CVII.—*The Carbohydrates of Barley Straw.*

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IN a previous communication (this Journal, **69**, 804), we showed that the furfuroïd constituents of the cereal celluloses, by a simple process of acid hydrolysis, can be sharply separated from a presumably normal hexose complex which is left as an insoluble residue, that is, a resistant cellulose. The furfuroïds in the solution had the characteristics of fully resolved groups (monoses), and their quantitative reactions were expressed by the formula,

representing a pentose monoformal.

The aim of these investigations, which so far was realised by the above results, was to extend the scope of the inquiry from empirical numbers to constitutional problems; that is, from furfural as an empirical and aggregate measure of a group of carbohydrates, to the actual constitution of those members of the group, for which, in the meantime, we employ the designation of "furfuroïds."

In the seasons 1894-95, using the methods then available, we endeavoured to show the relation between the furfuroïd constituents of these plants and the general assimilation and conditions of

growth. The conclusions we arrived at are recorded in previous papers (*Ber.*, **27**, [1], 1061; **28**, 2604, and *J. Amer. Chem. Soc.*, **17**, 286; **18**, 8), and appear rather in a negative form, since we were met at this stage of the inquiry by the assumption then prevalent, that the furfural-yielding carbohydrates are exclusively pentoses, and that they are not formed as direct products of assimilation or elaboration, but from hexoses previously assimilated, the process, regarded molecularly, being one of oxidation of the terminal CH_2OH group (*De Chalmot, Ber.*, **27**, 2722), and its elimination as carbonic anhydride, or as a CO derivative.

Without again discussing these conclusions, we may take advantage of the confirmatory evidence which has since accumulated to express them in a simpler and more positive form. It appears, in fact, that the furfuroïds of the cereals are elaborated as such, that is, that they are originally built up into tissue with the special constitution or configuration brought into evidence by the characteristic manner in which they afford furfural, the circumstance from which they take their group-name.

We may distinguish here, as a parenthesis, between "Assimilation" and "Elaboration."* Assimilation is the initial process of building up complex carbon compounds, for example, carbohydrates from carbon dioxide, whilst elaboration is the working up of previously assimilated matter into organised forms or living structures.

No doubt, these processes are concurrent in the living cell, cellulosic tissues being built up directly from assimilated carbohydrates, which have never existed outside the cell in a condition of lower molecular weight; on the other hand, the plant-cell works up into its permanent tissue, carbohydrate material which has been assimilated and stored in other forms, such as starch or sugars. The operations, therefore, are to be regarded as distinct, even when concurrent and apparently simultaneous.

This parenthesis is necessary to show that we disclaim the statement that the furfuroïds are assimilated as such. It is possible that it may be the case, but familiar as we are with the molecular mobility of the simple carbohydrates, we think it equally probable that a dextrose or fructose molecule undergoes an internal rearrangement in the process of elaboration to tissue material in which it then persists as a "furfuroïd."

That this is the case is a general conclusion to which all our observations converge.

To emphasise this we have recalculated our numbers for furfural

* This distinction has, perhaps, little significance to the physiologist, as in the process of "constructive metabolism" the supply of carbohydrate material is taken for granted.

estimations—the measure of the furfuroïds—on the basis of the carbohydrates alone. In our scheme of examination of the barley crops of 1895, we included, in addition to furfural estimations, determinations of ash and albuminoids; subtracting the sum of these from the total dry matter, and calculating the furfural percentages on the residue, we have a direct measure of the proportion of furfuroïds to carbohydrates in the plant at successive stages. The two sets of numbers are subjoined in parallel columns representing the proportion of furfural, (A) to the whole plant, (B) to the total carbohydrates of the plant.

Date.	Age of Crop.	A.		B.	
		Plot 1.	Plot 6.	Plot 1.	Plot 6.
May 15	6 weeks	6·6	5·8	10·3	10·0
June 18	11 „	8·0	7·6	9·6	9·8
July 16	15 „	12·1	10·6	13·6	12·0
Aug. 16	19 „	9·2	9·8	10·8	11·3
Sept. 3	22 „	10·5	10·2	11·6	11·4

The increases in the figures under (A) disappear to a large extent under (B); in other words, the plant elaborates its tissue in an approximately constant ratio of furfuroïds [Furfural $\times$ 2] to total carbohydrates, namely, 1 : 4.

