LXXXIX.—*The Carbohydrates of the Cereal Straws.*

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WE have in previous papers discussed the general relationships of the furfuraldehyde-yielding constituents (abbreviated to "furfuroids") of the cereal stems, to the pentoses and pentosans on the one hand, and to the hexoses and their aggregated forms (anhydrides) on the other.

The evidence so far available justifies the assumption that amongst these furfuroids there is a group of pentose derivatives of the general formula

That evidence we do not propose to discuss again in detail, but being well aware that the basis for this view was indirect and inferential, we have endeavoured to verify the hypothesis by more positive and direct proofs. In the search for such proofs, it is not surprising that we encountered considerable difficulties, the nature of which will be readily appreciated from the consideration of the literature relating to an analogous group of compounds of which piperonal is the type. This compound, the methylene ether of protocatechuic aldehyde,

was known for many years before this formula was suggested for it; and the proofs of this now generally accepted view of its constitution are still mainly indirect, although a recent synthesis of the compound by the interaction of protocathecuic aldehyde, alkali hydroxide, and methylenic iodide (Wegscheider, *Monatsh.*, 1893, 14, 382) affords important confirmation. The methods of resolution and synthesis successfully employed in determining the constitution of this substance are quite inapplicable to a pentose methylene ether by reason of the extreme sensitiveness of the pentose residue to the action of acids at high temperatures, and of alkalis at ordinary temperatures, actions which the aromatic residue of piperonal satisfactorily withstands.

The most direct proof of the hypothetical formula which we have proposed for the special group of furfuroids under investigation is the fact that the action of hydrogen sulphide on the solutions of these compounds obtained by acid hydrolysis of the cereal celluloses give rise to characteristic thioformaldehydes; these, however, are formed in small quantity only, and as the result of special experiments. As we shall subsequently show, it is extremely difficult, under the conditions of high temperature necessary for complete solution (hydrolysis) of these compounds, to avoid the further condensation of the products. In our recent work, we have obtained these products in various stages of further condensation as shown by their yielding, not the 38—42 per cent. of furfuraldehyde which might be expected from a pentose derivative of the simple formula $C_5H_8O_3 \begin{smallmatrix} \diagup O \diagdown \\ \diagdown O \diagup \end{smallmatrix} CH_2$, and as was obtained in our earlier experiments, but 50—64 per cent. of furfuraldehyde. Moreover, these condensed forms differ in their behaviour with phloroglucinol. The products described in our previous paper give a pure blue coloration on warming with the phenol in presence of hydrochloric acid, and in this respect show a well-marked distinction from the pentoses, which give the well-known and characteristic rose-red coloration. The series of condensed products, on the other hand, may be fairly said to give no colour reaction, in fact nothing but brown, amorphous products are produced when the heating is long continued.

We next have to consider these compounds in relation to alcoholic fermentation. In our last paper, we showed that the products of acid hydrolysis of the constituents of the tissues of the cereals taken at the earlier periods of growth were fermented by yeast in neutralised solution. As evidence of the fermentation of the furfuroids, we relied on determinations of furfuraldehyde (and other constants) before and after fermentation; this evidence is open, however, to the objection that these compounds might disappear from solution otherwise than by fermentation, for example, by an assimilating action of the yeast cell, such as has been shown by Bokorny to obtain for a large number of

carbon compounds (Dingl, *Polyt. J.*, 1897, 303, 115). We have therefore made a number of experiments for the purpose of revising our previous conclusions, and generally, also, in view of the importance of the question, with the object of dissecting and separately studying the various possible factors of such a phenomenon. We arrive at the following conclusions :

1.—We confirm Tollens in his statements that the pentoses, when examined alone, completely resist fermentation (Stone and Tollens, *Annalen*, 1888, 249, 267).

2.—That the pentoses in presence of hexoses are equally resistant, the latter alone undergoing the usual fermentation.

