L.—*Constitution of the Cereal Celluloses.*

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THE general bearings of the subject of this communication have been dealt with in previous papers (Trans., 1894, **65**, 472; 1895, **67**, 433; *Ber.*, 1893, **26**, 2520; 1894, **27**, 1061; 1895, **28**, 1940 and 2604; *J. Amer. Chem. Soc.*, 1895, **17**; 1896, **18**, 8; *Chem. News*, 1896, **73**, 307). We have since followed up the special problem of the constitution of the cereal celluloses, more particularly with reference to the identity of their furfural yielding groups.

These celluloses we have assumed to be a complex of hexose groups on the one hand, and of groups allied to the pentoses on the other. That pentose molecules are not present as such is extremely improbable by reason (1) of the resistance of these celluloses to alkaline hydrolysis, (2) of their not giving the characteristic reaction with phloroglucinol and hydrochloric acid.

It is probable also, from the mode of formation of these celluloses in the plant, and the severe conditions of the alkaline hydrolysis by which they are isolated from the straws, that the complex is not a mixture of two celluloses, but a uniform aggregate of the two groups of molecules. The first object of the investigation was to determine the conditions of acid hydrolysis which would give a satisfactorily sharp separation of the constituent groups. The process ultimately adopted leaves nothing to be desired in this respect; the furfuroïds are easily obtained in solution at the same time that the normal hexose groups are separated as a cellulose having the ordinary characteristics, and retaining only a fraction of the former group. Moreover, the furfuroïds are obtained directly in a condition of molecular simplicity, which very much facilitates the work of determining the constants by which their identity is established. For the purposes of this preliminary discussion, we may anticipate the experimental proof of their identity. We shall be able to show that the members of this characteristic group of plant constituents are formal derivatives of a pentose, probably xylose.

A xylose monoformal, aggregated to its cellulosic form, is an addition to plant physiology, explaining many hitherto obscure points in the relationships of the furfural yielding carbohydrates.

It will be observed that a pentose monoformal, which we may represent as $C_5H_9O_3 \begin{smallmatrix} O \\ \diagup \quad \diagdown \end{smallmatrix} CH_2$, has the empirical composition of normal cellulose, $C_6H_{10}O_5$. It appears also that the compound is formed in the plant from a hexose; and the process may be explained as the result of an oxidation of the terminal carbon atom, the

changes being formulated briefly as follows: . . . $\text{CH}\cdot\text{OH}\cdot\text{CH}_2\cdot\text{OH}$ becomes . . . $\text{CH}_2\cdot\text{OH}$ and $\text{H}\cdot\text{COH}$; formaldehyde is, however, not set free, but remains united by oxygen linking to the new pentose residue.

The oxidation is, therefore, one of internal rearrangement, that is without addition of oxygen from without. Transformations of this order are recognised as characteristic of the carbohydrates (Lobry de Bruyn, *Rec. Trav. Chim.*, 1894, **14**, 213), and it is unnecessary to enlarge on the probability of their occurrence in the plant. We have only to point out that the latter view of the origin of these compounds, is to be preferred, since all the evidence as yet available tends to negative the view that the pentoses are formed directly in the assimilating process (E. Fischer, *Ber.*, 1894, **27**, 3230; De Chalmot, *Ber.*, 1894, **27**, 2723), and further the association of the pentose and formyl residues in the original complex is quantitative, whereas on the former view there might very well occur a mixed complex of pentose or pentosan groups with the compounds in question.

Our present view, therefore, is that the terminal carbon atom of a hexose is first oxidised by internal rearrangement, and that the C-C union is then resolved, and oxygen linking substituted.

