

BRIEF PAPERS

THE SUGARS OF THE HEMICELLOSES OF CORNSTALKS¹

EMMETT BENNETT

(WITH ONE FIGURE)

Received August 5, 1950

Introduction

The qualitative detection and quantitative estimation of individual sugars in a hydrolysis mixture are often attended by some difficulty. The combination of paper chromatography and the refinements in micro-quantitative methods for reducing sugars have been of considerable value in this operation.

The investigation to be reported deals with a study of the sugars from hydrolysates of four hemicellulose fractions obtained from the holocellulose of cornstalks by solvents of increasing strength. The information obtained indicates the presence of two common sugars previously unreported from this source and lends some support to the theory concerning the origin of pentose sugars in hemicelluloses.

Experimental

Hemicellulose fractions indicated as A, B, C, and D in the order of resistance to extraction were hydrolyzed for 10 hours with 4% sulphuric acid in a weight-volume ratio of 1 to 50 in an all-glass assembly. Hydrolysis was brought about in a bath of boiling water. Nitrogen gas was bubbled through the mixture to reduce oxidation to a minimum.

The hydrolyzed sugars were brought into aqueous solution in the usual manner. Positive qualitative tests were obtained for xylose by Bertrand's reaction and by the dibenzylidene dimethyl acetal test (1). Arabinose was detected as the osazone and as the diphenylhydrazone. Glucose and galactose could not be detected with certainty by chemical means; mannose was absent. Recourse was then taken to a paper-chromatographic procedure (2). The physical system designed consisted of the bowl of an 8-inch desiccator, an 8 × 18-inch bell jar, a 6 × 18-inch precipitating jar, a glass plate 2 × 7 inches, and the cover of a butter dish. The inside bottom of the desiccator and the precipitating jar usually fit together too poorly to afford a level footing for the dish containing the solvent; hence, about 1 inch of sand was placed in the bottom of the desiccator, upon which the precipitation jar could be easily and firmly adjusted. The glass plate was placed across the top of the precipitating jar and bore the dish, which in turn contained the solvent. Paper sheets for the chromatogram were anchored to the bottom

¹ Contribution No. 757 of the Massachusetts Agricultural Experiment Station.

of the dish and draped over either edge as described later. The solvent system used consisted of three parts of ethyl acetate, three parts of water, and one part of glacial acetic acid (3). Satisfactory separations could be made in 46 hours at a temperature of about 25° C.

Whatman filter paper #1 in sheets $18\frac{1}{4} \times 22\frac{1}{2}$ were cut into strips $4\frac{1}{2} \times 21$ inches. A fold at right angles to the long dimension was made $2\frac{1}{2}$ inches from one end. This fold should be superimposed upon the edge of the dish containing the solvent. One-half inch below and parallel to the fold, three light pencil dots were placed in a straight line $1\frac{1}{8}$ inches apart and an equal

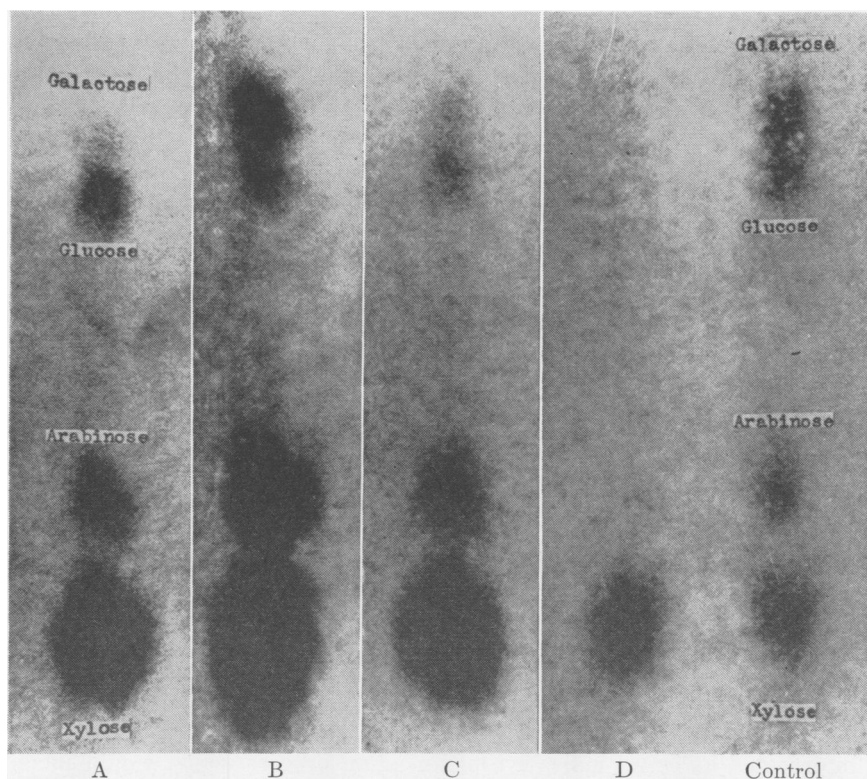


FIG. 1. The combined paper chromatogram of sugars from hemicellulose fractions A, B, C, D compared with a control.

