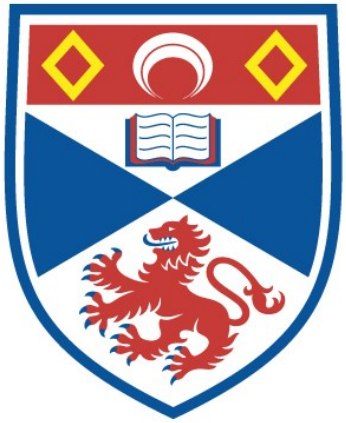
INVESTIGATIONS ON CELLULOSE

George James Robertson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1924

INVESTIGATIONS ON CELLULOSE.

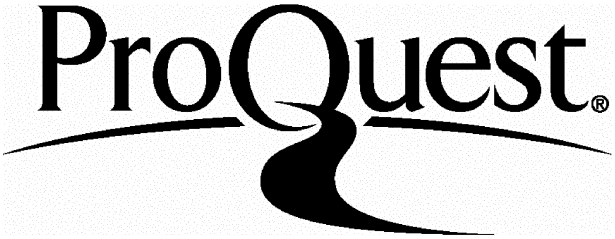
BEING A THESIS

PRESENTED BY

GEORGE JAMES ROBERTSON, M.A., B.SC.,

TO THE UNIVERSITY OF ST. ANDREWS

IN APPLICATION FOR THE DEGREE OF PH.D.



D E C L A R A T I O N.

I hereby declare that the following Thesis is a record of the results of experiments carried out by me, and farther that the Thesis is my own composition and has not previously been presented for a Higher Degree.

The Research was carried out in the Chemical Research Laboratory of the University of St. Andrews under the direction of Principal J.C.Irvine, C.B.E., D.Sc., Ph.D., F.R.S.

C E R T I F I C A T E.

I certify that Mr.George James Robertson,M.A.,B.Sc., has spent nine terms at Research Work under my direction, that he has fulfilled the conditions of Ordinance No 16 (St.Andrews),and is qualified to submit the accompanying Thesis in application for the Degree of Ph.D.

St.Andrews. Director of Research.

UNIVERSITY CAREER AND RESEARCH EXPERIENCE.

I graduated M.A.in the University of St.Andrews in 1921,and B.Sc.,with First Class Honours in Chemistry, in 1922.

I commenced the research on Esparto Cellulose,which is now being submitted as a Ph.D.Thesis,in February 1922,and was admitted a Research Student of the University from May 1922.

In 1922 I was awarded a Carnegie Research Scholarship which I have held for the past two years.

In addition to the work which is described in this thesis,I have been engaged on the investigation of the optical inversion of l-menthone and the associated problem dealing with the relationship of the various menthols and menthones.This work has been carried out under the supervision of Professor J.Read,Ph.D.

C O N T E N T S.

PART ONE.

THE NATURE OF ESPARTO CELLULOSE.

PART TWO.

THE STRUCTURAL BASIS OF COTTON CELLULOSE.

PART ONE.

THEORETICAL.

The name "cellulose" is a generic term applied to a class of substances rather than to an individual specifically characterised by its physical and chemical properties, and, as might be expected from the fact that cellulose forms the predominating constituent of plant tissues, there exists a wide range of divergent modifications, in conformity with the variety of natural elaboration. As a class, the celluloses have many properties in common. They are chemically inert, colourless and odourless bodies, which are represented empirically by the formula $(C_6H_{10}O_5)_n$, in which "n" is an integer of unknown magnitude. They are farther characterised by their insolubility in simple organic solvents and by their resistance, although in varying degree, to the processes of hydrolysis and oxidation.

It should be noted that the celluloses are not readily separated from the plant in a state of "purity", but are usually associated with small amounts of foreign material, which may be intimately mixed or even chemically united with the cellulose proper.

Such impurities are generally less resistant to hydrolysis and oxidation and may be eliminated by the action of alkaline hydroxides under pressure, followed by exposure to the action of chlorine gas at the ordinary temperature.The residue which results from this treatment,after thorough washing and drying, is regarded as a"normal"cellulose.

According to Cross and Bevan,("Cellulose", Second Edition,1910,P.78),"the celluloses of the plant world so far as they have been investigated from the point of view of chemical constitution, group themselves as follows:-

(a) Those of maximum resistance to hydrolytic action, and containing no directly active CO groups.

(b) Those of lesser resistance to hydrolytic action, and containing active CO groups.

(c) Those of low resistance to hydrolysis,i.e.more or less soluble in alkaline solutions and easily resolved by acids,with formation of carbohydrates of low molecular weight"

In an expansion of the above classification the same authors place the typical cotton cellulose along with the celluloses of Flax,Hemp,China Grass

and Sunn Hemp in group (a),and the celluloses obtained from wood,cereal straws and esparto grass in group (b). Moreover,they regard the celluloses derived from cereal straws and esparto grass as being strongly differentiated from the normal and characterise them as pronounced oxycelluloses.This view,with regard to esparto cellulose,will be referred to at a later stage.Group (c) is of little interest in relation to the present thesis,as it includes the heterogeneous class of cellular as opposed to fibrous celluloses, for which E.Schulze has proposed the term Pseudo-celluloses,and may be dismissed without farther reference.

THE CONSTITUTION OF COTTON CELLULOSE.

The attention of investigators was naturally first attracted to the constitutional study of cotton cellulose,which may be referred to as the typical cellulose,but many serious,almost insuperable difficulties lay in the path of the pioneers who attacked this intricate problem of carbohydrate chemistry. The very nature of cellulose,its insolubility in simple organic solvents,its uncertain,or rather unknown molecular magnitude and the possibility that as a

fibrous material it might be heterogeneous,were factors which called for no mean powers of ingenuity and resource in their elucidation.In addition to this, the early investigators were hampered by an inadequate knowledge of the simpler carbohydrates,a knowledge which has subsequently proved invaluable in constitutional studies of this type.In the face of such difficulties the early contributions to the subject are by no means insignificant,but it is not surprising that a violent conflict of opinion arose,with the result that progress was impeded for many years.

The literature on the constitution of cellulose is exceedingly extensive,but the results which were obtained in early investigations and the illogical speculations which were based upon them are so confusing as to render a summary undesirable and even unnecessary. In the light of recent researches carried out in this laboratory by J.C.Irvine and his collaborators, it seems more appropriate,as a preface to this essay, to outline the various steps by which the present conception of cellulose structure was arrived at.

The argument is based on two well-known and now well-established reactions.

"(1) Cellulose, $(C_6H_{10}O_5)_x$, on complete hydrolysis, is converted quantitatively into glucose.

(2) The graded hydrolysis undergone by cellulose during acetolysis may be arrested at a stage where the disaccharide cellobiose is a definite product."

(Irvine and Hirst, T., 1923, 123, 518)

It has long been maintained that the ultimate hydrolysis of cotton cellulose by means of mineral acids proceeds quantitatively in accordance with the equation

$$(C_6H_{10}O_5)_x + xH_2O \longrightarrow xC_6H_{12}O_6,$$

but until recently, the evidence adduced in support of the view that the product consisted entirely of glucose was by no means conclusive.

The validity of hydrolytic cleavage as a means to ascertaining structure may on first considerations be questioned, in view of the discovery of a third type of methylglucoside which was obtained as an uncrystallisable syrup, in addition to the crystalline α and β methylglucosides, by the action of methyl alcohol containing 1 per cent of hydrogen chloride on glucose. (Emil Fischer, Ber., 1914, 47, 1980) In the following year, Irvine, Fyfe and Hogg, (T., 1915 107, 524), shewed that this methylglucoside was a mixture

of isomerides derived from an entirely new variety of glucose,to which they ascribed an ethylene oxide structure.Other hexose derivatives,which shew the same characteristics,have subsequently been isolated.

The additional complpication which this discovery brings into constitutional considerations, and the increased caution which must be exercised in the interpretation of results from hydrolysis, are self-evident.A notable case is found in inulin. When inulin is hydrolysed either by dilute hydrochloric acid or by oxalic acid,ordinary laevo-rotatory fructose results.When,however,inulin is first methylated and the resulting trimethyl inulin is hydrolysed,a dextro-rotatory trimethyl fructose results.(Irvine and Steele, T.,1920,117,1474)The necessary inference is that fructose exists in inulin presumably as an amylene oxide type,which is converted into the usual butylene oxide variety on hydrolysis,unless such change is precluded by methylation.It is clear,however,that in determining the quantitative nature of the conversion of cellulose to glucose,the validity of hydrolysis is unimpaired.The internal structure of the resulting hexose molecule may indeed be changed,but the actual

identity and amount of the sugar are in no wise affected.

Many investigators have confidently claimed quantitative yields of glucose from cellulose by hydrolysis.Flechsig,(Zeitsch.phsiol.Chem.,1883,7,523), using concentrated sulphuric acid as the hydrolytic agent,claimed to have obtained a yield of glucose corresponding with 95-98 per cent of the theoretical quantity available from cellulose,a claim,moreover, which he based entirely on the copper reducing power of the solution obtained.

Working on similar lines,Ost and Wilkening, (Chem.Zeit.,1910,34,461),claimed almost quantitative yields of glucose by the action of 72 per cent sulphuric acid on cellulose.In this case the claim was based on polarimetric readings.Now it is surely clear,that while the above methods of estimation may afford valuable confirmatory evidence,they can scarcely be allowed as fundamental proof.

A few years later,Willstätter and Zechmeister, (Ber.,1913,46,2401),investigated the hydrolysis of cotton wool with 40-41 per cent hydrochloric acid. They used a 1 per cent solution of cellulose and followed the course of the reaction polarimetrically.

The yield of glucose thus indicated was equivalent to 96 per cent of the theoretical quantity,and this was apparently confirmed by copper reduction figures. The use of concentrated hydrochloric acid is,however, open to even stronger objections than the use of concentrated sulphuric acid.Fischer,(Ber.,1890,23,3687; 1895,28,3024),had previously shewn that in the presence of concentrated hydrochloric acid glucose undergoes condensation to form iso-maltose,but Willstätter and Zechmeister were of the opinion that no iso-maltose was formed during their hydrolysis owing to the low concentration of glucose present.This point has since been refuted by Davis,(J.Soc.Dyers and Coll.,1914,30,249), who proved that the action of hydrochloric acid in effecting the auto-condensation of glucose extends to solutions containing only 1 per cent of glucose.

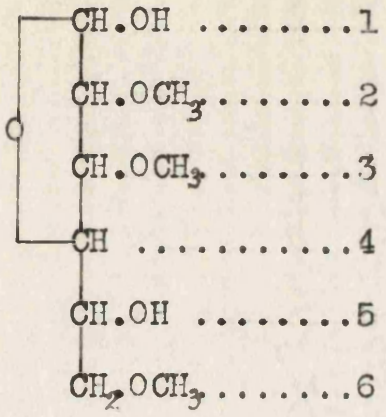
Realising the unsatisfactory nature of such investigations,Irvine and Soutar,(T.,1920,117,1489), attacked the problem from another angle.Cotton cellulose was first transformed into the corresponding triacetate by means of graded acetolysis with a mixture of acetic anhydride and sulphuric acid.The triacetate was then converted into crystalline methylglucoside by simultaneous

hydrolysis and condensation with acid methyl alcohol, and finally into the parent hexose.The yield of glucose thus obtained was 85 per cent of the theoretical amount obtainable from cellulose,calculated on the assumption that cellulose is entirely composed of hexose residues.In the above preparation of cellulose triacetate,a considerable quantity of soluble material remained in the aqueous liquors after precipitation of the acetate,the isolation of which involved a troublesome seriesof operations as well as unavoidable loss.A farther research on the subject was therefore undertaken,(Irvine and Hirst,T.,1922,121,1585),with a view to accounting for this divergence of 15 per cent from the theoretical value.

An improved method of acetolysis as described by W.L.Barnett,(J.Soc.Chem.Ind.,1921,40,8T),was made use of,and an excellent yield of uniform triacetate was obtained.No dissolved sugar was found in the aqueous washings and the operations referred to above were thus eliminated.On submitting the triacetate to treatment with methyl alcohol containing 1 per cent of hydrogen chloride in sealed tubes,a yield of methylglucoside was obtained corresponding with 95 per

cent of the theoretical amount.Evidence was also obtained that the whole of the material isolated was composed of methylglucoside without admixture with derivatives of other hexoses or of pentoses.It cannot be emphasised too strongly that the above claims are based upon the actual amount of analytically pure material isolated,and so for the first time the quantitative conversion of cotton cellulose to glucose was firmly established.

Beyond the fact that the cotton cellulose molecule is composed of glucose residues,the evidence described above gives no clue to the molecular structure. No indication is given of the hydroxyl groups which are involved in the linkage of the glucose residues, and no inference can be drawn as to the internal structure of each component glucose residue.This information was supplied by the methylation of cellulose and the subsequent hydrolysis of the alkylated product, which was first undertaken by Denham and Woodhouse. (T.,1913,103,1735). A methylated cellulose containing 25 per cent of methoxyl was obtained,which on hydrolysis yielded among other products a crystalline trimethyl glucose,which was shewn to have the following structure.

