.6 %)	45.0	− 22.7°
Alkali-extracted cotton	—	(96.8 %)	45.2	− 23.9°
<i>Fagus sylvatica</i>	1.013	1.784 g. (97.3 %)	45.5	− 19.6°
Thiriena pulp	4.00	7.00 g. (98.4 %)	47.4	− 18.4°
<i>Quercus sessiliflora</i>	4.5	7.8 g. (94.5 %)	46.9	− 11.9°
Sitka spruce	1.048	1.802 g. (96.7 %)	46.8	− 10.4°
<i>Quercus pedunculata</i>	2.2	3.85 g. (97.2 %)	47.4	Inactive

Theory for $C_6H_7O_5(COCH_3)_3$; $COCH_3$ 44.7 %.

Simultaneous de-acetylation and methylation of cellulose triacetates.

The process was exactly similar to that described previously for the intact celluloses [Bell, 1932], with the exception that the acetates, prior to treatment with the methylating reagents, were stirred for 3 hours with acetone and 30 % sodium hydroxide solution in amount slightly in excess of that required to effect de-acetylation.

(a) *Wood triacetate (Thiriena pulp)*. 35 g. of material after 10 treatments yielded 22 g. of a fine white powder; yield 91.7 % of theory, methoxyl content 39.2 %. Six further treatments failed to raise the methoxyl content. The material was dissolved in chloroform and shaken for 10 minutes with a little norite charcoal. After filtration through a bed of solid barium carbonate a perfectly clear, pale yellow solution was obtained which, on evaporation under diminished pressure, yielded a tough, very pale yellow film. The specific rotation of this material (5 % solution in chloroform) was $+2.3^\circ$.

(b) *Cotton cellulose triacetate*. 65 g. of the triacetate were treated as in (a). After 9 methylations 38 g. (85.5 % yield) of product with a methoxyl content of 38.6 % were obtained.

19 g. of this substance were subjected to 5 more methylations, the final product amounted to 18 g. and showed a methoxyl content of 43.7 %.

Further treatment with methyl sulphate and alkali failed to produce any increase in the methoxyl content; treatment of a pyridine solution of the material was likewise without avail. Purification was effected as in (a), but as the solution could not be freed from a trace of cloudiness, the specific rotation could not be determined with accuracy. A 3 % solution gave $[\alpha]_D -1.7^\circ (\pm 0.5)$ and the substance melted at $233-240^\circ$.

The action of Purdie's reagents on the above product.

3.475 g. of the methylated depolymerised cellulose were dissolved in the minimum of dry chloroform and treated with 15 g. methyl iodide and 15 g. silver oxide. The product was isolated in the usual way and treated twice as before. The final product gave a perfectly clear solution in chloroform; $[\alpha]_D -1.2^\circ$ in 5 % solution. The methoxyl content was 43.9 % and the m.p. $233-240^\circ$.

Cleavage of the methylated depolymerised celluloses.

The cleavage was effected under the same experimental conditions as described for the treatment of the methylated intact celluloses [Bell, 1932].

(a) *Material from Thiriena pulp*. 5 g. were treated at 80° for 400 hours; a solid residue was found at the end of this time amounting to 0.701 g. (14.0 %) and having a methoxyl content of 29.9 %. The cleavage products weighed 4.351 g. These were separated into 4.029 g. of trimethyl-methyl-glucoside, $n_D^{17} 1.4587$, and 0.317 g. (7.2 %) of residual material, $n_D^{18} 1.4792$, of methoxyl content 36.5 %.

3.246 g. of the above trimethyl-methylglucoside yielded 2.585 g. (84.7 %) of pure crystalline 2:3:6-trimethylglucose, M.P. 119–120°.

(b) *Material from cotton (OMe, 43.7 %)*. 2.443 g. were treated as in (a). Complete hydrolysis took place, the cleavage products amounting to 2.658 g. (93.0 %). Separation was effected into 2.273 g. trimethyl-methylglucoside (corresponding to 85.6 % of the methylated cellulose; theory, calculated from the methoxyl content, 88 %), and 0.368 g. of residual material (13.3 % of the methylated cellulose).

Examination of the cleavage products for tetramethylglucose.