distance from one edge in order to mark the position to be occupied by the test drops. Drops of uniform size are essential and were assured by use of a micropipette. The latter was placed in a fixed position, free from air currents and vibrations. Each drop was equivalent to about 0.007 ml. of water. Stock solutions and unknown solutions are well controlled when the concentration of each sugar is about 1% (2).

Control tests were made to determine the order of separation of D-glucose, D-galactose, L-arabinose, and D-xylose. Thereafter each sheet carried three drops; two drops were from an unknown hydrolysate, the third from

a mixture of known sugars which would serve for qualitative and orienting purposes. After the sugars had been separated, their location was determined as described by FLOOD *et al.* (2). The results obtained are shown in figure 1. The chromatogram for fraction D indicates only the presence of xylose. Greater concentrations, however, reveal the presence of arabinose, glucose, and galactose, in addition.

A second orienting experiment was conducted relative to quantitative procedures (2, 4). It was found that all procedures in this connection could be carried out on a quantitative basis. Data thus obtained were used to determine the molar ratio of the sugars which are shown in table I.

The content of L-arabinose reached a peak in fraction B by the above procedure and by the diphenylhydrazone method. In general, the content of xylose increased as the fractions became more difficult to extract.

TABLE I

THE MOLAR RATIO OF L-ARABINOSE, D-XYLOSE, AND D-GLUCOSE AND D-GALACTOSE IN THE HYDROLYZATES OF HEMICELLULOSE FRACTION.

Fraction	L-arabinose	D-xylose	D-glucose & D-galactose
A	1.0	7.4	1.7
B	2.3	6.3	1.0
C	2.3	9.6	1.0
D	1.0	13.0	1.0

Discussion

It appears significant that the same four sugars are present in all four hemicellulose fractions but in different proportions. A logical treatment of the observation would include first, a statement regarding the origin of the pentose sugars; second, a brief attempt to justify the variation in proportion of the pentoses. If one considers the theory of the origin of pentoses based on the decarboxylation of the uronic acid of the corresponding hexose, it will be evident that qualitatively the proper sugars are present.

If the tendency toward pure xylan in the fraction most difficult to extract is interpreted as being indicative of an aging process which would include qualitative changes and increases in their chain length, one must then account for the elimination of arabinose and the general increase of xylose. The most obvious approach would be to assume that arabinose was never present to any extent in such fractions; furthermore, that the presence of arabinose in different proportions in other hemicellulose fractions represents a stable state. This general statement assumes that there may be distinct hemicelluloses in a given plant. One could also assume that in the type of plant under consideration, arabinose was to some extent a transitory product and disappeared by some such change as occurs in gummosis. The latter process is not fully understood, but it is known that changes in the stereochemical configuration of a sugar residue do occur. The general in-

crease of the content of xylose might be accounted for by this means, and by the decarboxylation process mentioned above.

Summary

Four hemicellulose fractions previously extracted from holocellulose of cornstalks by means of solvents of increasing strength were hydrolyzed. Arabinose, xylose, glucose, and galactose were detected in all fractions. The sugars were determined quantitatively by paper chromatographic procedures. The ratios of the sugars were variable, but in general the content of xylose increased in the fractions most difficult to extract. The presence of the pentose sugars is discussed.

EXPERIMENT STATION CHEMISTRY
UNIVERSITY OF MASSACHUSETTS
AMHERST, MASSACHUSETTS

LITERATURE CITED

1. BREEDY, L. J. and JONES, J. K. N. Quantitative estimation of xylose. *J. Chem. Soc.*, pp. 438-439. 1945.
2. FLOOD, A. E., HIRST, E. L., and JONES, J. K. N. Quantitative analysis of mixtures of sugars by the method of partition chromatography. Part I. Standardization of procedure. *J. Chem. Soc.*, pp. 1679-1683. 1948.
3. JERMYN, M. A. and ISHERWOOD, F. A. Improved separation of sugars on the paper partition chromatogram. *Biochem. J.* **44**: 402-407. 1949.
4. SOMOGYI, M. A new reagent for the determination of sugars. *J. Biol. Chem.* **160**: 61-68. 1945.