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.

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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

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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

hour and filtered on a Büchner funnel. The filtrate and washings, of pH 3.5, were neutralized with saturated barium hydroxide, centrifuged, and washed, in the high speed centrifuge. The supernatant and washings were concentrated on the water bath. This solution contained sugars, oligosaccharides, and aldobiuronic acids, as well as hemicellulose which had been dissolved from the holocellulose and which was not hydrolyzed or only partially so. The hemicellulose was precipitated by ethyl alcohol and separated and washed at the centrifuge. The precipitation by ethyl alcohol was repeated, with intervening concentration of the supernatant and washings, until there was no further precipitation of hemicellulose. The hemicellulose precipitates were combined, washed with ethyl alcohol and ether, and dried. The last supernatant and washings were evaporated to a small measured volume. This, referred to as the extract, contained free sugars, oligosaccharides, and some aldobiuronic acids.

The holocellulose residue from the above treatment was heated in the boiling water bath with 130 ml. of 0.01*N* sulfuric acid for 2 hours. The mixture was treated as described above. The pH of the filtrate was 2.5. The residue from the 2-hour treatment was heated in the same way for 4 hours, and the 4-hour residue for 16 hours. Extracts and hemicelluloses from each treatment, and the final residue, were analyzed.

For the determination of the constituent sugars, the holocelluloses, hemicelluloses, and residues were hydrolyzed by heating with 1*N* sulfuric acid for 6 hours. Analyses of the hydrolyzates, and also of the extracts, were carried out as described (7) except that no quantitative determinations of rhamnose were made.

Rhamnose separated readily beyond glucose with one 16-hour descending run with *n*-amyl alcohol, pyridine, and water. Comparatively large portions of the sample are required to show this sugar.

Aliquots of the extracts were heated with 0.25*N* sulfuric acid to hydrolyze the oligosaccharides which interfere with the identification of uronides on chromatograms. The mixtures were neutralized, concentrated, and chromatographed with two descending 24-hour runs with ethyl acetate, acetic acid, formic acid, and water for qualitative separation. As the material available was not sufficient for elution and identification, preparations were made from another portion of the brome grass holocellulose. After separation in quantity, the uronides were subjected to hydrolysis by methanolic-hydrochloric acid, and the products were identified by comparison with authentic samples. These uronides were compared qualitatively as described above.

Some added information as to the occurrence and nature of the oligosaccharides and uronides was obtained by two supplementary procedures: Separate 2-gram portions of the holocelluloses were treated as described above, but only once, and for 1, 2, 4, and 16 hours. Hemicelluloses were separated from the extracts as above. Samples of 0.04 gram of the holocelluloses and of the final residues and of 0.02 gram of the hemicelluloses just prepared were heated in small stoppered vials in the water bath with 1 ml. of 0.1*N* sulfuric acid. At the end of 1, 2, 4, 7, and 12 hours, portions of the hydrolyzates were spotted on paper for chromatography with three ascending runs with *n*-amyl alcohol, pyridine, and water (135:145:120). The oligosaccharides of the extracts were also separated, in this case by repeated descending runs with the same solvent. The oligosaccharides located by spraying with aniline hydrogen phthalate on side strips were further separated after elution with other solvents as needed: ethyl acetate, acetic acid, formic acid, and water (18:3:1:4); 1-butanol, pyridine, and water (6:4:3); and 1-butanol, acetic acid, and water (4:1:4) (organic phase). Finally the oligosaccharides were eluted, subjected to stepwise hydrolysis with 0.1*N* sulfuric acid, and their components were determined chromatographically. If more than one sugar appeared, their ratios were determined.

