323. *Polysaccharides. Part X. Molecular Structure of Cellulose.*

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MANY problems connected with the structure of cellulose may now be regarded as having attained solution. It is generally recognised that the mode of linking of pairs of glucose units in the polysaccharide corresponds with that obtaining in cellobiose, and this biose is held to be a preformed breakdown product of cellulose. No other authentic biose is derivable as a hydrolysis product. In the accompanying paper (Haworth, Hirst, and Ant-Wuorinen; in the press) it is demonstrated that the supposed cello*iso*biose does not exist and that it may be dismissed from the literature. The conception that cellulose is constituted on the basis of the "association" of small structural units of C_6, C_{12}, C_{18}, or C_{24}, a view held by one school (Hess and Trogus, *Ber.*, 1928, **61**, 1982; Pringsheim, *Ber.*, 1926, **59**, 3008; Irvine and Robertson, J., 1926, 1488; Irvine, *Chemical Reviews*, 1927, **4**, 203), is untenable inasmuch as cellulose breaks down progressively to cellodextrins having a chain-length which can be determined as $(C_6)_{25}$ . . . $(C_6)_{10}$ and finally to the more readily measured reducing sugar units $(C_6)_4$, $(C_6)_3$, $(C_6)_2$ (cellobiose), and to glucose. There is no step in the scission of cellulose which corresponds to any halting-place recognisable as a non-reducing "dissociated" molecular unit $(C_6H_{10}O_5)_{2 \text{ to } 4}$, and it has been shown (Freudenberg) that the methylated unimolecular unit $(C_6H_{10}O_5)$ possesses no associating properties.

The traditional view, that the residues of glucose are mutually linked in cellulose just as they are in the glucosidic union of a biose, is demanded by all the chemical evidence and it derives further sanction from the interpretations of *X*-ray data, first of Sponsler and later of Meyer, Mark, Astbury, and others. The structural basis of the modern formula rests on the determination of the con-

stitution of cellobiose (Haworth and Hirst, J., 1921, **119**, 193; Haworth, Long, and Plant, J., 1927, 2809) which is indicated by the 1 : 4-linking of two units of β-glucopyranose. The union of many such groupings as a continuous chain may be visualised in either of two ways (Haworth, *Ber.*, 1932, **4**, *A*, 58). The molecule may terminate in two end groups recognisable by their singular properties, or may exist as endless loops of two parallel chains having every C_6 unit identical, in which case no singular property is to be found in any C_6 component.

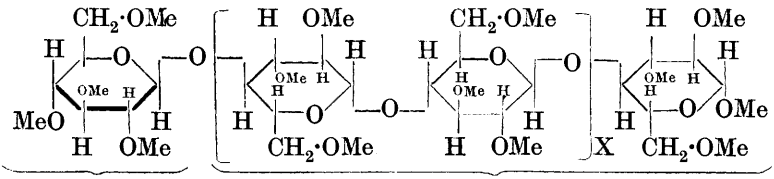
We have sought to investigate these alternative expressions for the finer molecular structure of cellulose by preparing, under stringently controlled conditions, a cellulose derivative which had suffered little if any degradation and is demonstrably free from the scission products of small-chain length which frequently occur during treatment of cellulose with chemical reagents. The acetone-soluble cellulose acetates of commerce usually contain lower breakdown products. Moreover, they are ill-adapted for subsequent conversion into the methyl derivative, since they require the application of several treatments with methylating agents to attain the condition of complete methylation.

During the past four years we have studied the preparation of an acetone-soluble cellulose acetate which is capable of undergoing complete substitution by methyl groups in an almost homogeneous medium after one treatment only with methyl sulphate and alkali at comparatively low temperatures. Over half a kilogram of this acetate was prepared, some of which was utilised for obtaining the methylated cellulose and the remainder was completely acetylated and fractionally precipitated from solvents in order to discover and remove low-molecular forms. The methylated cellulose, after treatment with several solvents, gave evidence of general homogeneity and we were satisfied that we were in possession of a product which consisted of groups of macro-molecules of similar properties and chain length. The acetate had a high viscosity, gave no reduction with Fehling's solution, and showed a negligible reaction with sodium hypoiodite when submitted to this test under Bergmann and Machemer's conditions (*Ber.*, 1930, **63**, 316, 2304).