The question of the origin of the pentoses proper in the plant has been dealt with by De Chalmot in a series of publications (*Amer. Chem. J.*, 1894, **16**, 589; and also by Tollens, *Kohlenhydrate*, **2**, 60). De Chalmot regards the pentoses as formed by the destructive oxidation of the terminal $\text{CH}_2\cdot\text{OH}$ group of a hexose and elimination of the carbon as carbonic anhydride, but has been somewhat sceptical in regard to our contention that this oxidation may occur by stages, and that the intermediate terms are represented in plant tissues. Tollens has more recently dealt with the subject experimentally, and one of his recent communications (*Zeit. angew. Chem.*, 1896, **2**) contains the results of a preliminary investigation of possible solutions of this problem suggested by the introduction of a second CO group into the molecule of a hexose. By the action of potassium permanganate on starch, he obtained, in addition to formic acid in considerable quantity, an oxidised product which yielded furfural when boiled with dilute hydrochloric acid, and gave a characteristic purple reaction with phloroglucinol and hydrochloric acid. This colour reaction, which is quite distinct from that of the pentoses, is characteristic of the products of acid hydrolysis of the cereal celluloses, that is, of the formal-pentoses. There is ample ground for expecting the formation of similar products. With the more positive indications of identity, furnished by the results detailed in this paper, it will be easy to ascertain the presence, in complicated mixtures such as were obtained by Tollens, of intermediate terms of the

series, hexose : formal-pentose : pentose formate : pentose, so that a subject which for some time past has been dealt with by hypothesis framed from opposite but converging points of view, is now in the more satisfactory position of being attacked by definite experimental methods.

We proceed to a brief description of our experimental results, which supply a simple and clearly defined method of experimental enquiry.

The results group themselves under the following heads.

1. Investigations by methods of hydrolysis.

(a.) By treatment with sulphuric acid between the limits of concentration, $\text{H}_2\text{SO}_4, 2\text{H}_2\text{O}$ and $\text{H}_2\text{SO}_4, 3\text{H}_2\text{O}$. This process was found difficult of control, and therefore abandoned.

(b.) By treatment at high temperatures with very dilute acids (1 per cent. H_2SO_4), the following conditions of treatment being finally adopted. 1 part of cellulose, and 6 parts of 1 per cent. H_2SO_4 ; rapid heating to 150°C . (3 atmos. pressure), and 15 minutes' heating at that maximum.

2. Proof of sharp separation of furfuroïds (solution) from the residue of normal cellulose (insoluble).

3. Proof of molecular simplicity of furfuroïds in solution.

(a.) Maximum numbers for cupric oxide reduction.

(b.) Maximum yields of osazones.

(c.) On fractionation, the melting points and composition of the osazones indicated the formation of osazones of pentoses.

4. Isolation of dissolved furfuroïds. Empirical composition, $\text{C}_6\text{H}_{10}\text{O}_5$, with high yields of furfural. Differentiation of reaction from those of pentoses.

5. Presence of formyl residue proved by oxidation with hydrogen peroxide, and quantitative estimation of the carbon dioxide formed, the results being confirmed by the synthesis, from pentoses and formic acid, of compounds which gave the characteristic reactions of the furfuroïds isolated from cereal celluloses.

In describing the experimental results, we shall find it convenient to use abbreviated expressions for "cupric reducing power" and for the yield of furfural when boiled under the standard conditions with hydrochloric acid (1.06 sp. gr.), namely, K for the percentage reduction referred to dextrose = 100; whilst F = percentage of furfural referred to 100 parts by weight of carbohydrate, or, in cases of solutions, calculated to 100 parts of dry solids (free from ash) in solution.

1. (a.) Process of solution in sulphuric acid.

The reactions of the normal celluloses with sulphuric acid are well

known. The concentrated acid behaves differently with the members of the two other sub-groups of fibrous celluloses (Trans., 1895, **67**, 439), the reaction being complicated by condensations, with formation of dark-coloured products. These condensations, we may remark incidentally, are now accounted for by the presence of the formaldehyde residues in combination. In the hydrates of the series $\text{H}_2\text{SO}_4, 2\text{H}_2\text{O} - \text{H}_2\text{SO}_4, 3\text{H}_2\text{O}$, that is, of 1.65—1.55 sp. gr., on the other hand, the cereal celluloses dissolve to almost colourless solutions, and without constitutional change. We cite a typical experiment.

One gram of wheat-straw cellulose was dissolved in 6 grams of sulphuric acid of 1.65 sp. gr., and allowed to stand two hours in the cold, then diluted, and the reprecipitated cellulose collected, washed, dried, and weighed. The furfural was then estimated in both precipitate and filtrate. The results were as follows.