3.—But the pentoses in contact with yeast do, under certain circumstances, *disappear* without any attendant fermentation phenomena ; this disappearance, which is shown by determinations of furfuraldehyde in the original solution containing a weighed quantity of a pure pentose, and after contact with yeast, seems to be due to the assimilation of the pentose by the yeast. We have observed decreases from the normal 48 per cent. to 17—24 per cent. The necessary condition for this result appears to be a low vitality of the yeast, such as can be brought about by washing it and leaving it for 24 hours in water. In the process of assimilation, it is to be noted that the pentose undergoes a constitutional change, for the amount of furfuraldehyde obtainable from solutions thus treated is the same, whether in determining the residual furfuroid the yeast is previously filtered off, or included in the process of distillation for furfural.

4.—We have carried out a number of fermentations with solutions of the furfuroids under investigation, and obtained by acid hydrolysis from various sources. The constants of the solutions have been determined before and after fermentation ; in some cases, also, the amount of alcohol produced has been determined.

We are now in a position to affirm on a more positive basis that there is a group of furfural-yielding carbohydrates, pentose derivatives, in fact, susceptible of undergoing fermentation by yeast with production of alcohol and carbonic acid. Some recently published investigations of Tollens and Glaubitz (*J. fur Landw.*, 1897, 106—107) have similarly shown that, in the “lager” beer fermentation, the soluble furfuroids of the malt are fermented in considerable proportion. This is an important biological fact, as well as of special significance in relation to the more purely chemical or constitutional problem which we have in hand. It may be taken in conjunction with the closely related question of animal digestion.

A large number of experimental investigations have been made in relation to the question, “Are the ‘pentosans’ digestible?”—the observers in this field having used, and still continuing to use, this term

as including all furfural-yielding carbohydrates. The answer is uniformly in the affirmative, and it is now fully established that the furfuroids, as we prefer to term them, of fodder plants are in large proportion digested in their passage through the animal organism. We think it should be noted that the question as regards the pure pentoses is still undetermined, although the investigations on record go to show that they are not assimilated.

Before we proceed to a record of the experimental work upon which the above conclusions are based, we may briefly review the main points in the evidence previously advanced for the specialised constitution of the group of furfural-yielding carbohydrates, constituents of the permanent tissues of cereals, and obtained from the latter by the action of acids at high temperatures.

1.—The distinctive colour reactions with the characteristic reagent of the group, namely, phloroglucinol.

2.—The exceptionally high numbers for cupric reduction, which are much in excess of those of the pentoses, these, again, being 10—15 per cent. in excess of those of the hexoses. This indicates the presence of a second CO-group in the molecule, or of a considerable proportion of such dicarbonyl compounds.

3.—The presence of a group easily oxidised by hydrogen peroxide in acid solution with production of carbonic acid.

4.—The susceptibility of these products of hydrolysis to the action of yeast, confirmed by the results of the present paper, and more definitely established as a case of true fermentation.

5.—Lastly, if we consider the raw material from which many of these products have been obtained, namely, the pure celluloses isolated by the severe treatments employed by the paper-maker, we must concede that resistance to the action of alkali hydroxides at high temperatures indicates a constitution very different from that of the pentosans, which are easily dissolved by dilute alkalis in the cold, and decomposed at high temperatures.

This is briefly a statement of the case for the recognition of a group of furfural-yielding carbohydrates, which are neither pentoses nor pentosans, although closely related to them; and, consequently, for extending the use of the term "furfuroid," so as to include the entire group of pentoses, pentose derivatives, and certain dicarbonyl compounds, which give furfuraldehyde as a characteristic product of decomposition on treatment with acids.

EXPERIMENTAL.

Hydrolysis of Celluloses.

The celluloses of straw and esparto have been treated with acids under various conditions, the results of an experiment being expressed by the proportion of the insoluble residue, and an estimation of the furfuraldehyde which it will yield; and as regards the solution, the total dissolved matters, the furfuraldehyde yielded, and its cupric-reducing power; the latter being chiefly a measure of the proportion which has been fully hydrolysed (monoses). The following results are typical.