The hydrolysis of this specimen of methylated cellulose at a low temperature with saturated aqueous hydrochloric acid effected complete cleavage into the methylated glucose components. The latter were converted into the methylglucosides and submitted to an exhaustive separation by fractional distillation. We isolated 1·1 g. of tetramethyl methylglucoside from these distillates. This value is subject to a 10% correction for experimental losses. The final estimate of the tetramethyl glucose content, from 200 g. of

methylated cellulose, represented 0·6% of this residue in the hydrolysis products.

Taking the following as the expression of the structure of the macro-molecules of methylated cellulose, it is seen that a quantitative assay of the left-hand terminal group (all other groups giving rise to 2 : 3 : 6-trimethyl glucose) establishes the existence of thread-like molecules which terminate and are not endless, and furnishes a measure of the chain length of our cellulose derivative. From the



Hydrolysis gives tetramethyl gluco-pyranose.

Hydrolysis gives 2 : 3 : 6-trimethyl glucose and MeOH.

percentage of the tetramethyl glucose component which is found, the value of x in the above expression can be estimated and the general average length of the molecules assigned. We reach the conclusion that this cellulose preparation consists of not fewer than 100 and not more than 200 β-glucose units. This corresponds to a molecular weight of 20,000—40,000 or a mean value of approximately 30,000, which is similar to the values (35,000) found for numerous proteins by the ultracentrifuge method of Svedberg and his co-workers. A minimum molecular weight of 20,000 for native ramie cellulose has been found by measuring the breadth of the lines taken along the fibre-axis in the *X*-ray diagram (R.O. Herzog and Krüger, *J. Physical Chem.*, 1926, **34**, 466; Hengstenberg, *Z. Krist.*, 1928, **69**, 271). Since our values are attained by the study of a derivative of cellulose, prepared by chemical methods, any degree of scission of the larger molecules, however slight, would be reflected in this estimate of chain length. We therefore regard our value as the average lower limit of the size of the macromolecule. Although it may be of interest, it is of no vital concern whether this value is to be increased to 100,000 for native cellulose. With either value the general structure and conformation of the polysaccharide are satisfactorily envisaged. We regard the alternative calculations of higher molecular weight, determined by Staudinger from viscosity measurements of native cellulose in solution, as being of a similar order of magnitude. The truth may lie reasonably near to either value. Cellulose is therefore to be considered as an extraordinarily large assemblage of β-glucopyranose components linked through positions 1 : 4 and continued,

by reason of the stereochemical arrangement of its primary valency bonds, as a straight chain of six-atom ring units. These chains terminate at a lower mean length of 100—200 glucose units and the colloidal properties and organised structure of cellulose are fully explicable from the conception which is here presented from the standpoint only of chemical behaviour.

EXPERIMENTAL.

Preparation of an "Acetone-soluble" Cellulose Acetate "C" (Modification of Barnett's method).—In a 1½ litre glass jar 50 g. of a good specimen of cellulose, which had been exposed to moist air, were soaked for ½ hr. in 360 c.c. AcOH into which a gentle stream of Cl gas had previously been passed at 15° for 2¾ mins. With this product was gradually mixed, in small portions with stirring and cooling (ice–salt mixture), 180 c.c. of freshly distilled Ac_2O into which SO_2 had previously been passed for 6 mins. During the mixing of the reagents the temp. was not allowed to rise above 5°. After 1 hr. a thick, gelatinous, but clear solution was obtained and the temp. was then about 15°, and this was kept for 3—4 hrs. Thereafter it was gradually heated, with good stirring, and maintained at 35° for 1½ hrs. Then, with stirring and cooling (ice-water), a mixture of 75 c.c. AcOH, 30 c.c. H_2O, and 7·5 c.c. conc. H_2SO_4 was admitted so slowly that the temp. did not rise above 15°. After being stirred for another hr. at this temp., the mixture was kept in a stoppered vessel at 18° for 20—24 hrs. This effects partial deacetylation.

