Water-Soluble Hemicelluloses of Grass Holocellulose

J. T. SULLIVAN, T. G. PHILLIPS, and D. G. ROUTLEY

U. S. Regional Pasture Research Laboratory, University Park, Pa., and New Hampshire Agricultural Experiment Station, Durham, N. H.

Hemicelluloses were prepared from five species of forage grasses by extracting them from the holocelluloses by hot water. They were constituted in descending order of abundance by xylose, glucose, arabinose, galactose, and rhamnose, and by some uronic acids. Slight quantitative differences existed among species and stages of growth, particularly with respect to glucose, the most variable constituent.

FORAGES DIFFER from one another in nutritive value, and species and stage of maturity are important factors in this respect. An objective in the study of hemicelluloses is the uncovering of differences which might be associated with these variables. Binger, Sullivan, and Jensen (*1*) found that almost three fourths of the total hemicelluloses of orchard grass could be extracted from chlorite holocellulose by hot water. It seemed probable that this fraction of the hemicelluloses might show differences associated with species and stage of growth.

Materials and Methods

Water-soluble hemicelluloses were prepared from five grasses by the method described by Routley and Sullivan (*6*). It consists of preparing chlorite holocellulose, removing the hemicelluloses by boiling water, and recovering the hemicelluloses from the water by concentration, dialysis, and precipitation by alcohol.

The hemicelluloses were analyzed for their component sugars as follows: A 0.1-gram sample was heated in a vial with 5 ml. of 1*N* sulfuric acid in a boiling water bath under an air condenser for 6 hours, cooled, transferred to a 50-ml. centrifuge tube, and neutralized to the mixed methyl red–bromocresol green indicator with calcium carbonate. After centrifuging and washing the solid matter the solution was made slightly acid, concentrated on the steam bath, diluted to 5 ml., again centrifuged to clarify, and preserved with thymol. The method of hydrolysis described gave the maximum yield of reducing sugars, with little destruction of xylose. Biuronides, but not oligosaccharides, were found in the hydrolyzate.

The component sugars were separated by paper chromatography, using sheets of Whatman No. 1 filter paper, 7.5 inches wide. A 1.5-inch strip was marked off on each side for staining, and an additional 1.25-inch strip for a paper blank. Spottings of the hemicellulose hydrolyzate were made on the side strips and on the remaining 3.25 inches. Xylose, arabinose, glucose, and galactose were separated by three 16-hour descending runs, with drying between, with a slight modification of the solvent proposed by Masamune and Maki (*4*), *n*-amyl alcohol, pyridine, and water, 135 : 145 : 120. Rhamnose, which moved more rapidly with this solvent, was separated by a single run of 16 hours. The 1.5-inch side strips were removed with shears and dipped in a solution composed of 100 ml. of isopropyl alcohol (85%), 1.66 grams of phthalic acid, and 0.75 ml. of aniline. The strips were blotted and heated at 100° to 102° C. for 15 minutes. The sheets were reassembled. With the stained outer strips as guides the center strips containing the intact sugars and a paper blank 3.25 inches long were cut out and eluted by the Dent method, essentially as modified by Dimler *et al.* (*2*). Between 0.5 and 1.0 ml. of eluate was collected, diluted if necessary to 1.0 ml., directly into observation test tubes of the Klett-Summerson photoelectric colorimeter marked at 1 and 8 ml. Determination of reducing power was made in the same tubes by the Nelson colorimetric modification (*5*) of the Somogyi method, with dilution to 8 ml. Readings were made with the No. 50 filter. Equations for each sugar were developed, 5 to 50 γ in 1-ml. volume. The accuracy of the method was checked with mixtures of 40 γ each of the sugars which were separated on papers, eluted, and determined as above. Recoveries were 94 to 104%.

Separation of uronic acids was attempted with the solvent system proposed by Fischer and Dörfel (*3*).

Table I. Sugars in Hemicelluloses of Orchard Grass at Three Stages of Maturity in Percentage of Total Sugars

Stage of Maturity	No. of Samples in Average	Rhamnose	Galactose	Glucose	Arabinose	Xylose
Pre-emergence	6	2.2	7.5	23.7	12.9	53.7
Half-emergence	5	1.6	6.7	25.4	12.5	53.8
Flowering	3	1.3	7.3	14.2	13.0	64.2

Table II. Sugars in Hemicelluloses of Different Species of Forage Grasses in Percentage of Total Sugars

Species	No. of Samples in Average	Rhamnose	Galactose	Glucose	Arabinose	Xylose
Orchard grass	14	1.8	7.2	22.4	12.8	55.7
Reed canary, 1st cut	3	0.8	6.5	10.1	11.1	71.5
Reed canary, fall cut	1	1.3	6.2	19.8	11.2	61.5
Tall fescue	2	1.6	4.7	11.1	9.6	72.9
Timothy	2	0.9	4.8	16.3	9.1	68 8
Kentucky blue	2	1.5	6.8	12.2	12.2	67.3

Results

Five sugars were found in all hemicellulose preparations. Xylose was always the most abundant, and in decreasing order were glucose, arabinose, galactose, and rhamnose, the last always being present in minor amount. The results with uronic acids were inconclusive. Spots corresponding in location

to galacturonic acid appeared by visual comparison to amount to 12 to 14% of the different hemicelluloses. This corresponds closely to the amounts found by Binger, Sullivan, and Jensen (*1*) in similar samples of orchard grass by densitometer methods. However, the reducing power of these spots after elution corresponded to only 1 to 3% as galacturonic acid. It is probable that these spots were of biuronides. Two or three other similar spots appeared on some chromatograms.