Esparto cellulose. Conditions of action: 80 grams of cellulose (dry), 500 c.c. of dilute sulphuric acid (1 per cent. H_2SO_4). Heated in a closed digester to the various steam pressures indicated, and maintained 15—30 minutes at that temperature.

Steam pressure in atm.	Residue.	Dissolved.	Constants of dissolved products per 100.	
			Furfur- aldehyde.	CuO reduction (dextrose = 100).
1	81.3	21.3	34.9	105
2	85.6	18.8	39.6	119
3	80.6	11.9	61.0	135

These numbers show that, up to a certain point, the hydration of the anhydride (cellulose) is measurable by the increase in total weight of the products; at the higher temperature, dehydration is shown by a considerable deficiency in the sum of the weights of the products, the condensation being equally evident from the increase in the furfural number. The increase in the cupric reduction is also noteworthy, for, according to our observations, furfuraldehyde itself is without sensible action on alkaline cupric solutions under the ordinary conditions of a "sugar estimation." The solutions, it may be observed, all contain small quantities of free furfuraldehyde.

A similar series of digestions was carried out with straw cellulose, of which the following are the results:—

Steam pressure in atm.	Residue.	Dissolved.	Constants of dissolved products per 100.	
			Furfur- aldehyde.	CuO reduction (dextrose = 100).
2	86.1	11.0	61.3	148.0
3	84.7	11.0	64.5	138.0
$3\frac{1}{2}$	66.7	17.4	43.5	142.8

We have to call special attention to this series, and especially to the result obtained at $3\frac{1}{2}$ atm., where the heating lasted for 30 minutes. Under these conditions, the condensation of the dissolved (hydrolysed) products is rapid; in fact, a large quantity of furfuraldehyde is formed. The extent of the dehydration is shown by the very large difference

between the sum of the products and the amount of original cellulose (100 parts). The furfural number is calculated to the fixed residue obtained by evaporating to dryness the neutralised (with BaCO_3) and filtered solution.

The particular feature of this result is the clear indication it affords of the conditions of temperature and duration of heating leading to further condensation of the furfuroids.

The solution obtained in this experiment gave a considerable precipitate with hydrogen sulphide, which was much increased on adding hydrochloric acid; the precipitate had the characteristic odour and properties of trithioformaldehyde.*

It will be noted that the numbers for cupric reduction are exceptionally high in this series, much higher than those accounted for on the hypothesis that the products of resolution are pentoses. This was also negatived by the solutions failing to give any colour reaction with phloroglucinol. In carrying out this qualitative test, it is necessary to allow the reaction of the phenol with the small quantity of free furfuraldehyde to become complete at the ordinary temperatures, and after removing the dark coloured condensation product by filtration to heat the clear filtrate in the usual way.

No red or blue colour reaction was obtainable, and on continuing to heat, brown, amorphous products were produced by condensation. It is evident, therefore, that, under these conditions of hydrolysis and subsequent condensation, a series of products can be obtained from a soluble carbohydrate to furfuraldehyde, on the one hand, accompanied on the other by formaldehyde and insoluble products resulting from their interaction.

It is our intention to follow up these results by operations on a very much larger scale so as to facilitate the separation of the several products.

It should be noted that the furfuroids of esparto cellulose are more resistant to acid hydrolysis than those of straw cellulose, more especially at temperatures corresponding with a steam pressure of 2 atm. They are dissolved (hydrolysed) at that temperature with much lower numbers for cupric reduction, and also for furfuraldehyde. At the higher temperatures, these differences disappear.

Fermentation of the Products of Hydrolysis (Celluloses).

Esparto and straw cellulose were treated under the conditions above given, the duration of heating at the maximum temperature being,

* This solution, when saturated with hydrogen sulphide in presence of hydrochloric acid, gave a precipitate of thioaldehyde amounting to 15 per cent. of the weight of the dissolved products, and the filtrate from this yielded as much furfuraldehyde as the original untreated solution.

however, reduced to 15 minutes. The solutions were then neutralised with calcium carbonate and fermented with yeast, the constants being determined before and after fermentation.