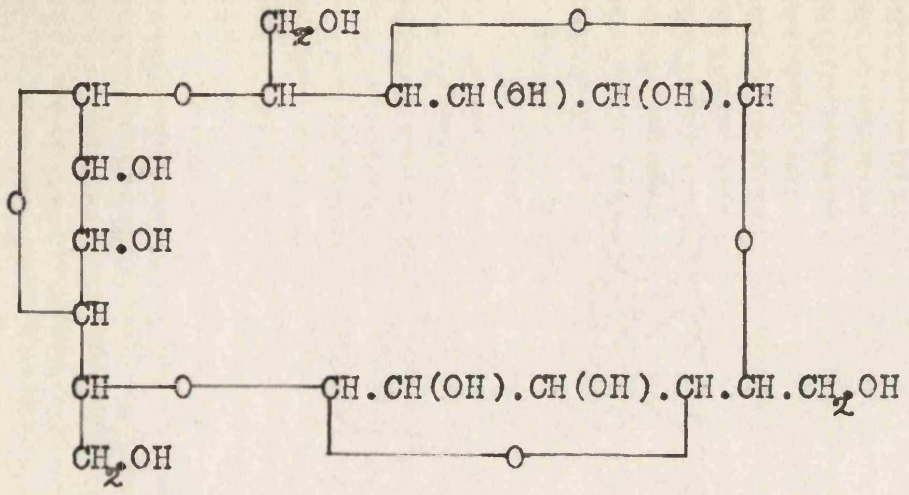


This constitution was confirmed by Irvine and Hirst. (T.,1922,121,1213)

From this it follows that cellulose is a derivative of glucose in which the positions 1 and 5 are replaced by one or more anhydroglucose residues, since these positions are obviously protected from methylation.It was clearly desirable to methylate cotton cellulose to the maximum extent,and at the request of Dr. Denham,the task was undertaken by Irvine and Hirst.(T.,1923,123,518).As was to be expected the product of maximum methylation corresponded with a trimethyl cellulose,and all efforts to increase the methoxyl content farther were unsuccessful.On hydrolysing the alkylated product the simplest of all possible results was obtained,as no sugar other than 2:3:6-trimethyl glucose was isolated.The cellulose molecule is therefore composed entirely of identical anhydroglucose

residues which are linked through the positions 1 and 5.

The remaining point to be considered is the formation of cellobiose octacetate by the graded acetolysis of cotton cellulose.The linkage in cellobiose is one which occurs only in natural carbohydrates,and this fact,in conjunction with the evidence that cellobiose,on methylation and subsequent hydrolysis,yields 2:3:6-trimethyl glucose,(Haworth and Hirst,T.,1921,119,193),points to the idea that cellobiose forms part of the cellulose molecule,as experimental evidence is entirely opposed to the view that cellobiose may be formed during acetolysis by the auto-condensation of glucose.If the cellulose molecule consists of two anhydroglucose residues linked through positions 1 and 5,100 parts of cellulose should yield 105 parts of cellobiose,but the highest recorded yields of the disaccharide are of the order of 50 to 60 per cent.The following molecular structure, which satisfies all the requirements outlined above, and would give yields of cellobiose approximating to the lower figures quoted above,has been suggested by J.C.Irvine.



It should be pointed out that the three anhydroglucose residues in the above formula may be arranged in other ways and still fulfil all the requirements, but as such a rearrangement would entail the possibility of cellulose yielding disaccharides other than cellobiose, the above is at present preferred as the simplest representation of a molecule, which, polymerised in unknown numbers, would represent cotton cellulose as a chemical entity.

THE ULTIMATE HYDROLYSIS OF ESPARTO CELLULOSE.

As a part of the experimental programme of this laboratory, a research, parallel with that already described in the case of cotton cellulose, was commenced on "esparto cellulose"; a term which is applied to the product which is obtained from esparto grass by heating under pressure with alkali and thereafter removing lignin and colouring matter by treatment with chlorine. Esparto cellulose is a fibrous material somewhat similar to cotton cellulose in appearance, but it differs markedly in that it gives a yield of furfural, corresponding with 18 to 20 per cent of pentosan, on distillation with 12 per cent hydrochloric acid. The following investigation, which involved the identification of the pentose present in esparto cellulose, along with the acetylation of the cellulose and the subsequent hydrolysis of the acetate, was carried out by Irvine and Hirst. (T., 1924, 125, 15).

A number of pentose estimations were carried out on esparto cellulose and the various results which were obtained lay between the limits of 18 and 20 per cent of pentosan. In the course of subsequent experiments, however, it was found that the extraction

of the cellulose with boiling sodium hydroxide resulted in solution of the pentosan constituent.It is probable, therefore,that the preliminary extraction of the esparto grass with sodium hydroxide solution was accompanied by a loss in pentose content,from which it follows that the maximum amount of pentosan present in esparto cellulose may be slightly higher than the results quoted above serve to indicate.

Before dealing with the preparation and hydrolysis of esparto cellulose acetate,it is convenient to state that the pentosan constituent was identified as xylan by the actual isolation of xylose from esparto cellulose.This was carried out by boiling the cellulose with a 12 per cent solution of sodium hydroxide, and thereafter precipitating the pentosan from the alkaline liquors by the addition of 90 per cent ethyl alcohol.The resulting pentosan was a white powder easily decomposed by heat,and on hydrolysis with 2 per cent sulphuric acid,gave a good yield of crystalline xylose.

The acetylation of esparto cellulose by Barnett's method did not proceed so readily as with cotton cellulose,but with slight modifications in the

experimental conditions, almost quantitative yields of a uniform acetate were obtained, calculated on the basis of a mixture of a triacetate of $(C_6H_{10}O_5)_x$, and a diacetate of $(C_5H_8O_4)_x$, in the ratio of 82 per cent to 18 per cent. The simultaneous hydrolysis and condensation of esparto cellulose acetate with acid methyl alcohol was carried out in a manner similar to that described in the case of cotton cellulose acetate and the product which was isolated after hydrolysis proved to be a mixture of methylglucoside and methylxyloside.

As regards the quantitative nature of this series of experiments, the over-all yield of glucose amounted to 91 per cent and that of xylose to 76 per cent of the quantities theoretically obtainable. The low yield of xylose was doubtless due to the destruction of pentose during the sealed tube treatment. It should also be stated that the above results are not based on any one single experiment, but have been confirmed in duplicate. We may therefore infer that esparto cellulose is, to the extent of 90 per cent at least, a definite chemical substance in which glucose residues and xylose residues are present together

in the ratio of 80 per cent to 20 per cent.

It has already been mentioned that Cross and Bevan,in their book "Cellulose",regard esparto cellulose as a pronounced oxycellulose,and the yield of furfural obtained on distillation of the cellulose with 12 per cent hydrochloric acid,as a direct measure of the degree of oxidation.They farther state that esparto cellulose may be deoxidised by prolonged exposure to neutral or alkaline reducing agents,and by solution as thiocarbonate followed by regeneration of the cellulose by heating the solution at 80° to 100°. They found that when esparto cellulose was exposed for some time to the action of a solution of zinc sodium hyposulphite,prepared by the action of zinc dust on sodium bisulphite,the yield of furfural was reduced from 12.6 per cent to 8.9 per cent,and that esparto cellulose regenerated from solution as thiocarbonate yielded only 2 per cent of furfural,while the weight of the regenerated cellulose amounted only to 80 per cent.of the weight of cellulose originally dissolved.

It is now clear that esparto cellulose must be regarded as a mixed cellulose rather than as an

oxycellulose.The yield of furfural obtained on distilling the cellulose with 12 per cent hydrochloric acid has been shewn to be a quantitative measure of the amount of pentosan present as xylan,and the effects which Cross and Bevan attribute to deoxidation may be more correctly explained as being due to the partial removal of the xylan component of the mixed cellulose.

The present work,which was designed as an extension of the researches outlined above may be conveniently divided into two parts.

(A) The first series of experiments was designed with two main objects in view:-

(1) To determine whether the hexosan and pentosan components of esparto cellulose are in chemical combination,or alternatively,mechanically mixed or forming a solid solution.

(2) To determine the nature of the hexose residue in esparto cellulose,and to compare it with the hexose residue derived from cotton cellulose.

(B) The second part of the paper deals with the possibility of obtaining true depolymerisation products of cellulose by means of acetolysis with a mixture of acetic anhydride and sulphuric acid.Evidence is adduced which

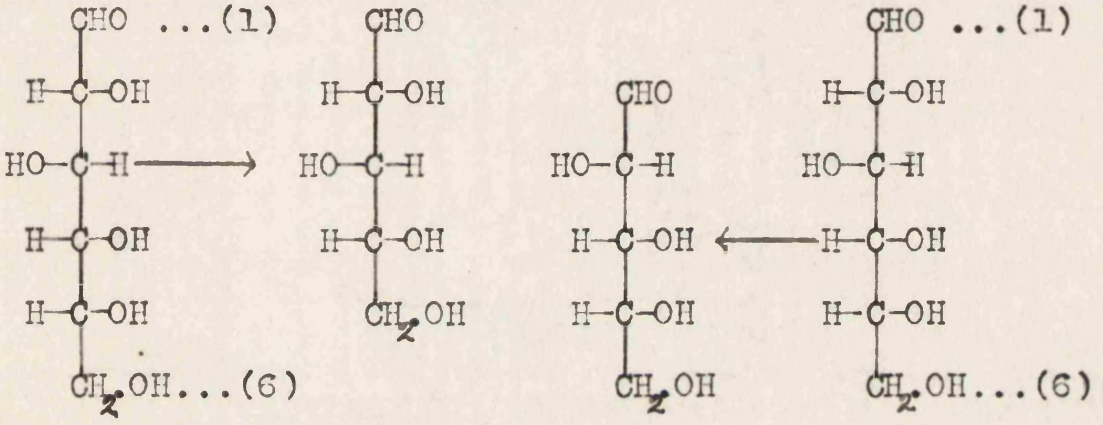
points to the idea that the structural basis of the complex cellulose molecule is a trianhydro-glucose.

THE COMPOSITION OF ESPARTO CELLULOSE.

A matter of no small interest to the investigator of esparto cellulose is the presence of the pentosan constituent which is composed of xylose residues. The question of the mechanism of the formation of pentoses in plants is still to a great extent shrouded in obscurity, but Ravenna, (Atti.R.Accad.Lincei, 1909, (v), 18, ii, 177), concludes that the simple sugars rather than the complex carbohydrates exert the major influence on their formation. The pentose component of esparto cellulose may be elaborated independently but in the same manner as the hexosan constituent, but on the other hand it may result as a degradation product of the hexosan constituent.

It is possible, by the degradation method of Wohl, to obtain D-arabinose from D-glucose, and in the same way D-xylose may be obtained from D-gulose. If, then, the pentoses formed in nature are degradation products of hexoses, we might on first considerations expect to find arabinose rather than xylose associated

with glucose in plants.On examining the configurations of glucose,arabinose and xylose,however,it becomes evident that another type of degradation is possible.



D-glucose D-xylose D-arabinose D-glucose

It is clear that by the method of Wohl the carbon atom which is eliminated from glucose during degradation to arabinose is that which is denoted by number (1) in the above formula.If,however,it were possible to elaborate a scheme by which degradation would proceed with elimination of the carbon atom denoted by number (6),the resulting product would be xylose and not arabinose.Such a process of degradation has not yet been achieved by chemical means,but it is by no means impossible that such a transformation may be carried out in the plant.

It appeared probable that some information concerning the composition of esparto cellulose might

be obtained by submitting it to graded acetolysis, for,as has already been pointed out,this process when applied to cotton cellulose,may be arrested at a stage when the octacetate of cellobiose is a definite product of the reaction.If,therefore,the hexosan and pentosan components of esparto cellulose are in chemical combination,it seems reasonable to expect that,by the application of the same process, at least traces of a hexose-pentose compound,of the same type as cellobiose octacetate,might be formed.

The method which was adopted was acetolysis by means of a mixture of acetic anhydride and sulphuric acid as described by Haworth and Hirst in the following preparation of cellobiose octacetate from filter paper. (T.,1921,119,193). "Twenty grams of filter paper were stirred into a water-cooled mixture of 80cc.of acetic anhydride and 11cc.of concentrated sulphuric acid. At this stage the temperature of the viscid mixture was kept just below 20°.Under the prescribed conditions a viscid paste was formed after stirring the paper with the reagents for five minutes.A bath,containing calcium chloride and water,having been heated in readiness to a temperature of 120°,the vessel containing

the viscid paste of impregnated filter paper was heated by this means,and the contents thoroughly stirred.Rapid disintegration and solution of the paper occurred;the mixture darkened in colour;and, at about 112°,assumed the form of a dark-red mobile liquid,which began to boil.This marked the critical stage in the acetolysis process.Immediately the red solution appeared to be changing to black,the whole of it was poured into one and a half litres of cold water,and a pale-yellow precipitate of crude cellobiose octacetate separated out after ten minutes."