Straw Cellulose—F = 13.2, resolved into

Normal Cellulose insoluble.

Soluble products.

64.0 per cent. F = 0.9

36.0 per cent. F = 12.4

(The F, or furfural numbers, of the products are calculated to the original straw cellulose.)

It appears, therefore, that the furfuroïd constituents may be sharply separated by this process, and without constitutional change. On the other hand, this molecular condition in solution is still complex, as is indicated by the low cupric reduction, K being found = 13, which was raised to 28 only after two hours boiling of the highly diluted solution.

On neutralising with barium carbonate and filtering, a pale-coloured solution was obtained, which, on evaporation, left a nearly colourless, gummy residue, giving no pentose reaction (phloroglucinol), and containing traces only of barium compounds.

This method was then tried on a large scale, but the results were much less satisfactory. Preparations were obtained, as described, containing a large proportion of barium compounds, with low furfural numbers (F = 12—20), and low cupric reduction (K = 20—30).

These operations, on the scale of 1 kilo. of raw material, were kindly carried out for us by Mr. William Chattaway, of the Apothecaries' Hall. To him and the Corporation of the Apothecaries' Hall we are much indebted for this kind assistance.

The differential attack of the acid was therefore not maintained under the changed conditions of mass. Further careful investigation showed that small variations in the concentration of the acid, in the temperature and in the duration of digestion, produced large variations in the results. We cite one or two typical series in which the

effect of a particular factor was investigated by causing it to vary, the other factors remaining unchanged.

(1) Concentration of acid—

	Sp. gr.	Cellulose reprecipitated on dilution.	Constituents dissolved.
$\text{H}_2\text{SO}_4, 3\text{H}_2\text{O}..$	1.55	71 per cent.	39 per cent.
$\text{H}_2\text{SO}_4, 2\frac{1}{2}\text{H}_2\text{O}.$	1.60	20 "	80 "
$\text{H}_2\text{SO}_4, 2\text{H}_2\text{O}..$	1.65	3 "	97 "

(2) Temperature—

	Cellulose reprecipitated on dilution.	Furfural numbers.	
		Precipitate.	Solution.
3°	96.0 per cent.	7.46	4.65
23	62.5 "	2.20	9.55

(3) Proportion of acid to cellulose—

	Cellulose reprecipitated.
3 : 1	23.0 per cent.
5 : 1	13.5 "
10 : 1	10.0 "

It needs no further evidence to show that the balance of conditions for the selective hydrolysis of the furfuroïds is difficult to control. The process was also unsatisfactory from the point of view of the condition of the furfuroïds in the dilute solution, the cupric reduction indicating that the CO-groups are largely unresolved. And again in the process of neutralising with barium carbonate there is a relatively large loss of the furfuroïds. This method of isolating the furfuroïds was therefore abandoned, partly on account of the difficulties thus experienced, chiefly, however, for the reason that experiments carried out simultaneously with the highly dilute acid (1 per cent. H_2SO_4) at elevated temperatures had given altogether satisfactory results.

(b.) The best conditions of digestion were determined by a series of comparative experiments with "brewers' grains," a closely allied substance, which has been investigated, especially with regard to the furfuroïd constituents, by Tollens. As the criterion of maximum effect, the cupric reducing power of the resulting solution was taken. The following table of results (next page) needs no further explanation than that the numbers in the last column represent the cupric reduction calculated in terms of dextrose and of the original weight of "grains."

With this material, as with the cereal celluloses, it was found that the furfuroïd constituents are satisfactorily exhausted and converted into soluble derivatives.

We shall refer subsequently to the composition of the products obtained in the above experiments, but for the present we confine

ourselves to drawing the one obvious conclusion from the numbers, namely, that for the maximum effect the essential conditions of the treatment are (1) a highly dilute acid (1 per cent. H_2SO_4 has been adopted); (2) a temperature of $133\text{--}143^\circ$, corresponding with 3—4 atmos. steam pressure; the lower limit has been adopted.

Invariable Conditions.