Test portions were withdrawn and pptd. in H_2O. The ppt. should be flocculent and asbestos-like. The whole can then be mixed gradually with a large excess of ice-water. The white product was sol. on agitation in cold acetone and gave a clear, non-reducing, very viscous solution. A product is sometimes obtained in long, hard, matted fibres, but this form is not so appropriate for the purposes of this work, as it is sparingly sol. in acetone. In such a case the partial deacetylation had not proceeded far enough; but the process is readily controlled with practice. The object is to obtain a partly acetylated cellulose which has just reached the stage (by partial loss of acetyl groups) where it becomes completely acetone-soluble. On the other hand, products less viscous than those described or which show appreciable reduction of Fehling's solution are to be rejected, as they contain products of degradation owing to improper control of the procedure just outlined.

Preliminary purification (from partly acetylated products) was effected by pouring the colourless acetone solution into well-stirred ice-water. The easily separated, white, asbestos-like ppt. was washed repeatedly with ice-water and finally with hot H_2O, treated with EtOH–acetone and then with Et_2O, and placed in a vacuum oven at 35° containing $CaCl_2$, a current of dry air being admitted. Yield, 70—80%. Mean $CH_3{\cdot}CO_2H$ content of acetate, 56—58%. $[\alpha]_D$ − 18° in $CHCl_3$. This material is designated "C."

Search for Degradation Products in the "Acetone-soluble" Cellulose Acetate "C."—In order to determine whether this acetone-soluble cellulose acetate contained lower break-down products, 200 g. of it were completely acetylated, in 5 lots of 40 g. with 3000 c.c. Ac_2O and 300 g. NaAc, by 2 hrs.' heating on a boiling water-bath. The solid dissolved completely after 5 mins. to a colourless solution, which became light yellow with continued heating. Slight

degradation to lower break-down products of the cellulose was unavoidable at this stage, but the material was intended for use only to control the extent of the total break-down products which were evaluated later. The material was isolated in the usual way and the crude cellulose acetate reduced Fehling's solution very faintly. $[\alpha]_D$ — 18° to — 19° (in $CHCl_3$ containing a little EtOH).

The iodine value of this specimen, determined under exceptionally drastic conditions (3 times the usual period of contact and 60 times the normal quantity of $N/10$-I_2 and $N/2$-NaOH) was calculated to be 0·42. Bergmann and Machemer (*Ber.*, 1930, **63**, 316, 2304) have applied these reagents with success under a variety of conditions, and on the basis of their calculations and assuming the homogeneity of the specimen we found the mean mol. wt. of this specimen of cellulose acetate to be 40,000. The completely acetylated cellulose was submitted to exhaustive examination for impurity: 170 g. of the completely acetylated cellulose (designated " D ") were treated with about 3 l. of boiling acetone under reflux, and at the onset of general swelling of the product 3 l. of EtOH were added and the whole was heated a further 3 hrs. just below the b. p. of the solvents. The whole of the acetate appeared to be pptd. On evaporation of the hot filtrate and thorough washing of the pptd. material with the same mixture of solvents (hot) there were obtained 1·4 g. of a yellow acetate which showed appreciable reduction with Fehling's solution. This on isolation showed $[\alpha]_D$ — 10·3° ($CHCl_3$). With the pptd. product a repeated search was made by a similar method for other break-down products of cellulose but without success. As will be seen later, this was the whole of the impurity (degraded forms) traceable in the material under examination. With the foreknowledge from other experience, we were aware that slight decomp. occurred under drastic conditions of heating of " C " with Ac_2O and NaAc at 100° for 2 hrs., but to the extent of 99·3% the crude re-acetylated cellulose " D " appeared to be uniform in character. We interpret this result as evidence of the much greater uniformity of the purified acetone-soluble cellulose acetate " C " which it was the purpose of these present experiments to investigate inasmuch as it was the specimen " C " which was subsequently utilised for the purpose of methylation.

Moreover fractional separations of 20 g. lots of this purified cellulose acetate " D " revealed no products which differed essentially from each other. These experiments were conducted by the gradual addition, to a $CHCl_3$ solution (containing a little EtOH) of the acetate, of acetone, followed by dry Et_2O, and the collection of the pptd. products (12·7 g.). The first fraction showed $[\alpha]_D^{21°}$ — 20° ($CHCl_3$ containing a little EtOH). With light petroleum as the precipitating medium the mother-liquors from the above gave 7 g. of a similar material, $[\alpha]_D^{21°}$ — 20°. The residue from the evaporation of all the solvents was negligible in quantity. The above second fraction was submitted to further fractional pptn., but this gave no material which affected hot Fehling's solution, and the rotation was unchanged.