Several experiments were carried out under conditions identical with those described above,but in each instance extensive charring of the material took place,and only very small yields of a discoloured amorphous powder were isolated.Haworth and Hirst lay some stress on the fact that the colour changes, apparent in the solution of filter paper during acetolysis,serve as a fair indication of the progress of the reaction.In the present case the initial solution of the esparto cellulose was accompanied by the development of a dark-brown colour,and it was found almost impossible to discern,by any deepening

of this colour, the most opportune moment for the precipitation of the dissolved products with water. The physical state of the cellulose appeared to have an important bearing on the reaction, and it was found that the amount of charring was slightly diminished by the use of cellulose in a very fine state of division. A farther series of experiments was now undertaken, in which the conditions of acetolysis were modified both with regard to the temperature of the reaction and the concentration of the reagents, but in every case the products which were isolated, although partly soluble in hot 90 per cent ethyl alcohol, proved to be entirely amorphous. No trace of a hexose-pentose compound was detected, but a fact of striking importance which will be dealt with later, was the entire absence of cellobiose octacetate from the products which were obtained in both series of experiments.

In conjunction with the foregoing, acetolysis experiments were carried out at room temperature by the same method, with a view to estimating the effect of the reaction on the pentosan constituent of the cellulose. The finely divided cellulose was added to

a cold mixture of 80cc.of acetic anhydride and 12cc. of concentrated sulphuric acid,and precautions against local heating during the initial stages of solution were taken.The cellulose dissolved very slowly with the development of the brown colour which appears to be typical of esparto cellulose.When the cellulose had dissolved completely the reaction mixture was poured into a large excess of distilled water,and the resulting creamy-white precipitate was washed and dried. In some experiments the reaction was continued for twenty-four or forty-eight hours after complete solution of the cellulose had been effected,in which cases the yields of solid products of the reaction were greatly diminished.The solid products of acetolysis were creamy-white amorphous powders,which,without farther purification,were shewn by elementary analysis to correspond roughly with triacetates of cellulose. Pentose estimations by the furfural method shewed that the pentosan content had fallen from 20 per cent to 11 per cent,and in some cases to 8 per cent.The acetolysis of esparto cellulose therefore results in the partial removal of the pentosan constituent, the loss of pentose being greater,the longer acetolysis

is allowed to proceed.Moreover,an examination of the mother liquors after precipitation of the solid products brought to light the fact that the pentosan component is not broken down to furfural,but is removed as soluble pentose or pentosan acetates.

This evidence,taken in conjunction with the fact that the pentosan constituent may be completely extracted from esparto cellulose in the form of xylan by treatment with boiling alkali,points to the idea that the pentosan and hexosan components are not in chemical union,but are mechanically mixed or form a solid solution.

THE HEXOSE RESIDUE IN ESPARTO CELLULOSE.

In the course of the experiments already described, in which esparto cellulose was treated with a mixture of acetic anhydride and sulphuric acid under varied conditions of concentration and temperature, no trace of crystalline matter was detected in any of the solid products of acetolysis. The entire absence of cellobiose octacetate from these products seems to indicate that the hexose residue present in esparto cellulose is of a different constitutional type from that present in cotton cellulose, but at the same time it is clear that the presence of a considerable pentosan constituent may introduce complications of a far-reaching nature into the process of acetolysis.

From such considerations it was considered desirable to repeat the experiments with esparto cellulose from which the pentosan constituent had been removed. "Normal" esparto cellulose was therefore submitted to repeated extractions with 12 per cent alkali at 90°, until the washed and dried residue yielded

Note.

"Normal" esparto cellulose is the term which is applied to the product obtained from esparto grass by heating under pressure with alkali and thereafter removing lignin and colouring matter by treatment with chlorine.

only traces of furfural on distillation with 12 per cent hydrochloric acid,and it was found that an "extracted esparto cellulose",containing only very slight traces of pentosan,might be obtained after three such treatments.The extracted material was used in a state of fine division in a series of experiments, duplicate with those already described in the case of the normal cellulose,and under suitable conditions, a yield of cellobiose octacetate,corresponding with 17.5 per cent of the weight of cellulose used,was obtained.Control experiments with cotton cellulose under the same conditions gave yields of cellobiose octacetate corresponding with 19 per cent of the weight of cellulose used.

This result affords valuable confirmatory evidence that the hexose residue which is present in esparto cellulose is of the same constitutional type as that present in cotton cellulose.It appears,however, that the formation of cellobiose octacetate from normal esparto cellulose is inhibited by the presence of the pentosan constituent.The reason for such an effect is obscure,but a little light may be thrown on this point by the fact that several experiments of

a qualitative nature shewed that the xylan,obtained by the extraction of esparto cellulose with alkali, was exceedingly resistent to acetylation.It seems not improbable,therefore,that as the pentosan constituent is uniformly distributed throughout the hexosan constituent,the resistance of the pentosan to acetolysis may impede or mask the hydrolysis of the hexosan constituent by a protective influence,which may be compared in effect with steric hindrance.

A systematic study of extracted esparto cellulose was now carried out.

The extracted material,which invariably contained a higher percentage of ash than the normal cellulose,was acetylated essentially in accordance with the method of Barnett,in which sulphuryl chloride is used as the catalyst,but the reaction took place very slowly and with great difficulty.Constant mechanical stirring was necessary,and the complete solution of the cellulose was not effected until the experiment had been in progress for over twenty hours, during which time several successive treatments with chlorine and sulphur dioxide were given to the mixture. In preliminary experiments,yields averaging 90 per cent

of the amount of acetate available on the basis of a triacetate of cellulose were obtained, while in a farther experiment, in which elaborate precautions against loss of material were taken, the yield corresponded with 95 per cent of the theoretical amount. The extracted esparto acetate differed from the typical cotton cellulose acetate in several respects. It was much bulkier; was not so hard and crisp; and was creamy in colour rather than pure white. An elementary analysis of the acetate shewed that it corresponded with a triacetate of cellulose, $[C_6H_7O_2(CH_3.COO)_3]_x$.

SIMULTANEOUS HYDROLYSIS AND CONDENSATION OF THE ACETATE WITH ACID METHYL ALCOHOL.

In order to convert the extracted esparto cellulose acetate into methylglucoside it was treated with acid methyl alcohol in sealed tubes, when it was invariably found to be much more resistent than either the normal cotton or esparto cellulose acetates. Concentrations of hydrogen chloride from 1 per cent to 3 per cent, and conditions of temperature ranging from 120° to 140° were employed in a long series of

experiments,but all efforts to effect complete solution of the acetate were unavailing.In every experiment partial solution of the acetate was accompanied by the development of a deep-brown colour and in many cases charring also took place.One of the most successful experiments,in which there was only a slight solid residue and development of colour, yielded a dark and only partially crystalline syrup, corresponding in amount with 83.5 per cent of the theoretical quantity of crystalline methylglucoside. It should be noted that the theoretical quantity was calculated on the assumption that the acetate was composed entirely of glucose residues.By recrystallising this syrup from ethyl acetate,the amount of pure crystalline methylglucoside which was obtained corresponded with 42 per cent of the theoretical quantity.

As it appeared probable that the physical state of the acetate might have a retarding influence on the reaction,an attempt was made to obtain it in a more reactive form.The acetate was dissolved in chloroform in a Carius tube,and an equal volume of methyl alcohol was added along with sufficient acid methyl alcohol to give a 1 per cent concentration of hydrogen chloride.The addition of the methyl alcohol

precipitated the acetate as a jelly-like mass,and it was considered that in this state the compound might react more readily with the methyl alcohol and hydrogen chloride.Very little colour developed in this experiment, but the solid residue was considerable,and the syrup which was finally isolated was again very dark in colour and only partially crystalline.

Treatment of the acetate with highly increased concentrations of hydrogen chloride in methyl alcohol was now resorted to.The finely powdered acetate was suspended in cold methyl alcohol and dry hydrogen chloride was passed into the solution until it was saturated.The mixture was kept in a stoppered flask with occasional shaking for six days,by which time the acetate had almost completely dissolved.After the elimination of the excess acid and neutralisation, the syrup which was isolated crystallised completely on nucleation with methylglucoside,and the yield in this case amounted to 64 per cent of the theoretical quantity.

From the general course of these experiments it is clear that the hexosan residue derived from esparto cellulose by the extraction of the pentosan is essentially different in type from cotton cellulose,

or that the prolonged treatment of the fibres of the esparto with boiling alkali has resulted in considerable changes in the cellulose.The latter view seems not unlikely when the drastic nature of such treatment with strong alkali is taken into account,and to decide this point,it appeared desirable to test the behaviour of normal cotton cellulose after a similar treatment with alkali.

ANALOGOUS EXPERIMENTS WITH COTTON CELLULOSE.

Cotton cellulose was accordingly heated with a 12 per cent solution of sodium hydroxide for nine hours at 90°,after which the washed and dried residue was acetylated as before.As far as acetylation was concerned, the cotton cellulose did not appear to have been appreciably altered by the treatment with alkali, for the reaction proceeded normally without the aid of mechanical stirring and gave a good yield of triacetate corresponding with 91 per cent of the theoretical maximum.

As in the case of extracted esparto cellulose acetate,this acetate was converted into methylglucoside by the agency of simultaneous hydrolysis and condensation

effected by means of acid methyl alcohol.The reaction was carried out on some occasions in sealed tubes at high temperatures,and on others by using high concentrations of acid at lower temperatures,and it immediately became apparent that a change from the normal had occurred,for whereas normal cotton cellulose acetate was completely hydrolysed by a 1.5 per cent hydrogen chloride concentration,the present acetate was not completely hydrolysed even with a 3 per cent concentration of hydrogen chloride.The highest yield of pure crystalline methylglucoside which was obtained in this series of experiments amounted to 84 per cent of the theoretical maximum.

The following synopsis facilitates a comparison of the results obtained in the present research with those previously obtained by Irvine and Hirst.The percentages quoted indicate the yields in terms of the theoretical maximum.

(1) Cotton Cellulose ⟶ Cotton Cellulose Triacetate 99.5% ⟶ Methylglucoside 95.5%

(Irvine and Hirst,T.,121,1585)

(2) Esparto Cellulose ⟶ Esparto Cellulose Acetate 97.2% ⟶ Methylglucoside 91.0%; Methylxyloside 76.0%

(Irvine and Hirst,T.,125,15)

(3)	Extracted Cotton Cellulose →	Extracted Cotton Cellulose Triacetate → 91.0%	Methylglucoside 84.0%
(4)	Extracted Esparto Cellulose →	Extracted Esparto Cellulose Triacetate → 95.0%	Methylglucoside 64.0%

The results obtained in the conversion of the hexosan component of esparto cellulose into methylglucoside, which are expressed in series (4) above, may be interpreted in two ways. The diminished yield of methylglucoside may be due to the presence in extracted esparto cellulose triacetate of some component (or components) unrelated to glucose and thus incapable of forming methylglucoside, or alternatively, purely physical causes may be responsible.

In the first place it must be remembered that no direct evidence whatsoever of the presence of any compound or compounds unrelated to glucose has been obtained, and such a supposition is mere speculation. Moreover, the results obtained by Irvine and Hirst in series (2), which prove that the hexosan component of esparto cellulose is composed of glucose residues to the extent of 90 per cent, render it highly improbable that the discrepancy of nearly 40 per cent

from the theoretical maximum, as shewn in series (4), can be accounted for other than by physical changes in the material.

When cotton fibre is submitted to the well-known process of mercerisation, in which it is brought into contact with a 20 per cent solution of sodium hydroxide at the ordinary temperature, the structural features of the fibre are instantly changed from a flattened ribband with a large central canal to a thickened cylinder in which the canal is more or less obliterated. Reasoning by analogy, it is almost certain that the drastic treatment with alkali, which is necessary to eliminate the pentosan constituent, will bring about similar profound physical changes in the esparto fibre. This view also receives indirect support from the fact that cotton cellulose, after similar treatment with alkali, presents the same resistance to hydrolysis, although, it must be admitted, in a much lesser degree than esparto cellulose. At present it would be imprudent to advance the definite opinion that physical causes are entirely responsible for such profound changes, but it may at least be stated that the evidence at our disposal points distinctly to the idea that this is the true explanation of the phenomenon.

PART ONE.

EXPERIMENTAL.

A generous supply of esparto cellulose was obtained through the courtesy of Messrs.Tullis,Russell and Co., of Markinch.It had undergone the usual preliminary extractions and had been boiled once with dilute sodium hydroxide,but had not been bleached.With the object ih view of using a starting material,which was, as far as possible,a normal cellulose,the crude esparto was bleached with chlorine,and the resulting white fibrous mass was thoroughly washed and dried.This material was used throughout the following investigation.