Table I. Recovery of Solids from 8 Grams of Holocellulose

Compound	Grams	%
Hemicellulose 1	0.430	5.4
2	1.128	14.1
3	0.358	4.5
4	0.129	1.6
Total hemicelluloses	2.045	25.6
Sugars in extracts, as anhydrides	0.330	4.1
Residue	5.000	62.5
Total recovery	7.375	92.2

Table II. Per Cent Sugars[a] and Ash in the Solid Preparations

Compound	Galactose	Glucose	Arabinose	Xylose	Ash
Holocellulose	1.5	4.2	5.0	21.3	3.8
Hemicellulose 1	3.2	11.6	8.3	33.6	22.3
2	3.9	10.4	4.9	46.9	13.6
3	2.2	9.7	2.4	41.6	17.6
4		17.1		21.6	27.7
Residue		2.6		8.8	4.8

[a] Liberated by heating 6 hours with 1*N* sulfuric acid.

Table III. Recovery of Sugars from the Holocellulose

	Galactose		Glucose		Arabinose		Xylose	
Compound	Mg.	%	Mg.	%	Mg.	%	Mg.	%
Holocellulose, 8 grams	123	100.0	336	100.0	396	100.0	1706	100.0
Extract 1	2	1.6			65	16.4	4	0.2
2	6	4.9			142	35.9	22	1.3
3	4	3.3			19	4.8	26	1.5
4	4	3.2	6	1.8	10	2.5	64	3.8
Extracts, total	16	13.0	6	1.8	236	59.6	116	6.8
Hemicellulose 1	14	11.4	49	14.7	36	9.1	145	8.5
2	44	35.8	114	34.0	55	13.9	531	31.1
3	8	6.5	35	10.4	9	2.2	148	8.7
4			22	6.5			28	1.6
Hemicelluloses, total	66	53.7	220	65.5	100	25.2	852	49.9
Extracted, total	82	66.7	226	67.3	336	84.8	968	56.7
Residue			129	38.4			436	25.6
Recovery, total	82	66.7	355	105.7	336	84.8	1404	82.3

Results

No qualitative differences were found between orchard grass and brome grass. Quantitative differences were slight, except for a noticeably higher glucose content in the orchard grass preparations. As this does not affect the conclusions to be drawn, the two sets of figures have been averaged in the tables.

The 7.8% loss of solids (Table I) could arise from the following: solids other than simple sugars in the extracts, including oligosaccharides and uronides; losses in the recovery of the hemicelluloses as quantitative transfer of these is difficult; and other losses in handling.

All the hemicelluloses precipitated from the extracts were contaminated with extraneous material (Table II). The ash content was especially high. No arabinose or galactose was found in the hemicellulose from the fourth extraction or in the residue.

Arabinose was removed rapidly, galactose more slowly, but both completely, as none of either sugar was found in the hydrolyzate of the residue (Table III). Xylose was split off much more slowly. The failure to attain quantitative recovery of these sugars may be accounted for in part by their occurrence in oligosaccharides and uronides, in part by destruction during hydrolysis, and in part by technical difficulties.

Probably, the treatment with dilute acid gradually changed some glucans to forms more easily hydrolyzed than they were in the holocellulose. This would account for the high recovery of glucose.

Oligosaccharides. Eight of these were separated in amounts sufficient for hydrolysis. From their position on chromatograms and their behavior during stepwise hydrolysis, an estimate can be made of the number of sugar units in each. They are as follows:

An arabinose disaccharide was present in extracts 1 and 2. It was hydrolyzed to arabinose by 0.01*N* sulfuric acid in 4 hours.

An arabinose-galactose disaccharide was present in extracts 1 and 2, but only a slight trace, or none, was found in extracts 3 and 4. It was hydrolyzed completely by 0.1*N* sulfuric acid in 3 hours.

An arabinose-xylose disaccharide was like the disaccharide above in occurrence and rate of hydrolysis.

An arabinose-galactose-xylose trisaccharide occurred in relatively small amounts in extract 2, with little or none in extracts 3 and 4. It was hydrolyzed by 0.1*N* sulfuric acid in 1 hour.

An apparent tetrasaccharide containing one arabinose, two galactose, and one xylose units was like the above trisaccharide in occurrence, but its R_f was much lower.