We were unable to effect any further separation of the completely acetylated cellulose (" D "). Even more important than the factor of unchanged sp. rotation was the character of the solubility of the product. It conformed to a good specimen of cellulose acetate free from short-chain molecules. The sharply degraded products of cellulose, such as the cellodextrins, give rise to acetates which have a varying sp. rotation of the order — 12° to — 15° and are easily soluble in acetone, giving solutions of low viscosity. They are

also sol. in 8% NaOH aq. and show marked reduction of Fehling's solution. With such preps. our product had nothing in common, nor were any of these cellodextrins observed as impurities. An extended study of cellodextrins has been carried out simultaneously with the present work and the results are given in a separate paper.

Acetone-soluble acetates are, after complete acetylation, practically insol. in this solvent and exhibit the phenomenon of very marked swelling with 8% NaOH aq. Our specimen of acetone-soluble cellulose acetate " C " possessed a remarkable capacity for film formation and proved eminently serviceable for the purpose of complete methylation by one treatment with methylating agents. It was quite stable towards 30% aq. or aq.-alc. alkali and became coloured only faintly yellow on heating. On the other hand, the cellodextrins of Hess do not withstand these reagents owing to the presence of reducing groups in increasing proportion to the length of shortened chain.

Methylation of the " Acetone-soluble " Cellulose Acetate " C."—A characteristic property of the material prepared as above described is that it is capable of complete methylation and simultaneous loss of acetyl groups by one treatment with Me_2SO_4 and alkali. This is not the case with commercial samples of " acetone-soluble " acetate which we have examined. This operation was conducted under the conditions described by Haworth, Hirst, and Thomas (*loc. cit.*) for the prep. of high-viscosity methylated cellulose. The acetone solution of the acetate was treated with Me_2SO_4 and aq. alkali, further quantities of acetone being added from time to time to replace losses and to maintain the acetate in solution during the greater part of the operation. After one treatment with the above reagents the product showed 45% OMe. This value was not increased by a second methylation process which we carried out in order to make quite sure that the end glucose-residues were completely methylated. Our further investigation showed that the completely methylated break-down products of cellulose of the type of the so-called Hess's biosan have different solubilities from the above methylated cellulose and are to be distinguished by this means. The methylated cellulose was thoroughly washed, with H_2O at 40—50°, followed by acetone, and then Et_2O, and a colourless product having OMe 45%, $[\alpha]_D^{20}$ — 9° in $CHCl_3$, was obtained (complete analyses were given by Haworth, Hirst, and Thomas, J., 1931, 821).

Hydrolysis of Methylated Cellulose.—200 G. of the above methylated cellulose were pounded into small particles and mixed, with stirring, with 1 l. of cold conc. HCl, the formation of lumps of material being avoided by bringing the solid and the HCl together in a large funnel and allowing the fine pasty mass to flow into a 2 l. flask. After 2 hrs. the solid had completely dissolved to give a very viscous yellow solution, which was cooled to — 20° and saturated with HCl (flask closed by a $CaCl_2$ tube). This solution was kept over-night surrounded by ice-water and then again saturated with HCl and kept overnight. Thereafter the temp. was allowed slowly to rise to 10°, and as much as possible of the HCl removed by aeration. Bone charcoal (2 g.) was added, followed by the requisite amount of $BaCO_3$ for neutralisation and 500 g. of ice. Finally the mixture was well stirred at 35—40° for 2 hrs. The faintly yellow filtrate and washings from this prep. were collected and the contained hydrolysis products were quantitatively estimated as follows. Preliminary attempts to obtain tetramethyl glucose by preferential extraction with $CHCl_3$ from the aq. solutions gave colourless products which contained much tri-

methyl glucose in the recovered syrup. This yielded a portion sol. in light petroleum, but no cryst. tetramethyl glucose separated from this solvent. It was evident, therefore, that the tetramethyl glucose was present in too low a concentration to admit of accurate estimation by this means. Freudenberg and Braun have shown, under other conditions, the impossibility of separating traces of tetramethyl methylglucoside from hydrolysis products of 30 g. only of methylated cellulose (*Annalen*, 1928, **460**, 288).