The cotton cellulose used in this series of experiments consisted of 10 per cent best grade Carolina cotton and 90 per cent best long staple Sakelereides Egyptian cotton.

THE ACETOLYSIS OF ESPARTO CELLULOSE.

Series (1).

Several experiments were carried out in accordance with the method of Haworth and Hirst,and have already been fully described in the first part of this paper.(Pages 21 and 22.)

Series (2).

The procedure was essentially the same as

in Series (1),but modifications were introduced with regard to the temperature of the reaction and the concentration of the reagents.

(a). 10 grams of finely divided cellulose were added to a mixture containing 80cc.of acetic anhydride and 12cc.of concentrated sulphuric acid at 15°,and the temperature of the reaction mixture was immediately raised to 115° by means of a bath containing a solution of calcium chloride.The cellulose dissolved immediately with the development of a brown colour,and when this colour had become almost black, the solution was poured into an excess of distilled water.A dirty brown precipitate was obtained which was subsequently shewn to be partly soluble in hot 90 per cent ethyl alcohol.The soluble product was examined microscopically and was found to be entirely amorphous.

(b). Experiment (a) was repeated at a temperature of 110°.The cellulose took an appreciably longer time to dissolve,but the result was the same as in (a).

(c). 10 grams of finely divided cellulose were treated with a mixture containing 90cc.of acetic anhydride and 9cc.of concentrated sulphuric acid at 110°.

(d). 10 grams of finely divided cellulose were treated with a mixture containing 80cc.of acetic

anhydride and 6cc.of concentrated sulphuric acid at 110°.

The products obtained in (c) and (d) were also entirely amorphous.

THE ACETOLYSIS OF ESPARTO CELLULOSE AT 15°.

Experiment (1).

5 grams of esparto cellulose were added to a mixture containing 27cc.of acetic anhydride and 4cc.of concentrated sulphuric acid.The cellulose dissolved very slowly with the development of the typical brown colouration,and after twenty-four hours the cellulose had completely dissolved.The solution was poured into an excess of distilled water,and a creamy-yellow precipitate was obtained.This solid product of acetolysis,after thorough washing and drying, was found to be slightly soluble in hot 90 per cent ethyl alcohol,but microscopic examination failed to reveal the slightest trace of crystalline material.

Pentose estimation on the solid product of acetolysis.

A weighed quantity of the product was distilled with an excess of 12 per cent hydrochloric acid,and a solution of phloroglucinol in water was added to the distillate,which contained the pentose as furfural.The resulting precipitate of phloroglucide which settled

out in about twenty-four hours was collected, dried and weighed.

0.848 grams gave 0.056 grams of phloroglucide, which corresponds with 0.0546 grams of pentosan.

Pentosan = 11.27%

Experiment (2).

Experiment (1) was repeated with 10 grams of cellulose, but 96 hours were allowed to elapse before the solution was poured into water. In this instance a larger proportion of the solid product of acetolysis was soluble in hot ethyl alcohol, but again no trace of crystalline material was observed.

Pentose estimation on the solid product of acetolysis

0.9843 grams gave 0.0362 grams of phloroglucide, which corresponds with 0.0369 grams of pentosan.

Pentosan = 8.26%

Examination of the aqueous filtrate from Experiment (2).

The total filtrate amounted to 425cc.

A solution of phloroglucinol was added to 25cc. of the filtrate, but no phloroglucide was formed. The filtrate was now boiled under reflux with 12 per cent hydrochloric acid for an hour, after which the addition of a solution of phloroglucinol effected immediate precipitation of phloroglucide.

50cc.of filtrate yielded 0.0688 grams of phloroglucide, which corresponds with 0.068 grams of pentosan.The total filtrate therefore contained 0.582 grams of pentosan,and the solid product of acetolysis contained 0.826 grams of pentosan,so that the total amount of pentosan accounted for is 1.408 grams,whereas the original material contained 2 grams of pentosan.It would therefore appear that a little pentosan is destroyed and lost during acetolysis.

THE ELIMINATION OF THE PENTOSAN CONSTITUENT OF ESPARTO CELLULOSE.

Normal esparto cellulose,containing 20 per cent of pentosan,was heated at 90° with a 12 per cent solution of sodium hydroxide for eight or nine hours.This treatment was repeated (three times) until the washed and dried residue yielded only slight traces of furfural on distillation with 12 per cent hydrochloric acid.

THE ACETOLYSIS OF EXTRACTED ESPARTO CELLULOSE.

(a) Two grams of finely divided cellulose were added to a mixture containing 20cc.of acetic anhydride and 3cc.of concentrated sulphuric acid.The temperature of

the mixture was raised to 120°,and the cellulose dissolved immediately.When the liquid was dark-red in colour,it was poured into an excess of distilled water and a dirty brown powder was precipitated. On dissolving a little of this powder in hot alcohol, and examining a drop of the solution microscopically, characteristic crystals of cellobiose octacetate were observed.The yield,however,was very small.

(b) Two grams of finely divided cellulose were added to a mixture containing 30cc.of acetic anhydride and 3cc.of concentrated sulphuric acid.In this case the reaction was carried out at 110°,and the solution was poured into water when it was black-red in colour. On microscopic examination of the solid product, numerous crystals of cellobiose octacetate were observed and the acetate was purified by crystallisation from ethyl alcohol as described by Haworth and Hirst. (loc.cit.).The yield of cellobiose octacetate amounted to 0.35 grams,or 17.5 per cent of the weight of cellulose used.

(c) Experiment (b) was repeated at a temperature of 115°,and the yield of cellobiose octacetate again amounted to 0.35 grams.

As the above experiments involved a new set of conditions for acetolysis,duplicate experiments with normal esparto cellulose were undertaken,but in no case was any cellobiose octacetate isolated.

THE ACETOLYSIS OF COTTON CELLULOSE.

For purposes of comparison,an experiment with cotton cellulose was carried out under conditions identical with experiment (c),and a yield of cellobiose octacetate was obtained which corresponded with 19 per cent of the weight of cellulose used.

THE EXAMINATION OF EXTRACTED ESPARTO CELLULOSE.

The extracted material,obtained by the method described above,was dried for several days at the temperature of the steam oven.

Pentose Estimation.

0.905 grams yielded 0.0012 grams of phloroglucide, from which it is clear that the percentage of pentosan is negligible.

Moisture.

The material was dried at a temperature of 115°,until a constant weight was recorded.

1.9764 grams gave a decrease of 0.058 grams.

Moisture = 2.9%

Ash.

A weighed quantity of material was incinerated in a platinum crucible.1.54 grams gave 0.0339 grams of ash.

Ash = 2.2%

THE ACETYLATION OF EXTRACTED ESPARTO CELLULOSE.

The acetylation was carried out essentially in accordance with the method of W.L.Barnett and a typical experiment is described below.

Experiment (1).

Ten grams of dry extracted esparto cellulose were added to 80cc.of glacial acetic acid through which dry chlorine had been bubbled for 30 seconds.80cc.of acetic anhydride were now added,and sulphur dioxide was passed through the mixture for one minute.The temperature of the mixture rose spontaneously to 30°, and after half-an-hour the temperature was raised to 75°and rapid mechanical stirring was employed.After five hours,during which time several additions of chlorine and sulphur dioxide were made to the mixture, the cellulose had not completely dissolved.The mixture

was accordingly left over-night,and on the following day the treatment was repeated for five hours before complete solution of the cellulose was effected.An equal volume of chloroform was now added to the clear viscid solution,followed by an excess of distilled water,and the chloroform was slowly evaporated off. During this operation the mixture was stirred occasionally,and the acetate separated as a white granular powder.The acetate was washed free from acetic acid and dried until a constant weight was recorded.The yield was 16.32 grams,while the theoretical maximum,calculated on the basis of a triacetate of $(C_6H_{10}O_5)_x$,is 17.78 grams.Allowing for 2.2 per cent of ash,the present yield is 93.6 per cent of the theoretical maximum.

Experiment (2).

19.93 grams of cellulose,containing 3 per cent of moisture and 2.2 per cent of ash,were acetylated as in Experiment (1).The yield of acetate was 30.78 grams, as compared with the theoretical 33.6 grams,and corresponds with 91.5 per cent of the theoretical maximum.

Experiment (3).

8 grams of cellulose,containing 1.5 per cent of moisture

and 2.2 per cent of ash,were acetylated as before and elaborate precautions were taken against loss of material.The yield of acetate was 13 grams,while the calculated yield was 13.7 grams,so that the percentage yield is 95 per cent.

THE EXAMINATION OF EXTRACTED ESPARTO CELLULOSE ACETATE.

The material which was examined was obtained in Experiment (1).

Moisture.

(a) 0.9526 grams,dried at 115°until a constant weight was recorded,gave a decrease of 0.0154 grams.

Moisture = 1.61%

(b) 0.6794 grams gave a decrease of 0.0098 grams.

Moisture = 1.44%

Mean of (a) and (b) Moisture = 1.50%

Ash.

(a) 0.9372 grams,incinerated in a platinum crucible, gave 0.0060 grams of ash.

Ash = 0.64%

(b) 0.6696 grams,incinerated in a platinum crucible, gave 0.0058 grams of ash.

Ash = 0.86%

Mean of (a) and (b) Ash = 0.75%

Acetyl Estimation.

Great difficulty was experienced in obtaining a reliable estimation of the acetyl content. In the first place the acetate was digested with standard alkali and the mixture was allowed to stand overnight in a stoppered flask, but after such treatment the reaction was obviously incomplete. Treatment with boiling alkali was next resorted to. A weighed quantity of acetate was boiled under reflux for an hour with an excess of standard alkali, but such a procedure resulted in partial decomposition of the acetate and this tended to give an apparently high acetyl percentage. It was hoped to obtain a correction factor by performing a blank experiment on the extracted esparto cellulose, but this was not accomplished owing to the fact that the cellulose did not neutralise any alkali even on boiling for an hour. This may be explained by the fact that the cellulose was prepared by boiling with 12 per cent alkali. The method finally adopted was prolonged treatment with standard alkali at room temperature. The acetate was kept in contact with a solution of sodium hydroxide for 72 hours.

0.4359 grams were digested with 50cc. of 0.206 Normal

sodium hydroxide.28.15cc.of 0.206 Normal hydrochloric acid wererequired to neutralise the mixture.The acetate had therefore neutralised 21.85cc.of 0.206 Normal sodium hydroxide.

Acetyl (as acetic acid) = 62.0%

Cellulose Triacetate requires Acetyl = 62.5%

Carbon and Hydrogen.

As the process of drying the acetate was difficult to carry out without slight decomposition of the material,a specimen of known moisture content was analysed directly,and the necessary corrections made for the weight of acetate taken and the weight of water obtained.

0.1667 grams,containing 1.5 per cent of water and 0.75 per cent of ash,i.e.0.1629 grams gave 0.2983 grams of carbon dioxide and 0.0848 grams of water. The corrected weight of water is 0.0823 grams.

Found:- C = 49.93% H = 5.61%

Cellulose triacetate requires:- C = 50.00% H = 5.55%

THE SIMULTANEOUS HYDROLYSIS AND CONDENSATION OF EXTRACTED ESPARTO CELLULOSE TRIACETATE WITH ACID METHYL ALCOHOL IN SEALED TUBES.

The acetate was subjected to treatment with acid methyl alcohol in sealed tubes,but was found to be extremely resistent,and in the majority of cases it only partially dissolved.

Summary of Experiments.

(1) 4.5426 grams of acetate were treated with 50cc. of methyl alcohol containing 1 per cent of hydrogen chloride.The tube was heated for 120 hours at 120°. A deep-brown colour developed and there was a large residue of undissolved material.

(2) 2.00 grams of acetate were treated with 50cc. of methyl alcohol containing 3 per cent of hydrogen chloride.The tube was heated for 200 hours at 130°. Charring took place and there was a large solid residue at the end of the experiment.The hydrogen chloride concentration may have been excessive,and the residue was probably not undissolved material, but humic acid which had been formed in the course of the reaction.

(3) 4.00 grams of acetate were treated with 75cc. of methyl alcohol containing 1.5 per cent of hydrogen chloride.The tube was heated for 70 hours at 120°. In this experiment very little colour developed and there was practically no solid residue.The solution was therefore neutralised with silver oxide,filtered and evaporated to dryness,when a dark and only partially crystalline syrup was obtained.This syrup weighed 2.2 grams and corresponded with 83.5 per cent of the theoretical maximum,an amount which was calculated on the assumption that the acetate was completely composed of glucose residues.By recrystallising this syrup from ethyl acetate,crystalline methylglucoside was obtained corresponding with 42 per cent of the theoretical maximum.

