XXI.—*A Reinvestigation of the Cellulose-Dextrose Relationship.*

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It has been assumed that in order to determine the structure of the cellulose molecule, an obvious course is to identify quantitatively the products of ultimate hydrolysis and from their known constitution to reconstruct the original complex. Hence the commonly accepted view of (cotton) cellulose as a polydextrose anhydride. Recent researches have, however, cast doubt on such interpretation of the results of hydrolytic resolution, for they have shown that a molecular structure is not necessarily that of its constituents after hydrolysis (Fischer, *Ber.*, 1914, **47**, 1980; Irvine, Fyfe, and Hogg, *T.*, 1915, **107**, 524; Haworth and Law *T.*, 1916, **109**, 1314). Moreover, and particularly as regards cellulose, observations of optical activity and reducing power of the products of resolution have been accepted as unquestionable evidence of identification, whilst the isolation of the substances having the recorded constants has even been regarded as superfluous. It seemed important, therefore, on this basis of criticism to reinvestigate the products formed from cellulose by the action of acids which have usually been regarded as giving maximum resolution, namely, sulphuric acid dihydrate, $\text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$, and hydrochloric acid of more than 40 per cent. concentration.

Two classes of cellulose have been selected for experiment, namely, cotton cellulose as the normal type, and esparto cellulose, which is characterised by a high furfuroid content, resembling in this respect the cereal celluloses, but being more resistant to hydrolysis. In the case of cotton cellulose, particular attention has been paid to the action of 40 per cent. hydrochloric acid, as the behaviour of the normal cellulose to sulphuric acid has already been studied by numerous investigators (compare Flechsig, *Zeitsch. physiol. Chem.*, 1883, **7**, 523; Stern, *T.*, 1895, **67**, 74; and Ost and Wilkening, *Chem. Zeit.*, 1910, **34**, 461). Esparto cellulose has not, however, previously been examined in this respect, but it has now been found that treatment with 73 per cent. sulphuric acid results in the formation of polysaccharose esters, which were usually obtained as barium salts. These compounds bear sufficient relation to the original complex to give a high, although diminished, yield of furfuraldehyde on distillation, but they are distinguished from the cotton cellulose sulphates prepared by Stern (*loc. cit.*), by the action of concentrated sulphuric acid, in having a much smaller

content of inorganic material. In the esparto products, the proportion of barium combined is approximately equal to that of SO_4 , so that the two are present in the ratio $\text{Ba}_2:(\text{SO}_4)_3$. This means that if either neutral or acid sulphates or both are formed with alcoholic hydroxyl groups, then an excess of barium is present which must be in union with hydroxyl groups of a sufficiently acid character to decompose barium carbonate, whilst the acid character of these hydroxyl groups is further emphasised by the stability of the compounds towards carbon dioxide, and this establishes them as genuine barium salts. The SO_4 residues are also in very stable union, as complete precipitation can only be effected by boiling with concentrated hydrochloric acid in the presence of barium chloride for some hours, and although alkaline hydrolysis with barium hydroxide solution greatly increases the barium content, the SO_4 groups are not entirely removed, and it is quite possible that some of them are present in the form of a neutral ester.

As regards hydrolysis by the action of 40 per cent. hydrochloric acid, the claim has been put forward that cotton cellulose under these conditions is quantitatively resolved into dextrose. It must be remembered, however, that this conclusion and the theoretical considerations to which it obviously leads were based, not on the yield of dextrose actually isolated, but, by inference, on the specific rotation and reducing power of a 1 per cent. solution of cellulose in the acid,* the yield of dextrose being calculated from the formula $c = a \times 100/[\alpha]_D$, where $[\alpha]_D$ is the specific rotation of dextrose in 40 per cent. hydrochloric acid (Willstätter and Zechmeister, *Ber.*,

* Willstätter and Zechmeister appear to have overlooked the elaborate investigation of J. König, and F. Hühn, "Bestimmung der Zellulose" (Berlin, 1912). In investigating celluloses isolated by the several hitherto recognised standard methods (Hugo Müller, Cross and Bevan, König, Tollens, Schulze, etc.) they employ the method of solution in the zinc chloride-hydrochloric acid reagent (Cross and Bevan) and polarimetric observations of the solutions. In this reagent, dextrose was found to have a maximum $\alpha_D + 60^\circ$ and xylose $+ 32^\circ$.