Fourteen samples of orchard grass represented three stages of maturity: pre-emergence, half emergence, and blooming. The weights of crude hemicellulose obtained from the three stages averaged 7.5, 10.7, and 11.2% of the grass, respectively, and of these approximately 70, 80, and 63%, respectively, were recovered as sugars. Weighted averages of the amounts of the sugars, in per cent of total sugars recovered, are given in Table I. Rhamnose declined slightly with maturity of the grass. A marked decrease in glucose also occurred between the second and third stage of maturity, while xylose was correspondingly higher. A similar decrease in the glucose content of the hemicelluloses of the stems and leaves of brome grass during the course of maturity was noted by Routley and Sullivan (*6*).

The weighted average composition by species appears in Table II. In reed canary grass one sample represented mixed late summer and early fall cuttings and it contained a higher proportion of glucose than the first cuttings, all of which were advanced in maturity and were very similar to one another in composition. In other grasses no great differences were found between samples of the same species, and the results reported for them are averages. In comparisons among species of grasses differences were noted only in the glucose and in the corresponding xylose contents. Orchard grass was distinctly higher than all the other grasses in the glucose content of the water-soluble hemicelluloses. A glucosan may be a constituent of the hemicellulose preparations, especially of those from orchard grass.

It is recognized that losses occurred both in the preparation of the holocelluloses and in the extraction and precipitation of the hemicelluloses and that these losses may have had some influence on the composition of the final hemicellulose preparations. The place of occurrence and the extent of these losses will be an object of future study.

Summary

Hemicelluloses were prepared from five species of forage grasses by extracting them from the holocelluloses by hot water. They were constituted in descending order of abundance by xylose, glucose, arabinose, galactose, and rhamnose, and by some uronic acids. Slight quantitative differences existed among species and stages of growth, particularly in respect to glucose, the most variable constituent.

Literature Cited

(1) Binger, H. P., Sullivan, J. T., Jensen, C. O., J. AGR. FOOD CHEM. **2,** 696 (1954).
(2) Dimler, R. J., Schaefer, W. C., Wise, C. S., Rist, C. E., *Anal. Chem.* **24,** 1411 (1952).
(3) Fischer, F. G., Dörfel, H., *Z. physiol. Chem. Hoppe-Seyler's* **301,** 224 (1955).
(4) Masamune, H., Maki, M., *Tôhoku J. Exptl. Med.* **55,** 299 (1952).
(5) Nelson, N., *J. Biol. Chem.* **153,** 375 (1944).
(6) Routley, D. G., Sullivan, J. T., J. AGR. FOOD CHEM. **6,** 687 (1958).

Received for review August 17, 1959. Accepted November 6, 1959. Contribution No. 164 of the U. S. Regional Pasture Research Laboratory, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, University Park, Pa., in cooperation with the 12 northeastern states, and Scientific Contribution No. 242 of the New Hampshire Agricultural Experiment Station.

GRASS HOLOCELLULOSE

Stepwise Hydrolysis of Grass Holocellulose

T. G. PHILLIPS, D. G. ROUTLEY, and J. T. SULLIVAN

U. S. Regional Pasture Research Laboratory, University Park, Pa., and the New Hampshire Agricultural Experiment Station, Durham, N. H.

Grass holocellulose was subjected to successive heatings with 0.01*N* sulfuric acid in a boiling water bath for 1, 2, 4, and 16 hours. Simple sugars, uronides, oligosaccharides, and hemicelluloses were extracted by each of the successive treatments. All the arabinose, galactose, and rhamnose, practically all the uronides, and 75% of the xylose were removed from the holocellulose. Glucans accompanied the other hemicelluloses, but glucose does not appear to be a constituent of the polyuronide hemicelluloses. The oligosaccharides found in the hydrolyzates indicate a considerably branched structure for the polyuronide hemicelluloses. The compounds recovered from the extracts are similar to those obtained by various workers from the hemicelluloses of other Gramineae.

MUCH OF THE PRESENT knowledge of the structure of the hemicelluloses of nonwoody plants has been obtained from the more or less highly purified xylan portion. Recently, however, partial or stepwise hydrolysis of the entire hemicellulose has produced compounds of low molecular weight which have provided some evidence as to the structure of the more highly branched and more readily soluble portion of the hemicelluloses (*6, 10*). As no forage grass hemicelluloses were included in these studies, it appeared desirable to compare the products of their stepwise hydrolysis with those of other plants, especially other Gramineae.

Material and Methods

Chlorite holocelluloses were prepared as described by Routley and Sullivan (*5*). They came from orchard grass, cut at the half-emerged stage, and brome grass, combined leaf and aftermath. Direct hydrolytic treatment of the holocellulose was used in order to avoid the losses entailed in extracting the hemicelluloses. To 8 grams of holocellulose in a 250-ml. Erlenmeyer flask, 130 ml. of 0.01*N* sulfuric acid were added. The mixture was heated under an air condenser in a boiling water bath for 1