This was carried out by first subjecting the cleavage products to the chloroform-water separation. The chloroform extract was distilled in a high vacuum (0.05 mm.) and the first runnings of the distillate, in which the pentamethylglucose becomes concentrated, collected separately and submitted to aqueous acid hydrolysis. The solution of sugar was extracted 20 times with chloroform and the extract worked up in the usual way. The extracted substance, if any, was weighed on the analytical balance, whereby 0.0005 g. of material could be definitely detected.

(a) *Material from Thiriena pulp*. 10.893 g. of syrup yielded 0.081 g. of colourless syrup which set to a hard mass of crystals after nucleating with 2:3:4:6-tetramethylglucose. After recrystallising once from light petroleum the substance melted at 87°. A mixed M.P. with 2:3:4:6-tetramethylglucose showed no depression. The amount of tetramethylglucose isolated corresponded to 0.5 % of the original methylate.

(b) *Material from cotton (partially methylated)*. From 6.730 g. of depolymerised methylated cotton, 0.048 g. of crystalline tetramethylglucose were isolated, M.P. 88°; a mixed M.P. with 2:3:4:6-tetramethylglucose showed no depression.

(c) *Material from cotton (fully methylated)*. No trace of tetramethylglucose could be detected, even after most careful examination.

SUMMARY.

1. Acetylation of different wood α -celluloses, under standard conditions, produced triacetates of specific rotations varying from -19° to inactivity.

2. Simultaneous de-acetylation and methylation of the acetate led to the formation of fully-methylated depolymerised cellulose (OMe, 39.2 %), completely soluble in most organic solvents, and having, in chloroform solution, $[\alpha]_D = -2.3^\circ$. The empirical composition is the same as that of the corresponding intact cellulose.

3. Cleavage of the above methylate yielded, as in the case of the intact methylate, 2:3:6-trimethyl-methylglucoside, and a "resistant portion," with 29.9 % OMe.

4. No tetramethylglucose was detected in the cleavage products of fully-

methyated intact wood cellulose, but examination of the cleavage products of the same cellulose, after depolymerisation and methylation, proved the presence of a small amount (0.5 % of the starting material) of 2:3:4:6-tetramethylglucose.

5. Control experiments on standard cotton cellulose showed that acetylation also effects depolymerisation, the trimethylated depolymerised cellulose being soluble in most solvents and having in chloroform solution $[\alpha]_D = -1.2^\circ$. Cleavage of this material was complete, and no tetramethylglucose was found.

6. Cleavage of depolymerised cotton methylated to the extent of 38.6 % OMe was complete, no resistance being encountered. In this case, however, 0.64 % of the starting material was isolated as 2:3:4:6-tetramethylglucose.

7. A definite relationship has been indicated between the optical activity in chloroform solution of the wood cellulose acetates and the proportion of "resistant material" isolated from the corresponding methylated intact celluloses.

8. It has now been shown that marked differences are to be found between cotton and wood celluloses; further, the wood celluloses do not display uniformity as individuals, but only as a class, possessing in varying proportions, a "resistant portion." The "resistant portion" has been shown to owe its inert properties to more fundamental reasons than degree of polymerisation.

The author wishes to express his gratitude to Sir J. C. Irvine for interest, help and criticism given to this work. Thanks are due to the Department of Scientific and Industrial Research for a maintenance grant for 1928-30, and to the Carnegie Trust for a Senior Scholarship for 1930-31.

REFERENCES.

- Barnett (1921). *J. Soc. Chem. Ind.* **40**, 8 T.
Bell (1931). (Unpublished work, with Rutherford, St Andrews.)
— (1932). *Biochem. J.* **26**, 590.
Haworth (1932). *Nature*, **129**, 365.
— Hirst and Thomas (1931). *J. Chem. Soc.* 821.
Hess (1921). *Ber. deutsch. chem. Ges.* **54**, 2867.
— and Schultze (1927). *Liebig's Ann.* **455**, 81.
— and Weltzien (1925). *Liebig's Ann.* **442**, 46.
— and Messmer (1923). *Liebig's Ann.* **435**, 1.
Irvine (1932). *Nature*, **129**, 470.
— and Macdonald (1926). *J. Chem. Soc.* 1502.
— and Robertson (1926). *J. Chem. Soc.* 1438.
— and Rutherford (1932). (Unpublished work.)
Ost and Kayama (1912). *Z. angew. Chem.* **25**, 1467.