Comparing these solutions of the celluloses (derivatives) there is a maximum for α_D of $+ 82.5^\circ$ in the case of cotton cellulose "purified" by the König method; the maxima are very variable over the special range of products investigated.

These authors do not draw the conclusion that dextrose is formed as such, or that the rotations, although apparently specific, are attributable to ultimate groups, namely, hexose (or pentose). What they state is that maximum of rotation is a criterion of maximum "purity" of cellulose. This conclusion has been examined in a communication dealing critically with the whole paper (Cross and Bevan: *Eighth Int. Congr. App. Chem.*, 1912, 13, 101). No doubt attaches to their experimental numbers and, being directly relevant to the issue raised by Willstätter and Zechmeister, they have to be taken into consideration.

1913, 46, 2401). Such data seemed entirely inadequate in view of the fundamental character of the issues involved, and it was therefore decided to repeat the polarimetric observations * and also to isolate the products in solution after definite intervals of

FIG. 1.

Hydrolysis of cotton cellulose with 40 per cent. hydrochloric acid.

time. The changes in optical activity were observed as nearly as possible every 30 minutes for 24 hours, and the results are shown graphically in Figs. 1 and 2. It will be observed from Fig. 1 (cotton cellulose) that there is a continuous increase in

FIG. 2.

Hydrolysis of esparto cellulose with 40 per cent. hydrochloric acid.

activity up to 600 minutes, but then a marked fall occurs, followed by a fairly regular rise to the value $[\alpha]_D + 45.6^\circ$ at the end of 1400 minutes, with a further increase of 10° during the next 13 hours.

* The polarimetric observations were made after dilution, owing to the doubtful condition of sugars when dissolved in strong acids. Mutarotation to the α -form assumed by Willstätter is not a satisfactory explanation of the remarkable increase in activity observed, for dextrose shows in acid containing 44.5 per cent. of hydrogen chloride a specific rotation 60° above that of the α -isomeride, and this suggests more profound constitutional change than merely isomeric transformation.

Esparto cellulose (Fig. 2) showed for the most part similar fluctuations in rotation, but the initial attack of the acid is less delayed, whilst more irregularities occur in the middle portion of the curve, and the final value for the specific rotation is somewhat lower. The hydrolytic solutions were diluted and neutralised at the end of 24 hours, when the esparto solution gave $[\alpha]_D +41.1^\circ$ and the cotton cellulose solution 45.6° , but in the latter case a separation of the products was also made at the end of 48 hours, since the specific rotation had risen to the value 56.5° , which is very nearly that of dextrose calculated according to the equation

The substances so prepared were lead salts of polysaccharose esters similar to the barium salts previously described, the higher molecular proportion of chlorine entering into combination according with the superior esterifying power of hydrochloric acid as compared with sulphuric acid. Dextrose could not be identified in any of the fractions, and, if present, it would appear and be concentrated in the more readily soluble portions, but these show a higher inorganic content and a lower specific rotation. The possibly misleading character of polarimetric observations when unsupported by the full identification of products is also emphasised by the fact that whilst solutions of the very divergent types of cellulose investigated show almost concordant optical activity, the esparto products are furfuroids, and their solution gives, throughout the hydrolysis, high yields of furfuraldehyde with a final loss of only 3 per cent., even if neutralisation is postponed for six days. Further, dextrose is not formed on subsequently heating the salts with dilute acid. Both the sulphates and the chlorides so treated show a fall in optical activity, whilst the products, isolated after the rotation has become constant, contain a diminished quantity of inorganic material, and, in the case of esparto, give a smaller yield of furfuraldehyde on distillation. Evidently, therefore, the main action of the acid is to cause further condensation of the original complex, and not hydrolysis to sugar. The same is even true of a sulphate prepared free from barium by the action of cold sulphuric acid on an alcoholic solution of the salt. It should be mentioned, however, that by the sulphuric acid hydrolysis of the barium salts, residual syrups are obtained which give glucosazones in small yield. The ester salts are further characterised by a marked reducing power and also by showing slight mutarotation which is uniformly downwards. These observations indicate that the compounds are correctly described as ester salts of polysaccharoses, and it is possible that they represent the initial results

of hydrolysis and are not "reversion products" (Ost and Wilkening, *loc. cit.*), as otherwise the fluctuations in optical activity would have been more marked. On the other hand, the main result of these experiments, that no quantitative resolution of cellulose to dextrose is in reality obtainable, reopens the whole question of the structure of the complex. This aspect of the matter is, however, dealt with in the following paper by Messrs. Cross and Bevan.

