

XLVIII.—*The Carbohydrates of Barley Straw.*

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IN the summer season of 1897, we have carried out the investigations suggested by the results of our previous work, namely, a comparison of the stem-tissues of the plant grown (1) under normal conditions, with that grown (2) under the abnormal condition induced by removing the ears when in full flower. The plants were cut at the usual harvest period, the ripe ears were removed from the former, and the stem-tissues of both were investigated by the methods previously described. It is hardly necessary to premise that the experimental plots were selected so that all other conditions of growth may be considered as having been equal, and therefore that the only variant is the suppression, in the case of (2), of the seed-bearing organs. The result of this variation, in regard to the most important of the factors under investigation, was mainly negative; the proportion of carbohydrates yielding furfuraldehyde ('furfuroids') to total carbohydrates was not affected, neither was the relative resistance of the two main groups of carbohydrates to hydrolysis with alkalis.

* For these investigations, we were again able to avail ourselves of the experimental barley plots of the Crawley Mill Farm of the Royal Agricultural Society.

On the other hand, the plant, with its energies relieved of the work of producing seed, made but little advance towards maturation; the 'cellulose' and 'permanent tissue' constants, remained very much as at the flowering period, and correlatively the stem-tissues contained a larger proportion of carbohydrates capable of yielding to hydrolysis with acid and alkali.

In reference to the comparative external features of the growth of the plant in the experimental plots, the following are the notes supplied to us by Mr. J. J. Forrester, the manager of the Royal Agricultural Society's farm, who kindly undertook the control of the cultivation experiments. "The general tendency on the plots when the ears were clipped was to throw up fresh shoots at the bottom. . . . All the plants from which the ears had been removed developed a striking rigidity in the stem, and gradually assumed a somewhat withered appearance. . . . These did not ripen so fast as those growing under normal conditions, and at the time of cutting were distinctly greener than the others."

The following are the results of the laboratory investigation of the stem-tissues, harvested as described.

	Plants grown normally.	Plants grown with ears removed.
Total dry matter	86.0	83.0
„ nitrogen	0.6	0.9
„ mineral matter (ash)	8.7	6.0
„ furfuraldehyde.....	13.9	13.2
„ "permanent tissue"	61.6	53.5
Furfuraldehyde from "permanent tissue"	16.0	15.0
Residue from alkali hydrolysis	50.9	42.6
Cellulose	43.3	33.0
Residue from acid hydrolysis	54.1	48.9
Furfuraldehyde from above residue ...	5.7	5.9

The products of hydrolysis by acid were subjected to fermentation with yeast in neutralised solution; the cupric reduction and furfural constants were determined before and after fermentation, the numbers being calculated to the original 'organic' solids of the solutions. The following results were obtained.

Acid Extract from Plants of Normal Growth.

Alcohol produced, 11.1 per cent.

	Before.	After fermentation.
Cupric reduction (dextrose = 100)	86.8	49.6
Furfuraldehyde	24.7 per cent.	16.9 per cent.

Acid Extract from Plants of Abnormal Growth.

Alcohol produced, 18.9 per cent.

	Before.	After fermentation.
Cupric reduction (dextrose = 100)	83.3	45.0
Furfuraldehyde	22.4 per cent.	17.0 per cent.

The only point of difference to be noted is the increased production of alcohol in the second case.

In all those constants which define the relationship of the furfuroids to the hexose-carbohydrates of the plant, no differences of any moment are observable.

In those constants which define to a certain extent the secondary changes summed up as 'maturation,' which consist, in large measure, of condensation changes with production of the more resistant forms of tissue-carbohydrates, the laboratory investigation confirms the observations of the agriculturist in regard to the history of the growth of the plants; the 'ripening' of the stem-tissue was largely arrested by the suppression of the seminal function, and the chemical results of this are that the carbohydrates remain in a more digestible condition, that is, a larger proportion are in the condition to yield to hydrolytic action; we may also note that the non-depletion of the stem as regards nitrogenous constituents is shown in the higher proportion (50 per cent.) of total nitrogen.

