S 41. *Pear Cell-wall Cellulose.*

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A cellulose isolated from pear cell wall has been shown to be identical with cotton cellulose. Hydrolysis with 72% sulphuric acid gave only D-glucose. Methylation in nitrogen gave a trimethyl derivative which on hydrolysis gave 2 : 3 : 6-trimethyl D-glucose (90% of theory) and 0·6% of 2 : 3 : 4 : 6-tetramethyl D-glucose. This corresponded to a chain length of 160 units. The chain length of the native cellulose was probably much longer because the drastic purification necessary to remove the other polysaccharides undoubtedly caused some degradation.

The structure of the cellulose of fruit cell walls is usually regarded as being identical with that of cotton cellulose, though the evidence for this belief is mainly based on analogy with other celluloses, such as the tunicin prepared from *Phallusia mammilaris* (Zechmeister and Toth,

Z. physiol. Chem., 1933, **215**, 267) and certain bacterial celluloses (Barsha and Hibbert, *Canadian J. Res.*, 1934, **10**, 170) which have been examined in some detail, and are believed to be similar in structure to cotton cellulose. This analogy is weakened by the fact that these celluloses can be obtained in a relatively pure condition without much chemical treatment, whereas the cellulose from fruit cell walls is very closely associated with xylan and lignin which has given rise in the past to the suggestion that the cellulose might contain xylose units as part of the molecule.

In the present study, a pure polyglucose (cellulose) was prepared from pear cell wall, and its structure examined in detail. The isolation was difficult because it was closely associated with lignin in addition to xylan, mannan, and galactan. Lignin was removed by repeated treatments of the cell wall with a 1·0% aqueous solution of sodium hypochlorite followed by extraction with a hot 5% aqueous solution of sodium hydrogen sulphite (Norman and Jenkins, *Biochem. J.*, 1933, **27**, 818). The xylan and other polysaccharides were separated from the cellulose by dissolving the residue in phosphoric acid (*d* 1·75) and then pouring into three volumes of water. The precipitate of regenerated cellulose was almost pure. The last traces of lignin were removed by a single mild treatment with sodium hypochlorite (Norman and Jenkins, *loc. cit.*), and traces of non-cellulosic polysaccharides by extraction with cold 4% aqueous sodium hydroxide.

By dispersing the crude cellulose in phosphoric acid the hydrogen bonds which are responsible for the close association of the xylan and cellulose are broken, making it possible to prepare a cellulose which is free from pentose.

A comparison of the properties of the pear cell-wall cellulose with those of cotton cellulose is given in the table.

Property.	Pear cell-wall cellulose.	Cotton cellulose.	Reference.
Yield of D-glucose after hydrolysis with 72% H_2SO_4 (% theory)	86	92	1
$[\alpha]_{4360}^{20°}$ (in cuprammonium)	−1000°	−1200°	2
Chain length, estimated from viscosity of a solution in cuprammonium	240	1030 (for regenerated cellulose prepared in the same manner as pear cell-wall cellulose, 220)	3
Copper number	1·33	0·37 (for regenerated cellulose, 2·0)	4
After methylation in nitrogen to the trimethyl derivative:			
$[\alpha]_D^{26°}$ (in chloroform)	−7°	−10°	5
Yield of 2 : 3 : 4 : 6-tetramethyl D-glucose on hydrolysis with 1·5N-HCl in 50% acetic acid	0·6% Chain length, 160	(when prepared from the triacetate the trimethyl derivative gave 0·5% of 2 : 3 : 4 : 6-tetramethyl D-glucose. Chain length, 200)	6, 7
Chain length estimated from viscosity in *m*-cresol	500	(Values up to 6000 have been recorded)	6, 7

[1] Monier-Williams, *J.*, 1921, **119**, 803. [2] Reeves, *J. Biol. Chem.*, 1944, **154**, 49. [3] Staudinger and Reinecke, *Papier Fabrikant*, 1938, **36**, 489. [4] Heyes, *J. Soc. Chem. Ind.*, 1928, **47**, 901. [5] Haworth, Hirst, and Thomas, *J.*, 1931, 825. [6] Haworth, Montonna, and Peat, *J.*, 1939, 1899. [7] Haworth, Hirst, Owen, Peat, and Averill, *J.*, 1939, 1885.