EXPERIMENTAL.

The Action of Sulphuric Acid Dihydrate, $\text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$, on Esparto Cellulose.—Sixty grams of cellulose, dried at 80° , were mixed with 300 c.c. of 73 per cent. sulphuric acid in a stoppered bottle. The bottle was mechanically shaken for six hours, and the very viscous solution allowed to remain for a further 18 hours, when it was diluted with 2 litres of water. A very small amount of regenerated cellulose was obtained, and the excess of sulphuric acid was removed by the addition of barium carbonate, followed by filtration. The syrup left after concentration of the filtrate was dissolved in 50 per cent. alcohol, and from this solution, after filtering, 37 grams of a white, amorphous product were obtained by the addition of 80 per cent. alcohol, whilst 8 grams of a second fraction were precipitated from the filtrate by means of absolute alcohol. Both fractions were very deliquescent and were only prepared in a solid form with difficulty. Drying operations had to be carried out at 50 – 60° , as charring easily took place, and the excessive swelling which accompanied it rendered elementary analysis impossible.

Fraction I.—Found: $\text{Ba}=8.6$; $\text{SO}_4=7.9$; $\text{C}_5\text{H}_4\text{O}_2$ (furfuraldehyde)=8.3. $[\alpha]_D + 47.8^\circ$ to 45.5° .

Fraction II.—Found: $\text{Ba}=8.6$; $\text{SO}_4=10.6$; $\text{C}_5\text{H}_4\text{O}_2=8.0$ per cent. $[\alpha]_D + 55.3^\circ$ to 51.6° .

Preparation of an Ester Free from Barium.—In attempting to isolate an ester free from barium, it was desired to avoid any further hydrolysis of the original product, and therefore the barium salt remained in acid alcoholic solution for some days in the cold. Barium sulphate was then removed by filtration, and the addition of absolute alcohol to the filtrate gave a white, amorphous precipitate entirely free from ash.

Found: $\text{SO}_4=5.3$; $\text{C}_5\text{H}_4\text{O}_2=6.2$ per cent. $[\alpha]_D + 41.6^\circ$.

Hydrolysis of Barium Salt with 2 per cent. Sulphuric Acid.—An 8.5 per cent. solution of the barium salt was made up in 2.3

per cent. sulphuric acid, and the following changes in optical activity were observed:

	Initial specific rotation	42.3°
After heating at 100° for 20 minutes.....		34.5
" " " " 40 "		32.8
" " " " 100 "		30.8 (constant)

At this stage in the hydrolysis, the acid solution was neutralised with barium carbonate, and after concentrating the filtrate, the syrupy residue was dissolved in 50 per cent. alcohol, and the solution gave a white, amorphous precipitate on the addition of absolute alcohol. The yield only amounted to 60 per cent.

Found: Ba = 5.3; SO₄ = 6.0; C₅H₄O₂ = 5.0 per cent [α]_D + 33.8°.

Hydrolysis of Barium Salt with 5 per cent. Barium Hydroxide Solution.—The barium salt was heated at 100° with the alkali for 7 hours. An immediate precipitation of barium sulphate occurred, but the resulting compound, isolated as usual, still contained a small percentage of SO₄. The barium content was greatly increased, and this probably indicates the presence of hydroxyl groups in the original complex, sufficiently acidic to combine with barium, but not able to decompose barium carbonate. The compound was too dark for polarimetric observations.

Found: Ba = 25.1. SO₄ = 2.4 per cent.

The Action of 40 per cent. Hydrochloric Acid on Cotton Cellulose. (a) *Changes in Optical Activity.*—A 5 per cent. solution of cotton cellulose in 40 per cent. hydrochloric acid was prepared, and the polarimetric readings were taken after the concentration had been lowered to 1 per cent. This gives an acid concentration of 8 per cent., in which, as shown by Willstätter, the specific rotation of dextrose is practically normal. The readings at 15°, from which the foregoing curve was constructed (Fig. 1), are given below.