Straw Cellulose (Extract).

	Furfuraldehyde.	Cupric reduction.
Before fermentation	39.7	140.0
After fermentation	26.1	72.5

Alcohol formed, 9.7 per cent. of dissolved solids.

Esparto Cellulose.

	Furfuraldehyde.	Cupric reduction.
Before fermentation	53.1	119.5
After fermentation.....	27.3	70.2

Alcohol formed, 13.7 per cent. of dissolved solids.

That these solutions contain no appreciable quantity of hexoses is shown indirectly by the high furfural numbers; confirmatory evidence was obtained by the absence of saccharic acid from the products of oxidation of a portion of the original solution by nitric acid, also by the study of the osazones, which in both cases were found to melt, after purification, at 139°, and contained no products of higher melting point.

Barley Straw.—Specimens of barley straw from the Rothamsted Plots 10 and 7.a (crop 1895) were hydrolysed by boiling for 2 hours with dilute sulphuric acid (2 per cent. H_2SO_4) under the ordinary atmospheric pressure. The original straw, containing furfuroids represented by 15—17 per cent. furfuraldehyde, yielded an extract containing 70—80 per cent. of the total furfuroids (represented by 12.6 of furfuraldehyde) calculated to the original straw. The neutralised solutions were fermented with the following results:

	Furfuraldehyde.	Cupric reduction.
Before fermentation	38.0	104.1
After fermentation	4.7	16.1

A large number of similar observations of fermentation have been made, in some of which the amount of alcohol formed has been determined. These operations, however, we are now carrying out on a very much larger scale, using 6—7 kilos. of raw material, so as to allow of a much more exact determination of all the products of fermentation. For this reason, and also from the consideration that certain errors are likely to invalidate conclusions drawn from observations on the small scale, it would serve no useful purpose to multiply records of such observations.

It is so far established that an ordinary alcoholic fermentation of hydrolysed tissue furfuroids does take place, and that, in consequence, they are constitutionally different from the pentoses.

Experiments in Investigation of the Relationships of the Pure Pentoses to Yeast.

In interpreting results of fermentation experiments, we have assumed that the disappearance of furfuroids, as measured by estimation of the furfuraldehyde before and after fermentation, may be set down to actual fermentation of the furfuroid.

In a series of fermentations, known mixtures of the pentoses (xylose and arabinose) and dextrose were employed. After fermentation, the furfuraldehyde was estimated and calculated to the known weight of the pure pentose taken.

In regard to the following statement of results, it must be remembered that the pure pentoses yield 48—50 per cent. of furfuraldehyde.

Mixture fermented.		Residual furfuraldehyde. calc. to pentose taken.
2 grams dextrose	0.225 xylose	41.3
2 " "	0.300 arabinose	36.7
1 " "	0.200 "	34.1
1 " "	0.500 "	22.7
1 " "	0.500 "	28.7

(A certain proportion of nitrogenous nutrient was also added).

These results show a variable and in some cases considerable disappearance of the furfuroid. It must be noted here that the conditions of fermentation were variable, not only as stated, but also in regard to the kind and condition of the yeast, the duration of the action, and in other respects, mainly for the reason that the results were obtained on the purely empirical basis of observation.

To determine more closely the cause of the disappearance of the furfuroid, further investigation was necessary. The pure pentoses were accordingly placed in contact with fresh brewers' yeast, the solutions containing 4 grams of pentose per 100 c.c. and a sufficient quantity of nutrient solution. A small evolution of carbonic anhydride was observed in certain cases, and a small diminution in the quantity of pentose corresponding with a fall in the furfural number to 43—46 per cent. In other cases, there was no action, and the furfural number remained unaltered. These observations sufficiently confirm Tollens' results that the pentoses themselves are not fermented.