(4) 4.00 grams of acetate were dissolved in 40cc. of chloroform in a Carius tube,and 40cc.of methyl alcohol were subsequently added,along with sufficient acid methyl alcohol to give a 1 per cent hydrogen chloride concentration.The methyl alcohol precipitated the acetate as a jelly and it was hoped that in this form the acetate would react more readily with the

acid methyl alcohol. After heating for 10 hours at 80° to 90°, very little change was noticed, and after a farther 40 hours at 140°, a considerable quantity still remained undissolved. Very little colour developed but the solid residue weighed 0.45 grams and appeared to be mainly regenerated cellulose. The neutralised solution yielded 2.17 grams of a syrup which again was very dark in colour and only partially crystalline. The methoxyl content of the syrup was 11.7 per cent, while methylglucoside requires a methoxyl content of 15.95 per cent.

THE HYDROLYSIS OF EXTRACTED ESPARTO CELLULOSE TRIACETATE WITH HIGH CONCENTRATIONS OF HYDROGEN CHLORIDE.

2.5298 grams of acetate were suspended in 100cc. of pure methyl alcohol, and dry hydrogen chloride was passed into the solution until it was saturated. The mixture was kept in a stoppered flask for six days, during which time it was frequently shaken, and at the end of this time only a very slight solid residue (0.1gram) remained, while the colour of the solution was golden-brown. In order to eliminate the excess of hydrogen chloride, the filtered solution was evaporated

to about half-bulk under diminished pressure, after which fresh methyl alcohol was added and the process was repeated. Neutralisation of the solution by means of silver oxide was followed by charcoal treatment in order to effect clarification, and on evaporating the solution to dryness in a tared flask, 1.0954 grams of a syrup were obtained, which crystallised completely on nucleation with methylglucoside. This yield is 64.3 per cent of the theoretical maximum.

Analysis of the methylglucoside obtained.

Zeisel Estimation.

0.1521 grams gave 0.1590 grams of silver iodide.

Methoxyl = 13.8%

Methylglucoside requires Methoxyl = 15.9%

Carbon and Hydrogen.

Found:- C = 43.1% H = 7.22%

Required:- C = 43.3% H = 7.22%

ANALOGOUS EXPERIMENTS WITH COTTON CELLULOSE.

For the purpose of comparison, cotton cellulose was now treated with a 12 per cent solution of sodium hydroxide for nine hours at 90°.

THE ACETYLATION OF EXTRACTED COTTON CELLULOSE.

The extracted cotton cellulose was acetylated in accordance with the method of Barnett, and the reaction was found to proceed normally without the aid of mechanical stirring, the cellulose dissolving completely in five hours. 9.68 grams of cellulose yielded 15.62 grams of dry acetate, and as the theoretical yield of a triacetate is 17.2 grams, this corresponds with 90.1 per cent of the theoretical maximum.

THE SIMULTANEOUS HYDROLYSIS AND CONDENSATION OF EXTRACTED COTTON CELLULOSE TRIACETATE WITH ACID METHYL ALCOHOL.

The experiments were carried out in the manner which has already been described for extracted esparto cellulose acetate.

Summary of Experiments.

(1) 1.0572 grams of acetate were heated with 50cc. of methyl alcohol containing 1 per cent of hydrogen

chloridefor 120 hours at 120°.A large residue (0.767gram) of undissolved material remained.The experiment was obviously incomplete and only 1.63 grams of crystalline methylglucoside,or 59.7 per cent of the theoretical maximum,were isolated.

(2) 4.0590 grams of acetate were heated with 50cc. of methyl alcohol containing 3 per cent of hydrogen chloride for 120 hours at 120°.Practically no colour developed in this experiment,but there was a slight gelatinous residue which weighed 0.2726 grams.The yield of crystalline methylglucoside was 2.2470 grams, or 82.2 per cent of the theoretical maximum.

(3) 2.7768 grams of acetate were treated with a high concentration of hydrogen chloride in the manner already indicated.The solid material which failed to dissolve was in this case negligible,and 1.5724 grams of crystalline methylglucoside,or 84.1 per cent of the theoretical maximum,were isolated.

PART TWO.

THE STRUCTURAL BASIS OF COTTON CELLULOSE.

The term "acetolysis" is,in general,applied to the process which accomplishes the hydrolytic cleavage of complex carbohydrates by means of acids in the presence of acetylating reagents which effect simultaneous esterification of the hydroxyl groups.The numerous acetolytic studies of cotton cellulose may well be described as a development of the fundamental observations made by Skraup and his collaborators,who first shewed that the series of reactions may be arrested at a stage when cellobiose octacetate is a definite product. Franchimont,(Ber.,12,1879,1941.),was the first investigator to isolate cellobiose octacetate,but he failed to recognise it as such,and characterised it as a triglucose which contained eleven acetyl groups.Some twenty years later,Skraup,(Ber.,32,1899,2413),who repeated the work of Franchimont,obtained the same crystalline compound, and questioned the constitution postulated by the latter.He suggested that Franchimont's "elffach acetylierte Triglukose"was an acetylated derivative of a hexose,a suggestion which he subsequently

contradicted,when,in collaboration with König,(Ber., 34,1901,1115),he definitely established the constitution of the compound as that of the octacetate of a biose. The degradation of cellulose to cellobiose octacetate by acetolysis is now a well-established reaction and it is of special importance,because investigations in this direction have thrown light on the mechanism of the processes of hydrolysis and acetylation.

The work of Klein,(Zts.ang.Chem.,1912,25,1409), who studied the acetolysis of cellulose by means of a mixture consisting of 80 per cent of acetic anhydride and 20 per cent of sulphuric acid,is of special interest in this connection.He claimed to have obtained yields of cellobiose octacetate corresponding with 60 per cent of the weight of cellulose used,and noted,that in addition to the octacetate,by-products of two distinct types were formed.He not infrequently found after precipitation and filtrationof the products insoluble in water,the aqueous mother liquors contained compounds which corresponded in weight with about 40 per cent of the cellulose used.Such compounds have not been systematically examined,but from a general study of their properties,Klein suggested that they

were probably aceto-sulphates of cellobiose or glucose. From the point of view of cellulose structure, however, more importance attaches to the other type of by-product, which was obtained from the products insoluble in water, after removal of cellobiose octacetate by crystallisation from ethyl alcohol. This by-product proved to be a mixture of cellulose dextrin acetates of varying complexity, and by a careful fractionation, Klein was able to shew that these acetates possessed specific rotations ranging from +11° to +34° in chloroform solution. From this it appeared probable that they were intermediate products in the disruption of the cellulose molecule.

Schliemann, (Ann., 1911, 378, 366), in a similar investigation, was also of the opinion that the amorphous by-products were the immediate precursors of cellobiose octacetate. From the general course of such experiments, acetolysis appears to act in a series of somewhat ill-defined steps, and the possibility of obtaining true depolymers of cellulose by this method is at least worthy of consideration.

The manner and numbers in which the "molecular units" of cellulose polymerise to give the complex molecule is unknown, but if for the sake of illustration

only,the cellulose molecule is regarded as a series of unit links,it was hoped that by a modified process of acetolysis,compounds might be obtained which would correspond with one,two,or several complete links in the fully acetylated condition.With this aim in view,several experiments were carried out to determine the most suitable conditions of time,temperature, and concentration of the acetolysis reagents,which would result in a slow smooth reaction,together with the maximum degradation of the cellulose molecule, short of the formation of cellobiose octacetate.

The scheme may be illustrated by reference to the following diagrammatic representation of the complete acetolysis of cellulose.

Cellulose ⟶ Acetylated Cellulose Dextrins

⟶ (X)

⟶ Cellobiose Octacetate

⟶ Glucose Pentacetate.

The investigation therefore deals with the exploration of the unknown stages indexed as (X).The experiments are described in detail in the experimental part of this thesis,and it is sufficient to state here,that in the method of procedure which was finally adopted,cotton

cellulose was treated with a mixture of acetic anhydride and sulphuric acid in the ratio of 5.9 parts by weight to 1 part by weight at a temperature of 15°.

EXAMINATION OF THE SOLID PRODUCT OF ACETOLYSIS OBTAINED UNDER THE CONDITIONS PRESCRIBED.

The solid product of acetolysis was a beautiful white amorphous powder,and numerous microscopic examinations, which were carried out on samples from different experiments,gave no indication of the presence of crystalline matter.The general properties of the product indicated definitely that considerable degradation of the cellulose molecule had taken place,and were in sharp contrast to the properties of a normal acetate of cellulose.The hard crispness,typical of cotton cellulose triacetate,had given place to a soft flakiness.Moreover, the degraded material shewed a much wider range of solubilities;possessed a distinct although ill-defined melting point;and was dextro-rotatory,in contradistinction to the normal acetate which is laevo-rotatory.
Elementary analyses gave results which corresponded closely with the data required for a triacetate of cellulose,but it was necessary to modify this view

having regard to the fact that the degraded material reduced Fehling's Solution.Quantitative experiments with Fehling's Solution were carried out before and after acid hydrolysis of the product,and the ratio of the copper oxide formed in the two experiments was as 2.2 is to 1.

It must be remembered,however,that in dealing with a degradation product obtained by the acetolysis of cellulose,the greatest caution must be exercised in the interpretation of analytical data.The theoretical possibilities are so numerous as to render the formulation of a definite hypothesis at this stage nothing more than speculation,and for this reason it appears desirable to pass from a discussion of the acetylated product, and to deal first with the constitutional study of the parent carbohydrate or carbohydrates.The nature of the acetylated product may then be discussed in the light of constitutional evidence and it is hoped that the argument contained in this thesis may thus be presented in a more coherent and logical manner.

THE HYDROLYSIS OF THE ACETYLATED PRODUCT.

As a preliminary to the constitutional study of the carbohydrates which form the basis of the acetylated product,it was considered necessary to eliminate the acetyl groups by hydrolysis,and thus isolate the parent compound.With a view to preparing the deacetylated product in quantity,experiments were undertaken in which aqueous caustic soda was used as the hydrolytic agent,but the problem was complicated by the significant fact that the deacetylated product was soluble in water.A separation of this soluble product,even if simplified by the use of barium hydroxide in place of sodium hydroxide as the hydrolytic agent,would involve a tedious and troublesome series of operations,and in order to avoid this,if possible,attempts were made to methylate the acetylated product directly by means of dimethyl sulphate and sodium hydroxide.It should be noted,however,that in addition to the main product of hydrolysiswhich was soluble in water,small amounts of insoluble material were isolated,which were shewn to correspond empirically with the formula $(C_6H_{10}O_5)_x$,and which undoubtedly represent higher stages in the degradation of the cellulose molecule.

THE DIRECT METHYLATION OF THE PRODUCT OF ACETOLYSIS.

The direct methylation of the acetylated product involved the elimination of the acetyl groups and their partial subsequent replacement with methoxyl in one operation,and for this purpose,the use of dimethyl sulphate and sodium hydroxide appeared to be eminently suitable.The experimental conditions which were adopted are fully described in the experimental part of this thesis,and the method proved to be quite successful in the case under discussion,but it is of interest to note that analogous experiments,carried out with glucose pentacetate,gave negative results.It would therefore appear that the experimental conditions must be carefully regulated to suit the specific characteristics of the particular acetate under examination,and the method cannot be claimed as having a general application in the preparation of methylated bodies from their corresponding acetates.

In the present case,the product which was isolated after the initial treatment with dimethyl sulphate and sodium hydroxide possessed a methoxyl content of 38.1 per cent,a fact which in itself indicated

that the material had undergone considerable degradation during acetolysis,as this figure is considerably higher than that required for a dimethyl cellulose. The partially methylated product was now subjected to three successive methylations and the methoxyl content was thereby increased to 45.6 per cent.After five additional methylations the methoxyl content was 45.8 per cent,and from this it was inferred that the experimental limit of methylation had been reached. The fully methylated product was a thick cloudy syrup which rapidly set to a hard glass on cooling,and which was separated into two constituents by means of repeated extractions with boiling ether.

The major part of the product,(94 per cent), was soluble in ether,and on removal of the solvent, was obtained as a beautiful clear golden syrup which again set to a hard glass on cooling.The residue, (6 per cent),which was insoluble in ether,was obtained as a faintly-coloured powder,and possessed a methoxyl content of 43 per cent.It appeared probable that this insoluble residue corresponded with the small amounts of insoluble material which were isolated after the hydrolysis of the acetylated material with sodium

hydroxide,and this view receives support from the fact that such dextrin-like bodies as were there described, would yield on methylation trimethyl dextrins with a methoxyl content of 45.6 per cent.Attention was therefore concentrated on the main or ether-soluble product which was systematically examined.

The alkylated product was readily crushed to give a fine white hygroscopic powder,and was easily soluble in benzene,chloroform and ether at the ordinary temperature.It did not reduce Fehling's Solution,and no condensation took place when a solution in acetone containing 0.5 per cent of hydrogen chloride was kept at 30° for several hours.The analytical data which were obtained on analysis corresponded closely with the values required for a trimethyl cellulose, with the exception of the methoxyl content which was 46.2 per cent,as compared with 45.6 per cent for a trimethyl cellulose.