The whole of the aq. solutions were now evaporated at 35° (diminished press.), precautions being taken to avoid difficulty with separated $BaCl_2$, and the separated salt was exhaustively extracted with hot $CHCl_3$ and the solution dried and concentrated at low temp. The resulting syrup was combined with that from the above preliminary experiment and the cold $CHCl_3$ solution stirred with a large vol. of light petroleum which was being gradually admitted. The lower layer, on keeping at 0°, deposited crystals of 2 : 3 : 6-trimethyl glucose (38 g.), and the mother-liquors with Et_2O washings were diluted with $CHCl_3$ and again treated as above with light petroleum. The lower layer was again separated and evaporated at 35° to give a syrup (A). The combined upper layers were also evaporated to give a syrup (B).

The procedure finally adopted for the greater bulk of the material was as follows: The neutralised filtered aq. solutions from the hydrolysis were extracted with 5—6 l. $CHCl_3$ and this gave on evaporation a syrup containing most of the tetramethyl glucose. By evaporation at 35° of the aq. portion and exhaustive extraction of the residue with hot $CHCl_3$ a syrup poorer in tetramethyl glucose was obtained. Both syrups were separately treated, with stirring, with a large excess of light petroleum in the previous manner. The upper layers in each case were richer in tetramethyl glucose, and these, were collected (B) and evaporated separately, and similarly with the $CHCl_3$ layers (A).

The syrups from (A) and (B) were separately converted into the methyl-glucosides (MeOH and 1% HCl under reflux for 10 hrs.) and submitted to exhaustive fractional distillation under 0·02 mm., the refractionations being carried out in a Widmer flask with column protected with thin asbestos, so that the b. p.'s could be accurately observed. The liquid distillates were colourless and their composition was further controlled on the micro-scale by the determination of (a) refractive index, (b) methoxyl content, (c) C and H analysis.

The following are typical results of this procedure after one fractional distillation of the syrup (B).

Fract.	Time of distill. (hr.).	Temp. (oil-bath).	Wt., g.	n_D.	Content, %. OMe.	C.	H.
1	1	125—140°	0·4	1·4459	61·0	52·65	8·85
2	½	140—145	0·45	1·4492	59·5	52·6	8·75
3	½	140—155	0·8	1·4570	51·5	52·45	8·32
4	½	155—160	1·1	1·4582	51·2	50·5	8·7

Refractionation was carried out in the usual manner by redistilling each fraction until a change in properties became evident, then adding the next fraction, and so on.

An initial distillation was always conducted from a Claisen flask and the individual portions were collected in a Widmer flask for the re-fractionation; all residues were conserved by this procedure. This was especially necessary in the distillation of the larger portion of syrup (A) in which most of the

trimethyl methylglucosides appeared, together with traces of dimethyl methylglucosides.

The fractions of lower b. p. from (A) were refractionated with selected fractions from (B) and in all 1·1 g. of 2 : 3 : 4 : 6-tetramethyl methylglucoside were collected. The error involved in the separation of tetra- and tri-methyl methylglucosides was investigated by taking an artificial mixture containing about 1% of the former and estimating how far the fractional distillations were effective. These control experiments showed an almost quantitative separation (95%). We determined also by control experiments the losses involved in the major processes of hydrolysis of a mixture of the methylglucosides, and regeneration of the methylglucosides by methyl-alc. HCl treatment and subsequent recovery, and estimated this to be of the order of 5%. The total of these two sources of error in our quantitative estimations is thus 10%.

Applying this correction, our estimate of the whole of the tetramethyl methylglucosides is raised to 1·2 g. from 200 g. of methylated cellulose, or 0·6% of the whole content of the methylated residues in the hydrolysis products. A portion of the above tetramethyl methylglucoside was hydrolysed and gave an almost quantitative yield of cryst. tetramethyl glucose showing $[\alpha]_D$ + 94° (after 5 mins.); + 83·5° (equilibrium val.) (Found: C, 50·6; H, 8·5. Calc.: C, 50·8; H, 8·5%). M. p. 89°.

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