Weight of "Grains" 80 Grams.

Volume of Solution, 500 c.c.

Experiments on varying Conditions.

Pressure.	Time.	Strength of acid.	Yield of reducing sugar.
No. 1, 9 atmos.	15 mins.	0.5 per cent.	17.19 per cent.
" 2, 9 "	15 "	1.0 "	17.39 "
" 3, 8 "	15 "	0.5 "	19.24 "
" 4, 8 "	15 "	1.0 "	19.49 "
" 5, 7 "	15 "	1.0 "	22.48 "
" 6, 7 "	15 "	0.5 "	20.58 "
" 7, 7 "	15 "	0.75 "	22.85 "
" 8, 7 "	15 "	1.5 "	22.48 "
" 9, 6 "	15 "	0.75 "	26.11 "
" 10, 6 "	15 "	1.0 "	27.6 "
" 11, 5 "	15 "	1.0 "	28.65 "
" 12, 4 "	15 "	1.0 "	35.67 "
" 13, 3 "	15 "	1.0 "	37.96 "
" 14, 2 "	15 "	1.0 "	30.48 "
" 15, 1 "	15 "	1.0 "	very slight
" 16, 3 "	15 "	1.5 "	37.96 "
" 17, 3 "	10 "	1.0 "	35.67 "
" 18, 3 "	30 "	1.0 "	37.0 "

The following further details and results of a typical experiment with a straw cellulose may be cited. 80 grams of the cellulose digested with 500 c.c. of 1 per cent. H_2SO_4 under the conditions described, gave the following.

The Products of Hydrolysis.

(a.) *Insoluble.*—The residual cellulose, which was only faintly coloured, weighed 55.5 grams, that is, 69 per cent. of the original.

On combustion it gave $\text{C} = 45.2$; $\text{H} = 5.6$ per cent.

On boiling with hydrochloric acid of 1.06 sp. gr., it yielded 2.3 per cent. of furfural, equal to 1.6 per cent. on the original cellulose.

On conversion into thiocarbonate, it gave a solution of normal characteristics, yielding tenacious films when evaporated upon a glass surface.

Comparing this residue with the one obtained by reprecipitation from solution in $\text{H}_2\text{SO}_4, 2\text{H}_2\text{O}$ in a successful experiment, we note the following differences. The latter, on combustion, gave the mean numbers $\text{C} = 43.0$; $\text{H} = 6.5$ per cent., and a furfural number $\text{F} = 1.6$.

The difference is merely a difference of hydration due to the corresponding difference in the conditions of treatment.

(b.) *Soluble*.—The solution was pale yellow, and smelt of furfural. The quantity of free furfural was, however, small, representing 0.1 per cent. of the total dissolved constituents. The total quantity of organic solids in solution represented 23 per cent. of the original solids. The furfural number was found to be $\text{F} = 39.5$. Taking, therefore, the furfural numbers of soluble residue and dissolved products, and calculating to the original cellulose, we obtain

$$\begin{array}{r} 70 \times 2.3 = 1.61 \\ 28 \times 39.5 = 11.06 \\ \hline 12.67 \end{array}$$

which satisfactorily accounts for the total furfuroïds of the original cellulose, for which $\text{F} = 12.8$.

The process, therefore, gives a satisfactorily sharp separation of the furfuroïds from the remaining groups which constitute a normal cellulose, and there is satisfactory evidence of the absence of constitutional change; it appears to be a process of simple hydrolysis.

3. That the soluble carbohydrates are in a condition of molecular simplicity, that is, fully hydrolysed, is proved.

(a.) By the cupric reducing power of the solution. A large number of such determinations have been made in the course of these investigations, both by volumetric and by gravimetric methods; the numbers show the unusual maximum, $\text{K} = 130$, with a range of variation between 110 and 130. These numbers further indicate the presence of a molecule or residue of pentose constitution, the C_5 aldoses having a cupric reducing power of $\text{K} = 105$ —115. Comparative observations were made with pure xylose and arabinose in solutions of the same concentration as the solutions of the furfuroïds in question, and under similar conditions. The numbers obtained were $\text{K} = 105$ —112.