It is clear also that the essential constitution of these compounds is preserved throughout the life of the tissue, although they may be subject to secondary changes of a minor order which will be subsequently dealt with.

In reference to the small variations in the numbers, it must be remembered that the plant is a complex of structural elements, which vary in relative proportion with its development. Thus, lignification, with an increasing proportion of lignocelluloses, is a prominent feature of its growth. In a previous paper (*J. Amer. Chem. Soc.*, 1896, **18**, 8), we have given a scheme of proximate analysis of the plant in respect of its main constituents, but as furfuroïds are present in large proportion in all of them, it is impossible to differentiate in respect of this factor. For the cereal stems, therefore, we must be satisfied with an aggregate and approximate demonstration of the constant chemical characteristics of these tissue constituents.

In dealing with a simple tissue, such as can be mechanically separated and examined after isolation, the same point may be, and, in fact, has been, much more satisfactorily demonstrated. In conjunction with our friend, Mr. A. Pears, we made in 1892–3 similar observations on the bast tissue (lignocellulose) of the jute plant, and showed that this much more complex compound is constant in composition throughout the life of the plant. [Pears, *Trans.*, **63**, 964; **65**, 470; see also *J. Imperial Inst.*, 1895, 398.]

That we find it necessary to insist on this point is due to the fact that the chemical history of the "permanent tissue" of plants is a comparatively neglected subject, and in reducing it to system it is of fundamental importance to distinguish between the constant and the variable features of composition; between the primary constituents of tissues and the products of secondary change. Thus, the lignocelluloses are primary products; they are not celluloses previously elaborated, on which encrusting substances (lignin) have been deposited as a result of secondary actions. The furfuroïd celluloses are not mixtures of a hexose complex, originally elaborated as such, with furfural yielding groups (pentoses) produced from the former by secondary oxidation. These characteristic features of composition are present from the first appearance of the tissue.

In reference to the latter group, which is the main subject of our present inquiry, we have already indicated that the furfuroïds of the cereal straws are subject to secondary constitutional changes of a minor order, of which we are able to give some account from the results of investigations of the growing crops (barley) of the past season.

In investigating the furfuroïds isolated from the cereal celluloses, the following are the methods which chiefly contributed to determine their constitutional features:

- (a) Copper oxide reduction (Fehling's solution).
- (b) Osazones: analysis and melting point.
- (c) Reaction with hydrogen peroxide at 80° , and estimation of the carbonic anhydride evolved.
- (d) Yeast fermentation and estimations of furfural and "sugar" before and after fermentation, showing the extent to which the furfuroïd is broken down, that is, fermented.

The application of these methods to the products isolated from the barley plant at successive stages of growth by the process of acid hydrolysis constituted our scheme of investigation of the 1896 crop. The numbers we have obtained give unmistakable evidence of a progressive constitutional change of the furfuroïds.

A complete series was carried out on Plot 6 (barley) of the Royal Agricultural Society's experimental station at Woburn, this plot representing maximum yields of straw and grain; the plants from Plot 1 (minimum yield) were also investigated in the later periods. The numbers of both are recorded in the subjoined table, which only requires a brief explanation. The green plants were under investigation within 24 hours of cutting; they were exhausted with boiling alcohol, and the well dried residue treated in a digester with five times its weight of 1 per cent. sulphuric acid at 3 atmos.

Plot.	Date.	Age of crop.	Total organic solids in acid extract. Per cent. of dry weight of tissue.	Fermentation investigation.						H ₂ O ₂ reaction. Per cent. of CO ₂ evolved.	Osazones. M. p.
				Furfural, per cent. of dry extract.*		CuO reduction.		Furfuroïds fermented. Per cent. of total.			
				Before fermentation.	After fermentation.	Before fermentation.	After fermentation.				
6	May 26	7 weeks	30.1	16.1	—	52.1	7.1	100	nil	185°	
"	June 16	10 "	30.2	24.1	—	81.1	5.4	100	nil	195	
"	July 14	14 "	53.0	20.4	3.2	49.6	8.8	80	trace	176	
"	Aug. 4	17 "	25.0	28.4	7.7	80.1	25.0	74	4.0	175	
"	Aug. 13	19 "	33.8	26.8	15.3	77.1	26.77	43	5.5	127	
1	July 14	14 weeks	50.0	21.4	4.4	59.5	13.0	79	trace	178	
"	Aug. 4	17 "	24.4	31.2	8.1	82.2	22.3	74	4.1	175	
"	Aug. 13	19 "	28.1	27.5	13.7	92.6	28.8	50	5.1	125	
Aug. 4 was the date of cutting. The period Aug. 4-13 is the time elapsing between cutting and carrying.											
Matured straw.....			22.8	42.0	25.7	110.0	57.0	39	11.7	145°	
Straw cellulose			17.0	48.0	23.5	118.0	57.0	51	21.0	148	