It will be remembered that the original purpose of these investigations was to obtain evidence as to the conditions of formation of furfuroids in the plant. We have already studied the plant under the widest range of variations of conditions of growth as regards soil, nutrition, and climate, and now we have added the effect of varying an essential physiological factor. In no case have we found any differentiation of the furfuroids from the hexose carbohydrates, such as to warrant the conclusion that they are formed from the latter by external chemical change (oxidation); or, to express the conclusion in positive terms, we find that the furfuroids rank with the hexose carbohydrates as primary products of assimilation. We are here confronted with a critical difficulty. The furfuroids of the cereal straws in their mature condition are undoubtedly (condensed) aldoses, and either pentoses or pentose derivatives. But an accumulation of physiological evidence has established that the pentoses do not rank with the hexoses in relation to assimilation, or to the constructional metabolism of the plant. The pentoses, unlike the hexoses, do not occur as such in the plant; the current theories as to the mechanism of the formation of carbohydrates in the growing cell also exclude any products intermediate

between those of the dimensions C_3 and C_6 . In our researches, we have given weight to this apparent conflict of evidence, and have searched for such evidence (1) as would differentiate the furfuroids of the cereal straws from the pentoses proper (or pentosans), (2) as would indicate a transition from the hexose to the pentose series. In both directions, we have arrived at results, but as these have been fully stated in our previous communications, it is needless to reproduce the evidence in detail here, especially as much fuller investigation is needed before the matter can be considered as finally settled.

On one point, however, we have some fresh evidence confirmatory of that recorded in our previous communication (Trans., 1896, 69, 1604). Whereas, under all conditions of hydrolysis by alkali, the two groups of carbohydrates are equally attacked, the same proportion between them being maintained in the residue of severe alkali treatment (cellulose), as in the intermediate stages, the attack by acids is more selective. This will be apparent at once from the results obtained with barley of 1897, taken (*a*) in the flowering stage and (*b*) after harvesting.

The conditions of acid treatment were as follows. Two hours boiling with dilute sulphuric acid (1.0 per cent. H_2SO_4) under ordinary atmospheric pressure. The furfuraldehyde in the dissolved products was estimated and calculated to the 'total organic matter' in solution. Whereas in (*a*) the proportion was 12.3 per cent., in (*b*) it amounted to 33 per cent. (average number for the two crops). Now the number for (*a*) differs but little from that of the entire plant substance (10 per cent.); in other words, in the earlier stages of growth, the two groups of carbohydrates are equally attacked by acids. In (*b*), on the other hand, the furfuroids are attacked by preference; and in our previous papers we have dealt with a number of cases, where, with furfural numbers at 40—50 per cent. of the dissolved solids, the attack by acid hydrolysis is more exclusively confined to the furfuroids. These results indicate that the furfuroids undergo some constitutional change with age by which they are differentiated from the hexose groups with which they are associated in intimate union. This fact is emphasised by the results of alcoholic fermentation. It is now well established that the pentoses are not fermented by yeast. In our last paper, we dealt with the causes of a disappearance of pentoses in the fermentation of mixtures of pentoses and hexoses, under certain conditions of the action of the yeast cell; we found that this occurred when there was a relative starvation of the latter, but *not* when the fermentation was vigorous and rapid.

The results given below of the fermentation of the acid extract (after neutralisation) of the immature plant (*a*), must be

compared with those previously recorded (see above) for the matured plants.

Alcohol produced, 19·4 per cent.

	Before.	After fermentation.
Cupric reduction (dextrose = 100)	49·6	0·00
Furfuraldehyde	12·3 per cent.	2·9 per cent.

The fermentation in this case was complete so far as regards the fully hydrolysed molecules; and the small proportion of residual furfuroids which are present in the still condensed form cannot be set down to resistance to the attack of the organism. Moreover, as the proportion of furfuroids to total carbohydrates was relatively low, the disappearance of 77 per cent. during fermentation is the more noteworthy.

These results add further confirmation to the conclusion that a constitutional change of the furfuroids accompanies maturation; a change from that of a furfural-yielding carbohydrate susceptible of fermentation by yeast to that of a pentose or pentosan. We have no evidence that this change is influenced by external conditions of growth, and there is nothing to show that the change is one of oxidation from without.

The only hypothesis we have so far found to be consistent with all the evidence is that of an internal constitutional change, the terminal $\begin{array}{c} \text{CH}_2\cdot\text{OH} \\ | \\ \text{CH}\cdot\text{OH} \end{array}$ groups of a hexose being rearranged with formation of a carbonyl group, that is, a process of internal oxidation. We have not yet sufficient positive evidence to take this suggested explanation out of the region of hypothesis; but our investigations in this direction are being continued, and we hope in a short time to communicate the results of a comprehensive study of the interaction of the hexoses with hydrogen peroxide, bearing directly on the questions herein discussed.

We wish to again express our obligations to Messrs. A. and E. Voelcker, whose laboratory has been at our disposal for the conduct of the chemical work; and to Mr. Forrester for his kind co-operation in the cultivation experiments.