(b.) The yields of osazones were also exceptionally high. Several determinations were made from time to time with different solutions, averaging 3 per cent. of total dissolved solids. The average yield of osazone was 30—40 per cent. of the weight of carbohydrate in solution.

Maquenne has determined the yields of osazones from typical

carbohydrates in dilute solution (1 : 100), and obtained the following numbers (*Compt. rend.*, 1891, **112**, 799).

Fructose, 70 per cent.; xylose, 40 per cent.; arabinose, 27 per cent.; dextrose, 32 per cent.

As our osazones were formed in 3 per cent. solutions, we made observations with xylose and arabinose in solutions of this concentration and under conditions identical with those which obtained with the carbohydrate under investigation; the yields of osazones were from xylose 25 and from arabinose 16 per cent. It is sufficiently evident, therefore, that in the soluble products of hydrolysis of the cereal cellulose we are dealing with a carbohydrate in its fully hydrolysed form.

We have now to record the experimental evidence for the actual identity of the compound.

The results already noted indicate a close relationship to the pentoses. The fractionation of the osazones revealed the presence of small quantities of osazones of low melting point (135—143°), but the main mass was obtained in fractions melting between the limits 146° and 153°. Not only in melting points, but in all respects the osazones were indistinguishable from those of the typical pentoses, xylose, and arabinose.

The results of ultimate analysis were also in close accordance with the requirements of a pentosazone formula. The following are the numbers obtained.

	(1).	(2).	Calculated for Pentosazone, $C_5H_8O_3(N_2HPh)_2$.
N	17.1	17.3	17.07 per cent.
C	62.5	62.3	62.2 ,,
H	6.4	6.5	6.1 ,,

As the osazone obtained from the soluble products of hydrolysis of straw cellulose was indistinguishable from xylosazone, it would appear that the carbohydrate in question is either xylose or a closely related compound.

4. The solution, on the other hand, did not give the reaction of the pentoses with phloroglucinol, but an equally characteristic reaction, namely, the development of a deep violet coloration with separation of an insoluble, blue-black compound.

We proceeded with the investigation of this divergence by attempting to isolate the compound by evaporation and crystallisation; for this purpose, the solution was first neutralised with barium carbonate and filtered. Traces only of barium compounds were present in the filtered solution, which was evaporated to a syrup, and kept for some weeks in a desiccator, but there were no signs of crystallisation. The compound was, therefore, dried at 100—105°; the residue was

an amorphous, gummy substance, brittle when dry, and almost colourless.

On combustion, we obtained the following numbers.

	I.	II.	Calculated for $C_6H_{10}O_5$.
C	44.2	44.5	44.44 per cent.
H	6.7	6.3	6.17 „

These numbers and the yields of furfural (38—42 per cent.) confirm the observation of divergence from the composition and properties of the pentoses and pentosans. We appear, therefore, to be dealing with a derivative of a pentose.

5. The effect produced on the compound by numerous processes of oxidation were studied; certain oxidising agents, when added gradually to the warm solution, determine the formation of formaldehyde, easily recognised by its odour and the reactions of the solutions obtained on distilling. The most characteristic oxidation, however, is that produced by hydrogen peroxide; on adding this reagent (20 vol. solution) in quantity corresponding with 1 atom of oxidising oxygen to 1 mol. of $C_6H_{10}O_5$, and warming, formaldehyde is liberated; effervescence then commences, and at the same time an amorphous compound is precipitated, but, on continuing to heat, this compound is dissolved again, with increased effervescence.

That the effervescence is due to the liberation of carbonic anhydride was proved by quantitative observations, the evolved gases being first passed through a drying tube containing calcium chloride, and then absorbed by potassium hydroxide. The results were satisfactorily constant, the following numbers being obtained in experiments in which the peroxide was added in excess (3—5 atoms of oxygen as H_2O_2 per molecule of $C_6H_{10}O_5$).

	I.	II.	III.
CO_2 estimated	19.5	20.5	20.1 per cent.

On this evidence we concluded that the compound $C_6H_{10}O_5$ is a compound of pentose and formic residues, the second CO-group being united by an oxygen linking to the pentose residue.