* The percentages of furfural are not calculated on the original plant-substance, but on the extract, including nitrogenous and mineral constituents.

steam pressure for 15 minutes. The determinations recorded in the table were carried out on the acid extract; in the fermentation experiments, this solution, previously neutralised with chalk and filtered, was employed, using washed yeast.

The results, taken together, have a very decided significance when compared with the numbers obtained with a fully matured straw and with straw cellulose, which are included in the table for comparison, and this notwithstanding the extracts from the plants in the earlier stages of growth are complicated mixtures.

That the extraction of the furfuroïds was satisfactorily complete, was established by estimations of furfural in the residues. The proportion of residue was from 45—55 per cent. of the original, yielding furfural amounting to 0.9 to 1.4 per cent. calculated on the original weight. This extraction is not selective, as it is in the case of the matured straw, for the furfuroïds in solution are accompanied by other constituents, and amount to only 33 to 50 per cent. of the whole extract. Whilst this fact lessens the value of the observations taken individually, we are able none the less to draw the following definite conclusions respecting the condition of the furfuroïd constituents of the plant when extracted.

(a) Up to the flowering period, the furfuroïds are in such a condition as to be capable of being completely broken down by yeast fermentation; but, with increasing age, a constitutional change ensues attended by a progressive resistance to the action of the yeast organism.

(b) In the early stages—in fact, until the period of ripening of the grain—the failure to react with hydrogen peroxide indicates the absence of a second CO group in the (assumed) C_6 unit, that is, the absence of the constitutional features which correspond with a pentose-formal.

(c) The osazones show a characteristic fall in melting point, such as would correspond with the change from the condition of a hexose to that of a pentose, or a pentose derivative.

As stated before, we regard these changes as of a minor or secondary order; they are not such as to affect the specific character of the carbohydrate; that is, the property of directly yielding furfural. This, no doubt, depends on a C_5 residue of special configuration which persists, while the sixth C group may be subject to structural changes.

These conclusions are in accordance with our anticipation that the plant furfuroïds will be found to represent a hexose-pentose series of which the intermediate terms can be recognised in mature cereal straws; the probable intermediate forms, that is, those existing in the condition of complex colloïds or celluloses, are, of course,

numerous; but when reduced to the simplest terms, that of a monose, the only stable compound would be the one formulated generally as a pentose-formal.

The formation of a pentose-monoformal from a hexose is most simply explained, if we regard it as produced by the oxidation of the terminal C atom of the hexose to formaldehyde by internal rearrangement, $-\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{OH}$ becoming $-\text{CH}_2\cdot\text{OH} + \text{HCOH}$, the formaldehyde and pentose residues remaining united by condensation. This is a species of fermentation change which cannot be regarded as an improbable occurrence in the plant; the evidence which we have now adduced strengthens the hypothesis that it does occur. It may be objected that this hypothesis involves the assumption of a hexose directly converted to furfural by the condensing action of acids; this, however, is not exactly the case. We have no criterion of configuration, and very little of constitution, in the amorphous and complex colloïd forms of the carbohydrates. It is not improbable that the mode of aggregation of hexoses to complex anhydrides may have an influence on their hydrolysis by dilute acid, or condensation by stronger acids. Without, however, labouring the argument, it is more consistent, with the evidence before us, to regard these tissue furfuroids as consisting of hexose groups which readily pass into pentose derivatives and, ultimately, into pentoses, by a series of internal changes, the latter occurring spontaneously in the life of the plant, or being determinable by the action of condensing acids. The alternative view that they are pentoses is inconsistent with the evidence fully set out in previous papers, and now strengthened by the observations contained in the present paper, namely, that the furfuroids in the early stages of growth are, when hydrolysed, completely fermented by yeast, yield osazones of high melting point, and change progressively with age in their reactions, developing the property of being decomposed by hydrogen peroxide with formation of carbonic anhydride; these changes, moreover, are most marked at the critical periods of the life history of the plant, that is, at flowering, and at the period of the ripening of the grain.