In order to gain more accurate evidence as to the nature of the alkylated product,it was converted into the corresponding methylated glucoside or glucosides by treatment with acid methyl alcohol,which effects hydrolysis and condensation of the products

with the solvent.The product of the reaction was fractionally distilled in vacuo,and analyses shewed that it corresponded empirically with a mixture containing 90 per cent of a trimethyl methylglucoside, and 10 per cent of a tetramethyl methylglucoside.

The mixture of methylglucosides was hydrolysed to the corresponding methylated glucoses by boiling with aqueous hydrochloric acid,and the product of hydrolysis was separated into two constituents,one of which was identified as crystalline 2:3:6-trimethylglucose,and the other as 2:3:5:6-tetramethyl-glucose.No trace of any other methylated glucose was detected.The amount of tetramethylglucose which best coincides with the analytical data previously obtained is 10 per cent,while 7 per cent was actually isolated in a state of purity.

In confirmation of the above results,the methylated product was hydrolysed directly to the methylated sugars by means of aqueous hydrochloric acid, and the product of hydrolysis gave,on analysis,figures which corresponded with a mixture containing 90 per cent of trimethylglucose and 10 per cent of tetramethyl-glucose.

DISCUSSION OF THE RESULTS.

The constitutional nature of the methylated product may now be discussed in the light of the evidence that it consists of 2:3:6-trimethylglucose residues to the extent of 90 per cent, and of 2:3:5:6-tetramethylglucose residues to the extent of 10 per cent.

The presence of tetramethylglucose naturally brings an added complication into the problem, and the theoretical possibilities are correspondingly increased. If the methylated product is a chemical entity, it must exist as an open-chain polysaccharide containing ten glucose residues, in which the nine oxygen-bridge linkages are through the 1 and 5 positions in the glucose molecules. The accumulated evidence, however, points distinctly to the idea that the methylated product is a mixture of at least two compounds, and the above very improbable case may therefore be dismissed without farther discussion, but it is of interest as shewing the maximum size of a molecule of which the tetramethylglucose may be a part.

It must be remembered, moreover, that the methylated product was primarily derived by the acetolysis of cellulose, in which case the possibility

that the tetramethylglucose was primarily derived from glucose pentacetate or cellobiose octacetate immediately suggests itself.This possibility is discounted on the following grounds.

(A) No trace of crystalline material was detected in the product of acetolysis.This is admittedly a qualitative statement and is only quoted as confirmatory evidence.

(B) It is highly improbable that either glucose pentacetate or cellobiose octacetate would survive the particular method of methylation which was adopted in the present series of experiments.This has been demonstrated by the writer in the case of glucose pentacetate,and by another worker in the case of cellobiose octacetate.

(C) If,however,glucose pentacetate and cellobiose octacetate were in part to survive the direct methylation as partially methylated glucose and cellobiose respectively, such traces would be eliminated during subsequent methylation experiments.It was found that after two methylations the partially methylated product separated from the alkaline reaction mixture as a pasty mass,

which could be filtered off,and the extraction of the reaction mixture with chloroform was therefore unnecessary.As partially methylated glucose and cellobiose would be soluble in the reaction mixture, and as the process of filtration was carried out in at least five methylation experiments,it follows that only the merest traces of such compounds could be present in the final methylated product.

With the elimination of glucose and cellobiose the number of possible explanations is very appreciably decreased.It is now clear that the tetramethylglucose must form part of an open-chain polysaccharide containing three,four,five,six,seven,eight or nine glucose residues,in which the oxygen-bridge linkages are through the 1 and 5 positions,since these positions are unaffected by methylation.It also follows that if this compound is a trisaccharide,30 per cent of the total methylated product is accounted for,if a tetrasaccharide,40 per cent,if a pentasaccharide,50 per cent and so on.

The fully methylated product yielded only two methylated glucoses on hydrolysis,and as the

tetramethylglucose has now been disposed of,it follows that the other component or components in the mixture are composed entirely of 2:3:6-trimethylglucose residues.The only possible structure for such compounds is that of a poly-trimethyl-anhydroglucose in which the oxygen-bridge linkages are through the 1 and 5 positions.A large number of possibilities exist. Although the presence of octamethyl cellobiose has been disproved,it does not follow that the corresponding di(trimethyl-anhydroglucose) is also absent,but this possibility is considered remote,in view of the fact that an open-chain polysaccharide containing more than two glucose residues is obviously present.There may therefore be in admixture with the open-chain polysaccharide, any one of the following compounds, or there may be a complex mixture of them or of their polymers.

A tri(trimethyl-anhydroglucose),

A tetra(trimethyl-anhydroglucose),

A penta(trimethyl-anhydroglucose),

A hexa(trimethyl-anhydroglucose), etc.

The following table gives a few of the simplest possible mixtures,all of which give practically identical

analytical data,and all of which agree empirically with the results obtained throughout the investigation.
(I) A mixture containing 70 per cent of a tri(trimethyl-anhydroglucose),and 30 per cent of a methylated trisaccharide.(Molecular Weight approximately 625)
(II) A mixture containing 60 per cent of a tetra(trimethyl-anhydroglucose),and 40 per cent of a methylated tetrasaccharide.(Molecular Weight approximately 834).
(III) A mixture containing 50 per cent of a penta-(trimethyl-anhydroglucose),and 50 per cent of a methylated pentasaccharide.(Molecular Weight approximately 1043).
(IIII) A mixture containing 40 per cent of a hexa-(trimethyl-anhydroglucose),and 60 per cent of a methylated hexasaccharide.(Molecular Weight approximately 1251).

The above are quoted merely as examples of mixtures which satisfy the entire range of analytical data,and it is thoroughly understood that the list may be extended to include a very large number of possibilities.It naturally followed that no inference could be drawn from analytical data,and it became necessary to determine the order of the molecular weight of the mixture.A series of molecular weight determinations,

which were carried out by two distinct methods, gave a long series of very consistent results, and an average value of 658. A mixture containing 70 per cent of tri(trimethyl-anhydroglucose), and 30 per cent of a methylated trisaccharide, (No. I in the above table), would give an average value of 625, and this is the only one of the many possibilities which gives a molecular weight approximating to the figure obtained. The following synopsis shews how well the hypothesis formulated above fits in with the analytical data obtained throughout the investigation.

A mixture containing

{70% Tri(trimethyl-anhydroglucose) and 30% Methylated Trisaccharide.}

Requires:- C = 52.9%, H = 7.86%, CH_3O = 47.4%

Found:- C = 52.7%, H = 8.00%, CH_3O = 46.2%

↓

A mixture containing

{90% Trimethyl-methylglucoside and 10% Tetramethyl-methylglucoside}

Requires:- C = 51.04%, H = 8.50%, CH_3O = 53.5%

Found:- C = 50.95%, H = 8.60%, CH_3O = 52.8%

↓

A mixture containing

{90% Trimethylglucose and 10% Tetramethylglucose}

Requires:- C = 48.87%, H = 8.15%, CH_3O = 42.9%

Found:- C = 48.90%, H = 8.32%, CH_3O = 41.1%

↙ ↘

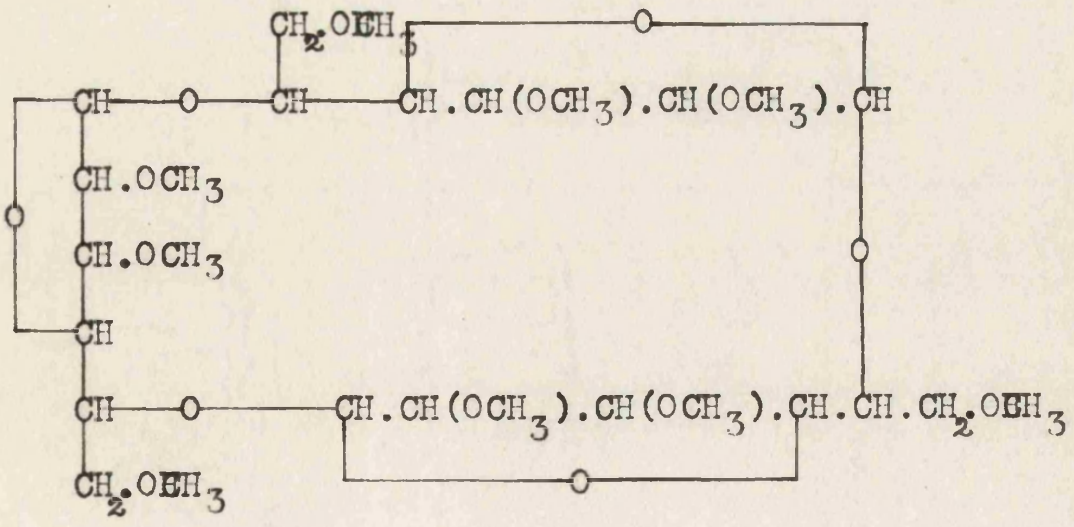
2:3:6-Trimethylglucose

(84% Actually Isolated.)

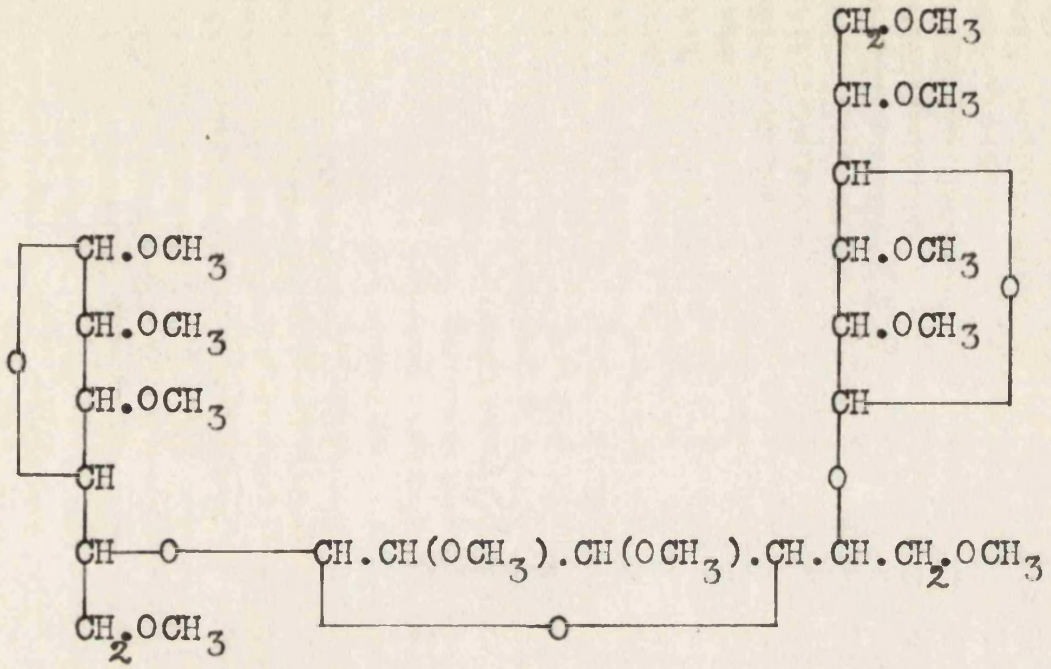
2:3:5:6-Tetramethylglucose

(7% Actually Isolated.)

. The methylated product therefore appears to be a mixture of tri(trimethyl-anhydroglucose) and the corresponding methylated trisaccharide, which is formed by the rupture of one of the oxygen-bridge linkages. The internal structure of such a trianhydro-glucose has been fully dealt with by Irvine and Hirst, (T., 1923, 123, 518), and their conclusions have been summarised in the introduction to this thesis.
The most probable structure for the tri(trimethyl-anhydroglucose) described above is therefore,



while the methylated trisaccharide would have the following structure.



It is desired to stress the fact that the conclusions which are drawn from this investigation are based entirely on the constitution of the methylated product, but it is now possible to advance an opinion concerning the nature of the product of acetolysis. The compounds which appear in the methylated product must have been present as their corresponding acetyl derivatives in the product of acetolysis, which may therefore be said to consist essentially of tri(triacetyl-anhydroglucose) and the corresponding acetylated trisaccharide. Evidence has also been described which points to the presence of small quantities

of cellulose dextrin acetates,and these conclusions are confirmed by the results obtained on analysis.

The present investigation therefore points to the idea that the carefully controlled acetolysis of cotton cellulose may be arrested at a stage when the main product of the reaction is the fully acetylated derivative of trianhydroglucose.It is true that neither trianhydroglucose nor its methylated derivative have been prepared in a state of chemical purity,but the evidence which has been adduced in favour of their existence is incapable of a satisfactory alternative explanation.

This leads directly to the idea that the structural basis or "molecular unit" of the complex cellulose molecule is a trianhydroglucose,in which the oxygen-bridge linkages are through the 1 and 5 positions in the three glucose molecules.The existence of hexa-amylose may be cited as an argument against such a proposal,but this is counterbalanced by the fact that Pictet,(Helv.Chim.Acta,1922,5,640),has isolated a compound of the formula $(C_6H_{10}O_5)_3$ from starch, while Pringsheim,(Ber.,1922,55,1414),claims to have isolated trifructose sodium,$(C_6H_{10}O_5)_3$,NaOH,from inulin.