In the native state, it is probable that the molecular size of the pear cell-wall cellulose is very similar to that of cotton cellulose. The treatment necessary to isolate the pear cell-wall cellulose in a pure state almost certainly causes considerable degradation (cf. van Ekenstein, *Ber.*, 1936, **69**, 549). The presence of an end group suggests that this cellulose has been modified in much the same manner as is cotton cellulose during acetylation (cf. Haworth *et al.*, *loc. cit.*).

Experimental.

Preparation of Crude Cell-wall Material.—Unripe pears (variety "Conference") (10 kg.) obtained from the Research Station, East Malling, in 1936 were peeled, cored, and then frozen at −20°. The frozen pears were ground to a flour, and the flour stored at −20° until required. The flour (5·5 kg.) at −20° was added slowly to boiling ethyl alcohol (20 l.) and the mixture heated under reflux for ¾ hour. A large galvanised iron dust-bin was used as the extraction vessel, the inverted lid being used as the reflux condenser. The residue was filtered off and then again heated under reflux with ethyl alcohol for 2 hours to remove traces of sugar. The insoluble residue was filtered off, washed twice with ethyl alcohol, and then dried in a vacuum. Yield 160 g. (Found : lignin, 18·5%).

Preparation of the Crude Cellulose.—The cell-wall material (200 g.) was extracted with boiling aqueous ammonium oxalate (0·5%; 4 l.) for 12 hours. The insoluble residue was filtered off, and the treatment repeated. The yield of pectin-free material was 134 g. (Found : lignin, 27·6; ash, 0·4%).

The depectinated cell wall (120 g.) was delignified by treatment with sodium hypochlorite following the procedure described by Norman and Jenkins (*loc. cit.*). Six treatments removed most of the lignin originally present. The residue was washed with distilled water, ethyl alcohol, and ether, and then dried

in a vacuum. Yield, 68·5 g. of a white fibrous material [Found : lignin, 0·2; pentose (calculated as xylan), 32%. Traces of mannan and galactan were also present].

Purification of the Crude Cellulose.—The crude cellulose (30 g.) was dissolved in phosphoric acid (*d* 1·75; 600 ml.). The mixture was stirred for 48 hours, and the temperature not allowed to rise above 15°. Moisture was carefully excluded. The solution was then centrifuged to remove a small amount of undissolved material, and the clear brown liquid was poured into 3 volumes of water. The undissolved residue consisted of highly lignified stone cells from the original cell wall. The cellulose settled down as a flocculent precipitate. The precipitate was separated from the supernatant liquid centrifugally, and then washed with water until no longer acid to methyl-orange. It was finally washed with ethyl alcohol and ether, and dried in a vacuum at 100°. Yield, 7·5 g. of a white powder [Found : lignin, 0·2; pentose (calculated as xylan), 3%]. The small amount of lignin which still remained was removed by a single very mild treatment with sodium hypochlorite. The residue was then stirred for 12 hours with aqueous sodium hydroxide (5%; 100 ml.), filtered, and washed with 10% aqueous acetic acid until free from sodium salts. It was washed with distilled water, ethyl alcohol, and ether, and dried in a vacuum at 100°. Yield, 5·5 g. of a white powder [Found : lignin, negligible; pentose (calculated as xylan), $<0·3$; phosphorus (calculated as P), 0·3%]. The naphtharesorcinol test for uronic acids was negative; $[\alpha]_{4360}^{20°} -1000°$ (*c*, 0·5 in cuprammonium). When hydrolysed with 72% sulphuric acid, the cellulose gave crystalline D-glucose (yield 86% of the theoretical). This was acetylated; the m. p. of the resulting penta-acetate was 113°, alone or mixed with authentic β-glucose penta-acetate. No trace of mannose or galactose could be detected in the mother-liquor. The yield of D-glucose from cotton cellulose, hydrolysed under similar conditions, was 92%.