Minutes.	[α] _D .	Minutes.	[α] _D .	Minutes.	[α] _D .	Minutes.	[α] _D .	Minutes.	[α] _D .
120	3.2°	385	14.8°	635	26.0°	900	28.4°	1245	42.4°
150	3.2	415	16.4	675	22.4	930	29.2	1290	44.0
180	5.6	455	19.2	705	22.4	1010	32.8	1330	44.8
210	6.0	485	20.0	735	25.2	1040	34.8	1375	44.8
240	8.0	515	21.6	765	24.8	1070	37.6	1400	45.6
295	10.4	545	22.4	795	26.4	1100	39.2	1435	45.6
325	11.2	575	24.2	825	27.6	1160	40.8	2250	56.8
355	12.8	605	26.0	870	28.0	1195	41.6		

It will be seen that, except for some irregularities in the period 635 to 825 minutes, the optical activity continuously increases to the final value +56.8° at the end of 2250 minutes. The values for [α]_D are calculated on the original weight of cellulose.

(b) *Yield of Furfuraldehyde*.—At the end of 24 hours, the acid solution was diluted to 12 per cent. and distilled, as in the ordinary procedure for a furfuraldehyde estimation. The yield only amounted to 1·2 per cent., calculated on the original weight of cellulose, so that evidently the high furfuroid content of the esparto solution is due to the persistence of the original complex and not to any specific action of the acid treatment.

(c) *Isolation of the Products formed after Hydrolysis for (1) 24 Hours, (2) 48 Hours*.—When the specific rotation had reached the value $+45\cdot6^\circ$ at the end of 24 hours, the acid solution was diluted to 1 litre and neutralised with lead carbonate. The filtrate was then concentrated under diminished pressure, and the syrupy residue freed from any lead chloride retained as impurity by precipitation with hydrogen sulphide and subsequent neutralisation with silver carbonate. Some lead sulphide was formed, but very little acidity developed, and it is therefore possible that a portion of the precipitated lead was in combination with an organic compound. After further concentration in a vacuum, a clear, colourless syrup was obtained which dissolved readily in 80 per cent. alcohol, and the addition of absolute alcohol precipitated from it 2·5 grams of a white, amorphous, deliquescent compound. A second fraction was obtained from the filtrate amounting to 3·1 grams, and there then remained 1·3 grams of residual syrup, which also contained lead and chlorine. The total yield of products is therefore only 69 per cent.

Fraction I.—Found: Pb = 7·9; Cl = 6·1; $C_5H_4O_2 = 1\cdot08$. $[\alpha]_D + 48\cdot2^\circ$ to $43\cdot0^\circ$.

Fraction II.—Found: Pb = 8·2; Cl = 7·3 per cent. $[\alpha]_D + 46\cdot7^\circ$ to $33\cdot4^\circ$.

(2) *Products Formed after 48 Hours' Hydrolysis*.—The products in solution after the more prolonged hydrolysis were separated as previously described, but their general characteristics were quite similar, although the specific rotation of the acid solution had increased to the constant value $+56\cdot8^\circ$. The yields from 10 grams of cellulose were: fraction I, 2·1 grams; fraction II, 3·9 grams with 0·9 gram of residual syrup, also containing lead and chlorine.

Fraction I.—Found: Pb = 8·3; Cl = 6·2. $[\alpha]_D + 42\cdot5^\circ$ to $34\cdot5^\circ$.

Fraction II.—Found: Pb = 9·4; Cl = 9·8 per cent. $[\alpha]_D + 26\cdot7^\circ$ to $17\cdot3^\circ$.

The Action of 40 per cent. Hydrochloric Acid on Esparto Cellulose. (a) *Changes in Optical Activity*.—A 5 per cent. solution of esparto cellulose was prepared as previously described in the case of cotton cellulose. The esparto solution darkened more quickly

and the precipitated cellulose was more discoloured. The darkening of the solution was due to the separation of humus substances which were not entirely soluble in alkali. The polarimetric observations were taken as before, at 15°, after dilution to 1 per cent. concentration, reckoned on the original weight of cellulose.