From a compound represented by the formula $C_5H_8O_3 < \overset{O}{\underset{O}{\text{C}}} > CH_2$, we may calculate the yields of the products of decomposition.

(1) CO_2 by oxidation of the formal group.

(2) Furfural by the ordinary process of condensation of the pentose, first liberated by hydrolysis as follows.

(1) $C_6H_{10}O_5$ yields $CO_2 = 27.1$ per cent.

(2) $C_6H_{10}O_5$ yields $C_5H_{10}O_5$ which yields 48 per cent. of furfural.

And this calculated to the original $C_6H_{10}O_5$ is $150 \div 162 \times 48 = 44.0$ per cent. Taking into account that we have been unable to

crystallise the product or otherwise to separate it in a condition of purity, and that these decompositions are not strictly quantitative, there is sufficient concordance between the experimental and calculated numbers to bear out our conclusion as to the identity of the compound.

The synthesis of compounds of formaldehyde with the carbohydrates does not appear to have been attempted. The aldehyde readily reacts with the corresponding alcohols, mannitol, for example, with formation of crystalline compounds, formals of considerable stability and resistance to acid hydrolysis (Schulz and Tollens, *Annalen*, 1895, **289**, 20). The reaction requires the presence of powerful condensing agents, and it may be, therefore, difficult to devise suitable conditions for the simple combination of formaldehyde with the alcoholic OH-groups of the pentoses. The subject is an attractive one, and we hope before long to be in possession of more direct verification of the results of this investigation, by comparing the "natural" with the laboratory products.

Formic acid, on the other hand, appears to react readily with the pentoses under conditions of condensation involving no fundamental change of the carbohydrate. On warming the pentoses, in solution, with formic acid in presence of hydrochloric acid, compounds are obtained giving the purple reaction with phloroglucinol, identical with that of the compounds under investigation. It is not probable, however, that the latter are formates or formic acid ethereal salts derived from the pentoses. In the first place, they could hardly survive the severe alkaline treatment by which the cereal celluloses are isolated from the straws; secondly, no acid groups are present among the products of hydrolysis whether this is effected by the concentrated or dilute acid; thirdly, the ultimate analysis of the products establishes a carbohydrate formula.

It is not improbable, however, that a formal derivative and a formate should give an identical colour reaction with phloroglucinol when warmed with hydrochloric acid of 1.06 sp. gr.

In the above account of experimental results, we have kept strictly to the positive evidence of identity of the particular furfuroïd under investigation. We regard this as established in general terms.

Certain minor issues of the work we have postponed until the main argument was complete. They may be dealt with *seriatim*.

1. The presence of one or more products of hydrolysis in the solutions obtained by the action of acids on the cereal celluloses.

We have assumed that the dissolved solids represent one compound only, namely, the formal-pentose.

The absence of hexose compounds has been established by investigation of the products formed on oxidation by nitric acid; neither

saccharic nor mucic acid is formed, but the proportion of oxalic acid is high, and nitroso-compounds are also formed in large proportion. A series of compounds of acidic nature, giving calcium salts insoluble in acetic acid were also obtained; these all contained nitroso-groups, and burned more or less explosively on heating to 200—300°. To obtain these compounds in quantity sufficient for complete investigation, we intend to operate on a large weight of the original substance.

The absence of hexose groups is in accordance with what we know of the degree of resistance of the normal celluloses to acid hydrolysis. Cotton cellulose, when treated under the conditions adopted for resolving the cereal celluloses, lost in weight 5·7 per cent., and the products in solution showed the maximum reduction of CuO, that is, were present as dextrose.

We regarded it as probable, therefore, that the hydrolysed furfuroïds would be accompanied in solution by a certain proportion of hexose compounds; but that the latter are actually present in only fractional proportion is shown (1) by the results of nitric acid oxidation, (2) by the non-formation of osazones of high melting points, (3) by the high furfural numbers of the solution calculated to the total dissolved solids.