The chain length of the cellulose was estimated from the viscosity of a solution in cuprammonium. The copper number was determined using Heyes's (*loc. cit.*) micro-method. The values obtained for the pear cell-wall cellulose and for cotton cellulose are as follows :

Cellulose.	Copper number.	Chain length (anhydroglucose units).
Untreated cotton wool	0·37	1030
Regenerated cellulose from cotton wool, prepared in the same manner as the regenerated cellulose from pear cell wall	2·0	220
Regenerated cellulose from pear cell wall	1·33	240

Methylation.—The pear cell-wall cellulose (10 g.) was methylated in an atmosphere of nitrogen. After 6 methylations the product was filtered off, washed with boiling water, and dissolved in chloroform (500 ml.) containing ethyl alcohol (100 ml.). The yellow viscous solution was filtered through a sintered-glass filter, and the methylated cellulose precipitated from solution by the addition of light petroleum (b. p. 40—60°). The resultant cream powder (9·8 g.) (Found : OMe, 43·6%) was dissolved in chloroform, and then reprecipitated by the cautious addition of light petroleum (b. p. 40—60°) so as to give several precipitates in succession. Each of these precipitates was examined in detail. They were all similar in physical properties, and the methylated cellulose was therefore homogeneous. It was insoluble in acetone, but was soluble in ice-cold water, glacial acetic acid, and in alcoholic chloroform. $[\alpha]_D^{26°} -7°$ (*c*, 0·78 in chloroform containing 10% ethyl alcohol), $\eta_{sp}^{20°} = 1·98$ (*c*, 0·4 in *m*-cresol) corresponding to a chain length of 500 units using Staudinger's formula with $Km\ 1 \times 10^{-3}$.

Hydrolysis of the Methylated Cellulose.—Methylated cellulose (8·1 g.) was dissolved in a mixture of equal volumes of glacial acetic acid and 3N-hydrochloric acid (80 ml.), and the solution heated on the steam-bath. $[\alpha]_D$ rose to $+71°$ in 4½ hours. The hydrochloric acid was neutralised with barium carbonate, and the solution concentrated to a syrup at 40° under reduced pressure. The syrup was dissolved in water (50 ml.), and the solution exhaustively extracted with ether in a continuous extraction apparatus. Concentration of the extracts gave a syrup (8·2 g.), which crystallised. The crystals were separated on a porous tile, and were purified by recrystallisation from acetone–ether–light petroleum (b. p. 40—60°). Yield, 5·9 g. The syrup obtained on concentrating the mother-liquors was added to the syrup obtained by exhaustive extraction of the porous tile. This syrup (2·2 g.) crystallised on keeping. The crystals were separated on a porous tile, and the crystalline sugar twice recrystallised from the same solvent. The crystalline sugar proved to be 2 : 3 : 6-trimethyl D-glucose, having m. p. 92° alone, or mixed with authentic material; $[\alpha]_D^{20°} +70°$ (in water at equilibrium). After the crystals had been removed, a syrup remained (0·3 g.) which was dissolved in water (50 ml.), and the aqueous solution extracted exhaustively with light petroleum (b. p. 40—60°). Concentration of the extracts yielded a syrupy mixture of 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl D-glucose (0·13 g.) which was dissolved in as small a quantity of water as possible. 0·25M-Sodium metaperiodate was added, and the solution left overnight at room temperature. The amount of sodium metaperiodate added was about twice that necessary to destroy the 2 : 3 : 6-trimethyl derivative; the 2 : 3 : 4 : 6-tetramethyl D-glucose was unaffected. Oxidation products and 2 : 3 : 4 : 6-tetramethyl D-glucose were extracted from the solution with chloroform, and the syrup (0·08 g.), obtained on concentration of the extracts, was boiled with methyl-alcoholic hydrogen chloride (25 ml.; 1% w/v) under reflux for 6 hours. The cooled solution was neutralised with silver carbonate, filtered, and concentrated. The residual syrup (0·06 g.), $n_D^{20°}$ 1·4438, was distilled under reduced pressure to give a syrup (0·05 g.), $n_D^{20°}$ 1·4430 (Found : OMe, 61·0%). This material (0·03 g.), after hydrolysis with boiling N-sulphuric acid, gave crystalline 2 : 3 : 4 : 6-tetramethyl D-glucose in good yield, m. p. 87° alone, or mixed with authentic material. The anilide had m. p. 136°. This yield of tetramethyl glucose indicates an average chain length of 160 glucose residues in the cellulose molecule.

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