Moreover, it appears rather improbable that a trianhydroglucose would result as the scission product of a hexanhydroglucose, and the conception of trianhydroglucose as the structural basis of cellulose is offered as the best explanation of the results recorded in the present investigation.

PART TWO

EXPERIMENTAL.

THE ACETOLYSIS OF COTTON CELLULOSE.

The following experiments were undertaken with the object of ascertaining the most suitable conditions of time, temperature, and reagent concentration, under which the acetolysis of cotton cellulose would proceed slowly and smoothly to give the maximum possible degradation of the molecule without the formation of cellobiose octacetate. From the general course of acetolysis experiments which were performed with esparto cellulose, it appeared probable that suitable conditions might be obtained by the use of a mixture containing acetic anhydride and sulphuric acid in the ratio of 5.9 parts by weight to 1 part by weight, provided that the reaction was carried out at 15°.

In the first experiment, five grams of cotton cellulose, which had previously been dried for several hours at 110°, were added to a mixture containing 50cc. of acetic anhydride and 5cc. of sulphuric acid at 15°. Precautions against local heating during the initial stages of the reaction were taken, and the cellulose

dissolved slowly and completely in 24 hours to give a golden-yellow solution.The solution was poured into an excess of distilled water,and a white powder was precipitated.The precipitate was thoroughly washed and dried,and examination shewed it to be soluble in chloroform,but only very slightly soluble in hot rectified spirit.

The experiment was now repeated,but in this case 96 hours were allowed to elapse before the solution was poured into water.A white powder,which displayed a tendency to coagulate to a plastic mass,was precipitated, and it proved to be completely soluble in hot rectified spirit.

A farther repetition of the experiment was carried out and 144 hours were allowed to elapse before the solution was poured into water.In this case the solid product of acetolysis was again completely soluble in hot rectified spirit,but the yield was very small and the major part of the cellulose had apparently been transformed into products soluble in water,probably acetosulphates of cellobiose or glucose.

From the results of the three experiments described above,it appeared likely that the conditions

of acetolysis which would lead to the maximum degradation of the cellulose molecule,and yet stop short of the rupture necessary for the formation of cellobiose octacetate,were represented in the second experiment. The solid material obtained in this experiment was therefore examined,and it proved to be an amorphous powder in which microscopic examination failed to discover any trace of crystalline matter.It shewed a fairly wide range of solubilities,being readily soluble in acetone,chloroform and hot rectified spirit,and rather sparingly soluble in benzene.It was farther characterised by a melting point,and elementary analysis shewed that it corresponded empirically with a triacetate of cellulose.Acetolysis experiments were now carried out on a larger scale and the procedure was standardised as follows.

40 grams of cellulose,which had previously been dried at a temperature of 110°,were added to a mixture containing 400cc.of acetic anhydride and 40cc.of sulphuric acid at 15°.It was not found necessary to prepare the cellulose in an extremely fine state of division,but precautions against local heating in the initial stages of the reaction were invariably required to prevent charring.

This was carried out by immersing the jar containing the reaction mixture in cold water and by frequent stirring of the pasty mass until the reaction had been in progress for some five hours.Under such conditions, the cellulose was noticably affected in about an hour and a half.It dissolved very slowly to give a pulpy mass,which,in the course of 24 hours,gave place to a clear but faintly yellow-coloured solution.In the subsequent 72 hours which were allowed to elapse before the solution was poured into water,the colouration deepened to orange-red,but the solution remained perfectly clear.On pouring the solution into an excess of water, (about four litres were used),a white powder was immediately precipitated.This powder shewed a distinct tendency to coagulate,but was disintegrated to a fine flaky powder by grinding with a pestle after farther dilution with water.After filtering through calico,the solid product was thoroughly washed and slowly dried at a temperature of 30°-40°.In order to remove minute traces of acetic acid which still persisted,the dry powder was dissolved in hot rectified spirit,reprecipitated by the addition of distilled water,and again washed thoroughly. The average yield of the solid product of acetolysis was 50 grams from 40 grams of cellulose.

EXAMINATION AND ANALYSIS OF THE SOLID PRODUCT OF ACETOLYSIS.

It was clearly necessary to make certain that the product did not contain sulphur in the combined condition and several qualitative tests gave entirely negative results.

Form. A small quantity was dissolved in hot rectified spirit and a drop of the solution, placed on a glass slide and covered with a cover-slip, was examined under the microscope. In previous experiments it had been found possible to detect quite small amounts of cellobiose octacetate by this method, but in the present instance no trace of crystalline matter was detected. The experiment was repeated several times with similar results, the product in all cases precipitating as an amorphous powder.

Solubility. The product was only slightly soluble in hot absolute ethyl alcohol, but readily soluble in hot rectified spirit. It was readily soluble in acetic acid, acetone and chloroform, and sparingly soluble in benzene at the ordinary temperature. In connection with solubility in acetone, it was interesting to note that when a solution was allowed to evaporate on a watch-glass, a film was not formed.

Moisture. 0.6311 grams of air dried material shewed a

decrease of 0.0085 grams after heating at 110° until a constant weight was recorded.

Moisture = 1.35%

Ash. The slight ash which was obtained by incinerating 0.6226 grams in a platinum crucible was found to be unweighable and a second experiment confirmed this result.

Melting Point.

The melting point of the degraded material was not constant, but varied between 120° and 160° in different preparations of the acetylated product. This points distinctly to a complex mixture.

Specific Rotation.

Variation was also noted in the specific rotation of material from different preparations and values ranging from +11° to +19° in chloroform solution were recorded. It was also noted that a higher positive rotation was always displayed in acetone solution than in chloroform solution.

Acetyl Content.

The finely-powdered product was suspended in an excess of 0.21N sodium hydroxide solution and the mixture was kept in a stoppered flask with occasional shaking for 72 hours. The material dissolved almost completely with

the development of a faint yellow colour.The acetyl content was estimated by difference,after titration of the residual sodium hydroxide with standard hydrochloric acid.

(a) 0.505 grams neutralised 25.9cc.of 0.21 N NaOH.

Acetyl = 64.6%

(b) 0.7034 grams neutralised 38.85cc.of 0.21 N NaOH.

Acetyl = 64.2%

Carbon and Hydrogen.

0.1659 grams,(dried in vacuo at 105°),gave 0.0864 grams of water and 0.3034 grams of carbon dioxide.

C = 49.87% H = 5.79%

A triacetate of cellulose requires:-

C = 50.00% H = 5.55%

and Acetyl = 62.5%

The Ultramicroscopic Examination of Solutions of Cellulose Acetates in Chloroform.

Specimens of normal cotton cellulose triacetate and the degraded product of acetolysis were dissolved in chloroform and examined under the ultramicroscope. In the case of the normal triacetate a distinct cone of light was visible and there was no doubt that the solution was colloidal.On the other hand,when a solution

of the degraded acetate was examined in the same way, only a very faint cone of light was visible, and there appeared to be a tendency towards true molecular solution. While such observations are admittedly of a very qualitative nature, they are by no means without interest, and at least give a fair indication that considerable degradation of the cellulose molecule has taken place in the case of the product under discussion.

Experiments with Fehling's Solution.

The degraded material reduced Fehling's Solution at the boiling point. In view of this fact two quantitative experiments were carried out.

(a) A weighed quantity of the degraded material was dusted into an excess of Fehling's Solution at the boiling point and the mixture was boiled for four minutes. The cuprous oxide was immediately filtered off, washed with water, alcohol and ether, and dried in a steam-oven. 0.3289 grams gave 0.1467 grams of cuprous oxide.

(b) A weighed quantity of the degraded material was dissolved in 60 per cent alcohol which contained 8 per cent of hydrogen chloride and the solution was boiled under a condenser for four hours. The major part of the

alcohol was now removed by distillation,and was replaced by aqueous 8 per cent hydrochloric acid.The mixture was again boiled and after eight hours only a very slight residue remained and the solution was practically colourless.After careful neutralisation the solution was added to an excess of Fehling's Solution at the boiling point,and the precipitated cuprous oxide was collected and weighed as before.

0.3289 grams gave 0.325 grams of cuprous oxide.

The ratio of the copper reduction figure after acid hydrolysis to the figure before acid hydrolysis is as 2.2 is to 1.

Fractional Precipitation of the Product of Acetolysis.

The material under examination melted at 125° and shewed $(\alpha)_D$=+19.

16.5 grams were dissolved in 250cc.of hot rectified spirit. Part of the material precipitated spontaneously from the solution on cooling and constituted fraction (1), 12 grams.

On adding distilled water to the mother liquors,270cc. were added before a farther permanent precipitate appeared.350cc.of water were added at this stage,and

after standing over-night the solid which had precipitated was filtered and dried. Fraction (2), 4 grams.

Fraction (1).

The melting point was indefinite, 130°-140°.

Fehling's Solution was reduced at the boiling point.

A solution in chloroform shewed $(\alpha)_D$ = +20'.3.

Analysis gave C = 49'.6% and H = 5'.75%

Fraction (2).

The melting point was indefinite, 140°-148°.

Fehling's Solution was vigorously reduced at the boiling point.

A solution in chloroform shewed $(\alpha)_D$ = +19'.4.

Analysis gave C = 49.6% and H = 5'.61%

The above experiments yielded no information beyond the fact that the degraded material was a mixture.

HYDROLYSIS OF THE DEGRADED PRODUCT.

4.8 grams were digested with an excess of 2-Normal sodium hydroxide solution and the temperature of the mixture was maintained at 25° by means of a water-bath. After three hours the acetylated product had completely dissolved and the solution was faint yellow in colour. The solution was carefully neutralised with hydrochloric

acid,and a white flocculent precipitate was obtained which rapidly darkened on washing and drying in vacuo. The precipitate weighed 0.4 grams.The main product of the reaction was soluble in water,and instead of attempting its isolation,experiments on the direct methylation of the acetylated product were undertaken.

The solid material which was isolated was, however,examined,and proved to correspond empirically with the formula $(C_6H_{10}O_5)_x$.The washed and dried material was glassy in appearance and was easily crushed to a powder which shewed signs of decomposition on heating to 220°.It was insoluble in all simple organic solvents, but was soluble in sodium hydroxide solution and in concentrated sulphuric acid.

Carbon and Hydrogen.

0.1464 grams gave 0.0848 grams of water and 0.2391 grams of carbon dioxide.

C = 44.5% H = 6.4%

$(C_6H_{10}O_5)_x$ Requires:- C = 44.4% H = 6.2%

It would therefore appear that this material is composed of cellulose dextrins and probably represents higher intermediate stages in the break-down of the cellulose molecule during acetolysis.

DIRECT METHYLATION OF THE DEGRADED PRODUCT.

In a preliminary experiment, 15 grams of the degraded material were suspended in 75cc. of 2-Normal sodium hydroxide solution, and thorough disintegration was ensured by means of rapid mechanical stirring. The temperature of the mixture was maintained at 30° by means of a water-bath and small equivalent quantities of dimethyl sulphate and 30 per cent sodium hydroxide solution were added at short intervals. When the major part, (three-fourths), of the reagents had been added, the temperature was gradually raised to 70°, and the remainder of the reagents was added at this temperature. In all, 40cc. of dimethyl sulphate and 40 grams of sodium hydroxide in 60cc. of water were used. The temperature was there-after raised to 100° for a little over half-an-hour, and after cooling, the reaction product was found to be entirely soluble in the cold reaction mixture, which was therefore extracted thoroughly with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and on distilling away the chloroform, 2.7 grams of a clear syrup were obtained, which, on cooling, rapidly set to a hard glass. (It should be noted that the 15 grams of starting material contained 64.0 per cent of acetic

acid and that only 6 grams of deacetylated material were available for methylation.)

A Zeisel estimation was carried out on the product obtained in the above experiment and it proved to have a methoxyl content of 38.1 per cent.The methylation of the degraded product was now carried out on a larger scale by the method outlined above.

In the first large scale experiment 46 grams of the degraded product,(i.e.27 grams of deacetylated material),gave 14 grams of the partially methylated product.....(A).

In a second similar experiment,13.2 grams of the partially methylated product were obtained.....(B).

The products (A) and (B) were now united and after three methylations,each of which was carried out with 60cc.of dimethyl sulphate and 60 grams of sodium hydroxide ~~solution~~ in 100cc.of water,24 grams of the methylated product (C) were isolated.During these experiments it was noted that an increase in the methoxyl content had the effect of rendering the methylated product insoluble in alkali,and it could be filtered from the reaction mixture as a pasty mass.

Zeisel estimation on Product (C).

0.1182 grams gave 0.4086 grams of AgI.

CH_3O = 45.6%

This value corresponds with the methoxyl content of a trimethyl cellulose, but as the results obtained from analyses of the acetylated product pointed to the idea that the degraded material was a mixture, it was considered necessary to subject the product to still farther methylations. Accordingly, the product (C) was subjected to five additional methylations, each of which was carried out with 25cc. of dimethyl sulphate and 25 grams of sodium hydroxide in 50cc. of water, after which 19.8 grams of a methylated product (D) were isolated.