In these experiments, we obtained indications that the second phenomenon, that is, the disappearance of the pentose without fermentation, observed in some instances, was dependent on the condition

of the yeast employed. A quantity of yeast was therefore set aside for some hours in water, with the addition of nitrogenous nutrient matter. The experiments with the pure pentose (arabinose) were then repeated. In this case, the furfural number fell to 22·7 per cent. In a second experiment, the yeast was "starved" for a period of 24 hours and the experiment again repeated. In this case, nearly 75 per cent. of the pentose disappeared, the furfural number falling to 11·35 per cent. The latter result was confirmed by a subsequent experiment in which the yeast itself was included in the solution in which the "residual furfural" was estimated. The result was the same. It is thus shown that the pentoses are, under certain circumstances, a yeast-food, and further, that their assimilation by the yeast cell is attended by a change of constitution. They are probably assimilated as hexoses.

To test the correctness of these conclusions, the original experiments with mixtures of hexoses and pentoses were repeated, using fresh brewers' yeast and smaller proportions of the pentoses, and estimating the residual furfuraldehyde when the fermentation was nearly complete. The following results were obtained:

Mixture fermented.		Residual furfuraldehyde calc. to pentose taken.
2·0 grams dextrose	0·2 gram arabinose	50·5
1·0 " "	0·2 " "	50·3
1·0 " "	0·2 " "	50·1

From these results, we conclude that in a vigorous fermentation, not unduly prolonged, the pentoses are not assimilated by the yeast cell.

These observations were sufficient for the purposes of our investigations, and the results indicate the precautions necessary to be observed in dealing with either pentoses or furfuroids.

To measure the fermentation by the fall of the furfural number only, is admissible only when these precautions are duly observed, and even then it is obviously advisable to determine in addition the true fermentation products, that is, the carbonic acid and alcohol. To find out what other products are formed is the purpose of investigations now in progress, and in order to carry out these we have been able to make provision for a very much larger supply of the products of hydrolysis. By working with kilos. in place of grams, the labour of investigation will be very much lightened.

By these and our previous investigations we are able to reduce the practical problem of isolating the furfuroids of constituents of permanent tissues (celluloses) to the following simple prescriptions.

As the hydrolysing acid, sulphuric acid is to be preferred. As regards

its more concentrated forms acting in the cold, the acid $\text{H}_2\text{SO}_4 + 3\text{H}_2\text{O}$ has a selective action on the furfuroids, the entire cellulose dissolving in the acid, but on diluting, the furfuroids remain in solution, the dextrose groups being reprecipitated in the colloid form (cellulose hydrate). With increased concentration, the whole cellulose complex tends to be equally attacked. At the concentration represented by $2\text{H}_2\text{SO}_4 + \text{H}_2\text{O}$, secondary actions of condensation take place as indicated by the discoloration of the solution. In all actions of the concentrated acid, however, the degree of hydrolysis is limited, that is, the specific cupric reduction of the products is relatively low. On boiling with dilute acid containing 2 per cent. H_2SO_4 , the rate of hydrolysis is low, and the limit attained is low.

The dilute acid (1—2 per cent. H_2SO_4) at the boiling temperature, under atmospheric pressure, attacks the furfuroids more rapidly than the dextrose-celluloses, but the action is not satisfactorily selective. The degree of molecular hydrolysis is also low. At high temperatures, however, corresponding to 2—3 atm. steam pressure, the furfuroids are selectively attacked, and dissolved with maximum resolution, that is, hydrolysed to monoses.

Where, therefore, the question of alcoholic fermentation is to be studied, the latter treatment is to be preferred.

In studying this question, it will be necessary to determine the following constants of the solution before and after fermentation. (1) Total dissolved solids; (2) furfuraldehyde; (3) cupric reduction; also to estimate the carbonic acid and alcohol produced. Further, having ascertained the conditions for maximum fermentation, to examine the solution for the other products of the fermentation.

Further investigations upon this experimental basis are in progress.

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