2. *The Oxidation by Hydrogen Peroxide.*—It may be objected that there is not sufficient evidence for the conclusion that the evolution of carbonic anhydride is due to the decomposition of a formyl residue. It has been stated by C. Wurster that the carbohydrates themselves are oxidised by the peroxide with liberation of carbonic anhydride (*Centralbl. f. Physiol.*, 1, 33), though the action is very slow; we have, therefore, kept this possibility carefully in view.

The following experiments on xylose and arabinose, under conditions of treatment identical with those employed in investigating the furfuroïds are of interest. No carbonic anhydride was evolved, but that a certain amount of oxidation had taken place was shown by the change in the fufural number.

No. of atoms O, as H ₂ O ₂ per l				
mol. pentose	0.	1.	2.	3.
Furfural obtained from product—				
Arabinose.....	49·0	35·6	34·4	34·4
Xylose.....	51·0	48·5	—	42·2

The action on xylose is therefore slight; on arabinose more pronounced, but reaches a limit, whilst the furfuroïd under investigation shows a behaviour differing from that of the pentoses of known constitution. Treated under the conditions mentioned above, and examined for furfural after oxidation, the following numbers were obtained.

	Original.	After oxidation (hydrogen peroxide) with		
		1 at., O.	2 at., O.	3 at., O.
Furfural	42.0	29.3	11.4	5.3

This more pronounced oxidation of the pentose residue is accounted for by the fact that it is a residue, and consequently the entrance of the oxidising groups is facilitated where the formaldehyde residue is split off; the divergence does not necessarily imply a constitution or configuration different from that of the known pentoses. The constants of the osazone, and the specific rotation of the original furfuroid ($[\alpha]_D = 26^\circ$) are almost identical with those of xylose. The evidence, therefore, is sufficiently strong to justify the conclusion that it is in effect a xylose-formal.

De Chalmont, in a paper on the formation of pentoses in the plant (*Ber.*, 1894, 27, 2723), points out, on the one hand, the similarity in configuration of *d*-glucose with *l*-xylose, and of *d*-galactose with *l*-arabinose; and, on the other, that these carbohydrates occur in plants and plant products in corresponding pairs. We have, therefore, this *a priori* presumption in favour of the xylose residue in the cereal straws.

3. *Action of Bromine.*—Bromine is rapidly absorbed by the acid solution of the furfuroid, and if the bromine be added until a slight excess is present the quantity taken up will be from 21 to 25 per cent. of the weight of the furfuroid; the bromine is found to be exclusively in the form of the hydracid. On adding bromine in like manner to the neutralised solution, removing the slight excess, and titrating the free acid present, the latter was found to correspond exactly with the amount of hydrogen bromide calculated from the weight of silver bromide directly precipitated. The action of the bromine is therefore one of oxidation, and no unsaturated groups are present. The bromine calculated to an equivalent of oxygen ($\text{Br}_2 = \text{O}$) works out to only a fractional molecular proportion of the furfuroid; the action, therefore, is at first selective, the formaldehyde being oxidised to carbonic acid. The yield of osazone is considerably increased, but is identical in composition and properties with that obtained from the original solution. These results generally confirm the conclusions as to the identity of the furfuroid.

4. Other comparisons were instituted with pentoses of known constitution, such as oxidations by permanganate and by chromic acid: the results were confirmatory. The action of dilute alkalis on this group of aldoses is also noteworthy. On adding sodium hydroxide in excess (0.5 per cent. NaOH on the solution), and warming to 70–80° the furfuroid is rapidly changed; the cupric reducing power falls

in a few minutes to one-fifteenth of the original, and a new complex of products is present, a characteristic feature of which is the large yield of iodoform obtained on warming the alkaline solution with iodine. The pentoses were also found to be very sensitive to the action of alkalis; this point is noteworthy from the fact that the cereal celluloses are themselves resistant to the action of alkalis, so far as regards the "furfuroid" configuration.

5. *Fermentation (Yeast).*—The solutions obtained from the cereal celluloses by the action of acids, when neutralised, ferment rapidly with fresh, washed yeast. A limit is, however, soon reached, and on investigating the unfermented residue it is found that the cupric reduction has fallen to 60—70 per cent. of the original, and the furfural number to 70—80 per cent. of the original; a partial fermentation of the furfuroids therefore occurs, with formation of alcohol and carbonic anhydride. We shall revert to this point in dealing with the question of the composition of the original straw. These observations further confirm the evidence furnished by the more important constants of the compounds in question.