Note:- In subsequent experiments it was found to be practically impossible to free the methylated product from chloroform by heating under diminished pressure at 100°, and the yields which are quoted above are therefore slightly higher than the true values.

Zeisel estimation on Product (D).

0.0667 grams gave 0.2316 grams of AgI.

CH_3O = 45.8%

From this result it appeared that the experimental limit of methylation had been reached.The final product (D) was now repeatedly extracted with pure dry ether and by this means it was separated into two constituents.One portion,which amounted to 94 per cent of the total material,was soluble in ether,while the portion represented by the remaining 6 per cent was insoluble in ether.A Zeisel estimation was carried out on the insoluble residue,from which it was proved to have a methoxyl content of 43 per cent.It therefore appeared probable that the insoluble residue represented higher intermediate stages in the degradation of the cellulose molecule,and attention was accordingly turned to the portion soluble in ether which represented the main product of the reaction.

THE EXAMINATION AND ANALYSIS OF THE METHYLATED PRODUCT.

The methylated product is a clear hard glass-like substance which is easily ground to a fine white hygroscopic powder, and is converted into a thick syrup on heating to about 40°.

Solubility.

It is easily soluble in ether, chloroform and benzene at the ordinary temperature and less soluble in methyl alcohol and rectified spirit. On attempting to dissolve it in water a cloudy solution was obtained.

Specific Rotation.

(a) 0.3564 grams, dissolved in 10cc. of chloroform, were examined in a 1-d.m.tube.

$$(\alpha)_D^{15^\circ} = +7^\circ$$

(b) 0.1682 grams, dissolved in 10cc. of benzene, were examined in a 1-d.m.tube.

$$(\alpha)_D^{15^\circ} = +10.1^\circ$$

Action on Fehling's Solution.

The substance did not reduce Fehling's Solution, even on boiling the mixture for several minutes.

Action of Acetone containing 0.5% Hydrogen Chloride.

0.5051 grams of the methylated product were dissolved in 15 cc. of acetone which contained 0.48 per cent of

hydrogen chloride.The solution was transferred to a jacketed 2-decimeter polarimeter tube and the temperature was maintained at 30° by means of a thermostat. Polarimetric readings were taken every hour.

Initial Reading α = + 0.53

After one hour α = + 0.53

After two hours α = + 0.54

After three hours α = + 0.53

After four hours α = + 0.53

After five hours α = + 0.54

$$(\alpha)_D^{30} = + 15.7°$$

This experiment proved that no free reducing group was present in the methylated product,as condensation with the acetone and a corresponding change in rotation would then have resulted.

Zeisel.

0.0934 grams gave 0.3278 grams of AgI.

$$CH_3O = 46.2\%$$

A mixture containing 70 per cent of tri(trimethyl-anhydroglucose),and 30 per cent of the corresponding methylated trisaccharide requires $CH_3O = 47.4\%$

Note:- The evidence accumulated in subsequent experiments points to the idea that we are in fact dealing with a mixture of the composition indicated above.

Carbon and Hydrogen.

0.1808 grams gave 0.1328 grams of water and 0.3490 grams of carbon dioxide.

C = 52.7% H = 8.0%

The mixture quoted above requires:- C = 52.9% H = 7.86%

SIMULTANEOUS HYDROLYSIS AND CONDENSATION OF THE METHYLATED PRODUCT WITH ACID METHYL ALCOHOL.

4.3552 grams of the methylated product were heated in a sealed tube with 50cc.of methyl alcohol which contained 1 per cent of hydrogen chloride,for 70 hours at 110°, after which time there was no residue and the solution was pale golden-yellow in colour.The solution was neutralised with silver carbonate,and after filtration, evaporated to dryness in a tared flask,when 4.562 grams of a colourless syrup were obtained.The yield,calculated on the assumption that we are dealing with a mixture of the composition already indicated,is 92 per cent of the theoretical maximum.

The syrup was distilled in vacuo and the following fractions were obtained.

(a) 0.48 grams B.P.113°/0.3mm. $n_D^{15^\circ}$ = 1.4502

(b) 3.39 grams B.P.115°/0.3mm. $n_D^{15^\circ}$ = 1.4570

ANALYSIS OF THE PRODUCT OF HYDROLYSIS.

On the basis postulated above, i.e., that the methylated product is a mixture containing 70 per cent of a tri(trimethyl-anhydroglucose), and 30 per cent of the corresponding methylated trisaccharide, the product obtained on hydrolysis ought to consist of a mixture containing 90 per cent of trimethyl methylglucoside and 10 per cent of tetramethyl methylglucoside.

Analysis of Fraction (a).

Zeisel.

(1) 0.0545 grams gave 0.2139 grams of AgI.

$CH_3O = 51.8\%$

(2) 0.0810 grams gave 0.3286 grams of AgI.

$CH_3O = 53.5\%$

Mean of (1) and (2) $CH_3O = 52.6\%$

The postulated mixture has $CH_3O = 53.5\%$

Carbon and Hydrogen.

0.1482 grams gave 0.1107 grams of water and 0.2768 grams of carbon dioxide.

$C = 50.93\%$ $H = 8.3\%$

The postulated mixture has $C = 51.04\%$ $H = 8.5\%$

Analysis of Fraction (b).

Zeisel.

0.0708 grams gave 0.2833 grams of AgI.

CH_3O = 52.8%

Required:- CH_3O = 53.6%

Carbon and Hydrogen.

0.1842 grams gave 0.1438 grams of water and0.3441 grams of carbon dioxide.

Found:- C = 50.95% H = 8.6%

Required:- C = 51.04% H = 8.5%

Fractions (a) and (b) were therefore identical in composition.

HYDROLYSIS OF THE MIXTURE OF TRIMETHYL AND TETRAMETHYL METHYLGLUCOSIDES WITH 8 PER CENT AQUEOUS HYDROCHLORIC ACID.

2.67 grams of syrup,(i.e.,the remainder of the distilled product after analyses had been carried out),were dissolved in 110cc.of 8 per cent hydrochloric acid,and a small quantity of charcoal was added to prevent the development of colour during hydrolysis.The solution was boiled under a condenser for 45 minutes,and the rotation was then observed in a 1-decimeter tube.

α = + 2.11

On boiling the solution for an additional 15 minutes, the observed rotation was α = + 1.80
The solution was again boiled for 45 minutes, after which the observed rotation was α = + 1.75
The constant rotation observed indicated that the hydrolysis was complete and the solution was accordingly neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure. The mixture of sugars was extracted from the solid barium chloride by repeated extractions with boiling acetone. The acetone was distilled away and the resulting syrup was redissolved in dry ether, and after filtration, the ether was in turn evaporated. The syrup which resulted from this treatment weighed 2.2 grams and on nucleation with 2:3:6-trimethylglucose, partially crystallised. The yield, calculated on the assumption that we are dealing with the hydrolysis of a mixture containing 90 per cent of trimethyl methylglucoside and 10 per cent of tetramethyl methylglucoside, is 87.6 per cent of the theoretical maximum.

SEPARATION OF THE CONSTITUENT SUGARS FROM THE MIXTURE.

As the evidence from analysis pointed to the idea that the syrup consisted of a mixture of a tetramethyl glucose and a trimethyl glucose, the syrup, which weighed 2.2 grams, was dissolved in water and the aqueous solution was thoroughly extracted with chloroform.

The aqueous solution was thereafter evaporated to dryness under diminished pressure and the resulting syrup was dissolved in absolute alcohol which was in turn evaporated. Finally, the syrup was dissolved in pure dry ether, and after filtration, the ether was slowly evaporated. The resulting syrup, on nucleation with 2:3:6-trimethyl glucose, solidified completely to a hard mass of crystals which weighed 1.85 grams.

The chloroform extract was dried over anhydrous sodium sulphate, and after distilling off the chloroform, 0.25 grams of a syrup were obtained which partly crystallised on standing.

The crystalline material obtained from the aqueous solution was identified as 2:3:6-trimethyl glucose by a study of its physical constants and by analysis.

Melting Point. The substance melted at 105°.

Specific Rotation.

0.1058 grams, dissolved in 10cc. of water, were examined in a 1-decimeter tube.

After 1 hour $(\alpha)_D^{15} = + 83.2^\circ$

After 60 hours $(\alpha)_D^{15'} = + 73.7^\circ$

After 108 hours $(\alpha)_D^{15'} = + 71.8^\circ$ (constant)

N.B. No catalyst was employed.

This is in agreement with the standard value for 2:3:6-trimethyl glucose, which is $(\alpha)_D^{15'} = + 72.^\circ$

Carbon and Hydrogen.

0.1501 grams gave 0.1088 grams of water and 0.2668 grams of carbon dioxide.

Found:- C = 48.5% H = 8.05%

2:3:6-trimethyl glucose requires:- C = 48.65% H = 8.11%

Zeisel.

0.0661 grams gave 0.2083 grams of AgI.

CH_3O = 41.6%

2:3:6-trimethyl glucose requires:- CH_3O = 41.9%

The other constituent, which was suspected to be tetramethyl glucose was dissolved in boiling petroleum ether, and from this solution, 0.1606 grams of crystalline material were obtained. This crystalline material was identified by melting point and mixed melting point

as 2:3:5:6-tetramethyl glucose.

A genuine specimen of 2:3:5:6-tetramethyl glucose melted at 88°-89°.

The material under examination melted at 87°-88°.

A mixture of the two melted at 88°-89°.

The amount of 2:3:6-trimethyl glucose which was isolated in a state of analytical purity was 84 per cent of the total product of hydrolysis, while the amount of 2:3:5:6-tetramethyl glucose was 7 per cent.

HYDROLYSIS OF THE METHYLATED PRODUCT WITH AQUEOUS HYDROCHLORIC ACID.

2.45 grams were dissolved in 50cc. of 8 per cent hydrochloric acid, and the mixture was boiled until a constant rotation was recorded.

After 10 minutes α = + 2.69

" 20 " α = + 3.41

" 30 " α = + 3.41 (1-dm. Tube)

After neutralisation with barium carbonate the solution was evaporated to dryness under diminished pressure and the solid residue of barium chloride was twice extracted with boiling acetone. The acetone was distilled off and the product was dissolved in boiling ether. After

filtration and removal of the solvent, a clear syrup was obtained and was analysed.

Zeisel.

0.1789 grams gave 0.5567 grams of AgI.

$CH_3O = 41.1\%$

90% Trimethyl glucose
10% Tetramethyl glucose } $CH_3O = 42.9\%$

Carbon and Hydrogen.

0.1815 grams gave 0.1364 grams of water and 0.3258 grams of carbon dioxide.

$C = 48.9\%$ $H = 8.32\%$

90% Trimethyl glucose
10% Tetramethyl glucose } $C = 48.87\%$ $H = 8.15\%$

MOLECULAR WEIGHT OF THE METHYLATED PRODUCT.

In the first series of experiments, the molecular weight was determined by the method of Rast, in which camphor is used as the solvent. A few milligrams of the substance under examination are fused with from 10 to 20 times their weight of camphor, and the melting point of the solidified "melt-cake" is determined. As the molecular depression constant for camphor is 40°, depressions of several degrees may be obtained and a Beckmann thermometer is unnecessary.

(1) 0.0068 grams in 0.0570 grams of camphor.

M.P. of pure camphor was 175.8°.

M.P. of the mixture was 167.8°.

Depression = 8°.

Molecular Weight = 596

(2) 0.0045 grams in 0.0632 grams of camphor.

M.P. of pure camphor was 175.8°.

M.P. of the mixture was 171.9°.

Depression = 3.9°.

Molecular Weight = 730.

A second series of experiments was carried out by the cryscopic method in which benzene was used as solvent.

(1) Weight of benzene used = 48.93 grams.

The molecular depression constant for benzene is 5000.

(a) 0.1127 grams depressed the freezing point 0.015°.

(b) 0.1202 " " " " " 0.019°.

(c) 0.1585 " " " " " 0.027°.

(a) M = 760. (b) M = 646. (c) M = 600.

(a)+(b) M = 700. (b)+(c) M = 619. (a)(b)(c) M = 656.

Mean Molecular Weight = 663.

As the depressions recorded in the above experiment were very small it was repeated with a greater concentration of material.

(2) Weight of benzene used = 38.55 grams.

0.5394 grams depressed the freezing point 0.109°.

Molecular Weight = 642.

In the above experiment the greatest precautions against super-cooling were observed, and it may be remarked that three readings of the freezing point for the pure solvent were identical, while three reddings which were taken after the addition of the substance, differed only by 1/1000°.

The average value for the molecular weight over the whole series of experiments is 658, while the approximate molecular weight of a mixture containing 70 per cent of tri(trimethyl-anhydroglucose), and 30 per cent of the corresponding open-chain trisaccharide is 625.

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