Minutes.	$[\alpha]_D$	Minutes.	$[\alpha]_D$	Minutes.	$[\alpha]_D$	Minutes.	$[\alpha]_D$	Minutes.	$[\alpha]_D$
130	8.4°	460	22.4°	750	30.4°	990	35.0	1330°	39.2°
155	9.2	490	24.8	780	29.6	1030	34.4	1405	40.0
185	9.6	535	26.8	810	29.6	1075	37.6	1490	41.1
215	10.8	570	27.2	840	30.4	1135	39.2	1620	46.3
245	11.6	600	28.4	870	32.0	1165	38.4	1770	45.6
290	13.6	630	29.2	900	31.2	1200	40.0	2035	46.3
320	15.2	670	31.2	930	35.1	1230	38.4	2250	46.3
370	18.0	735	32.4	960	35.0	1295	39.2	3000	54.4
400	19.6								

It will be seen that for the most part the changes in optical activity are parallel to those recorded in the case of cotton cellulose.

(b) *Variations in Yield of Furfuraldehyde*.---The yield of furfuraldehyde was determined at definite intervals by distilling a portion of the acid solution after the acid concentration had been reduced to 12 per cent.

Time from start.	$[\alpha]_D$	Yield of furfuraldehyde, per cent.
12 hours	29.5°	11.6
24 „	41.4	11.6
6 days	49.6	9.9

The yield of furfuraldehyde is calculated on the original weight of cellulose dissolved.

(c) *Isolation of the Products of Hydrolysis*.---At the end of 24 hours, the acid solution was treated as described under cotton cellulose. The products contained a complex which yielded furfuraldehyde, but otherwise they had the same general characteristics. As usual, drying operations had to be conducted with great care, as charring easily occurred, accompanied by marked swelling, which rendered an elementary analysis impossible.

Fraction I.---Found: Pb=7.9; Cl=5.6. $[\alpha]_D + 30.5^\circ$ to 22.2° .

Fraction II.---Found: Pb=8.2; Cl=8.6; $C_5H_4O_2=5.7$ per cent. $[\alpha]_D + 32.2^\circ$ to 25.2° .

The total yield was only 40 per cent. of the weight of cellulose employed.

Summary and Conclusions.

(1) The products of hydrolysis of the celluloses are not identifiable by the rotation constants of the hydrolytic solutions. The latest conclusions as to the cellulose-dextrose relation, based on

optical data, are now shown to be invalidated, for the following reasons: (a) Such widely different types as cotton and esparto celluloses give identical optical data on hydrolysis, but the ultimate products have necessarily divergent constitutions. (b) Solution in concentrated hydrochloric acid produces constitutional changes even in the simple hexoses of known configuration, as shown by the marked fluctuations in optical activity accompanying slight variations in acid concentration. The products obtained in acid solution from cellulose may therefore be the result of several concurrent reactions. Even when these are reduced to a minimum by sufficient dilution, there is still the ascertained complication of esterification.

(2) No quantitative resolution of cellulose to dextrose has yet been obtained, and the claim made by Willstätter and Zechmeister to have effected this conversion, since it rested solely on polarimetric observations and the equally non-specific data of cupric reduction, would seem inadmissible.

(3) Isolation and identification of the products of hydrolysis or their immediate and characteristic derivatives must be regarded as the only trustworthy means of quantitatively studying the resolution of highly complex colloidal carbohydrates such as cellulose.

(4) Under the action of both sulphuric and hydrochloric acids, cellulose forms esters of polysaccharoses which contain acidic hydroxyl groups. In the case of hydrolysis by the latter acid especially, attempts to isolate dextrose, if present, by fractionation only resulted in the production of compounds having a higher content of inorganic material and a lower specific rotation, whilst the effect of subsequent heating with dilute acid is to produce, not hydrolysis, but further condensation.

I wish to express my indebtedness to the Carnegie Trustees, who provided the opportunity for carrying out this research, also to Professor J. C. Irvine and Mr. C. F. Cross, who have given me valuable advice, and, lastly, to Messrs. Tullis, Russell and Company, who kindly supplied the esparto products used in the experiments.

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[Received, February 7th, 1918.]