We have only now to point out the physiological significance of this diagnosis of the constitution of the cereal celluloses. The evidence as to assimilation leads to the conclusion that the higher plants build up, in the first instance, *d*-glucose and *d*-fructose and their polysaccharides, if not exclusively, at least in overwhelming proportion (E. Fischer, *Ber.*, 1894, **27**, 3230). Assimilation is followed by the indefinitely wide range of secondary changes which accompany and determine the play of individual and group characteristics of structure and composition.

Next, in order of chemical significance, to hexose-formation is the appearance of a second CO-group in the C₆-molecule. We have endeavoured, in the investigation cited above, to trace this molecular change, the occurrence of which is very clearly indicated in the constitution of the lignocelluloses. We followed, in the first instance, the hypothesis of a process of direct or extrinsic oxidation. The results of a two years' study of tissue formation in the barley plant tended to negative this hypothesis (*Ber.*, 1895, **28**, 2604). The results of the study of the cellulose basis of the cereals now indicate that the origin of a second carbonyl may take place by a process of intrinsic change, that is, the migration of an oxygen atom within the hexose molecule. To this view we ask the careful attention of physiologists. It is consistent with all that is known of the chemistry of the carbohydrates considered as isolated compounds, and as they have an obviously increased mobility within the arena of cell vitality, the occurrence of such a process might well have been predicted. It is more satisfactory, however, to have arrived at the conclusion by way of

positive evidence, for that furnished by our investigations is certainly definite, if not finally conclusive, in respect of the actual constitution and configuration of the typical product. These points are under further investigation. Chemists will readily appreciate the increased range of possible transformations, provided for by the duplication of the CO-group of an aldose. Without, however, speculating on probabilities, these considerations find an immediate application to the chemistry of lignification. The characteristic groups of the lignocelluloses are undoubtedly of quinonic type; and they are also invariably associated with furfural-yielding groups. The ultimate purpose of these investigations is to trace the probable genetic relationships of these typical groups; and we have, therefore, extended to the province of the lignocelluloses the methods and conclusions dealt with in this communication. The results of this further investigation will form the subject of a separate paper, and we, therefore, merely outline, in conclusion, some of the more important results arrived at.

(a.) The lignocelluloses of the cereals, for example, the straw and seed envelopes (as obtained in brewers' grains) are similarly attacked by acid reagents, their furfuroid constituents being hydrolysed to soluble forms and separated from the complex of cellulose and unsaturated (keto-hexene) molecules.

(b.) These furfuroïds have characteristics similar to those isolated from the celluloses: but the hydrolysis of the parent molecule proceeds to a lower limit; the cupric reduction of the soluble furfuroïds is relatively low, and the osazones are of lower melting point (120° — 130°).

(c.) In regard to alcoholic fermentation, however, the furfuroïds are obtained in a more sensitive condition. Thus, in the case of barley-straw treated with $\text{H}_2\text{SO}_4, 2\text{H}_2\text{O}$, the solution, after dilution and filtration from the reprecipitated ligno-cellulose complex, was examined for the above constants, then neutralised and fermented, and the constants of the unfermented residue determined. The following are the results.

	K.	F.
Before fermentation	43.5	32.3
After fermentation	2.3	8.1

The soluble furfuroïds were therefore, to a very great extent, fermented. It appears, also, from the researches of W. E. Stone, published in the *American Journal Agricultural Science*, 1893, 7, 6, that of the total furfuroïds in a typical selection of fodder plants, a large proportion, 60—80 per cent., are digested on passing through the alimentary tract of herbivora.

On the other hand, it is positively established that the pentoses

proper are not susceptible of alcoholic fermentation, neither are they assimilated in the process of animal digestion.

The results, therefore, further indicate that in the cereal stems the furfuroïd constituents are not pentosans, but products representing stages intermediate between the hexoses and pentoses.

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