A xylose disaccharide, a xylose trisaccharide, and a xylose tetrasaccharide appeared in all the extracts. They were hydrolyzed completely by 0.1*N* sulfuric acid in 7 hours.

Uronides. These were present in the holocelluloses and in all the hemicelluloses and extracts. They were absent from, or present in only slight traces in, the residues.

Five uronides were separated from brome grass hemicellulose. Their constituents as determined by hydrolysis were: galacturonic acid, at least two units; galacturonic acid and rhamnose; glucuronic acid and galactose; glucuronic acid and xylose; and methyl glucuronic acid and xylose.

The first two were present in very small amounts. They may have come from nonhemicellulose contaminants. Free galacturonic acid was also present.

Rhamnose. This sugar was present in the holocelluloses, in increasing amounts in extracts 1 to 4, and in decreasing amounts in hemicelluloses 1 to 4. None could be detected in the residue. In all cases the amounts of rhamnose were small.

Discussion

Treatment of holocellulose with dilute acid produced two distinct results, a dissolving action and a hydrolyzing action which may interact. By far the greatest part of the dissolved material was removed by the combined action of the 1- and 2-hour treatments. As the holocellulose neutralized some of the acid used in the first extraction, the final pH was 3.5. This treatment was far less effective than the second, or 2-hour extraction, in which the pH was maintained at 2.5.

The treatments removed from the holocellulose all the arabinose, galactose, and rhamnose, practically all the uronides, and three fourths of the xylose. The xylose remaining in the residue would appear to be essentially in the form of pure xylan.

A considerable part of the material dissolved by each of the successive treatments was hemicellulose, precipitated from the neutralized extract by ethyl alcohol. These four hemicellulose preparations showed changes in composition as to the relative amounts of the three sugars, galactose, arabinose, and xylose, in them. Whether this resulted from fractionation of the hemicelluloses or only from the gradual removal of galactose and arabinose by hydrolysis is uncertain.

Uronides were present in all four of the hemicelluloses, as well as in the extracts. Thus they were more resistant to removal by hydrolysis than were the sugars except xylose and glucose.

The action of dilute acids for a limited time would be expected to affect mainly the side chains of the hemicellulose molecule rather than its central core (*8*). Thus, the simple sugars and the uronides in the extracts probably formed parts of such side chains.

The uronides isolated from the holocellulose are similar to those prepared from some other hemicelluloses (*1–4, 9*). Small amounts of the uronides appeared in the extracts, but more remained in all four of the hemicellulose preparations. Since only a trace of uronides was found in the residue, it appears that the hemicelluloses containing them are more readily soluble than are the xylans free from uronides, which include the xylose remaining in the residue.

The most direct evidence as to the structure of the hemicelluloses lies in the oligosaccharides occurring in the extracts, especially those which appeared in the earlier treatments and which were easily hydrolyzed. These give some indication of the nature of the side chains attached to the main xylan chain of the polyuronide hemicellulose.

Finding an arabinose disaccharide in the products of mild hydrolysis of the grass holocelluloses shows that longer arabinose side chains may occur, unless a separate araban is present. The oligosaccharides containing arabinose and galactose, arabinose and xylose, and arabinose, galactose, and xylose also indicate side chains of some length. From the large amounts of free arabinose in the extracts it seems probable that more and perhaps longer side chains containing this sugar may occur.

The series of xylose oligosaccharides may have come either from side chains of branched molecules or from the main chain broken at random. The former would appear to be the more probable result of the mild treatment.

The behavior of glucose was quite different from that of the other sugars. Only a very small amount of it appeared in the extracts and that only in the final one, resulting from 16 hours of exposure to the hot, dilute acid. Each of the hemicellulose preparations, and also the residue, yielded glucose on hydrolysis with 1*N* sulfuric acid for 6 hours. No oligosaccharides containing glucose were found in the extracts. It seems probable, therefore, that glucose is not combined in the same molecule as are the other sugars and the uronic acids, but arises from some glucan resembling the other hemicelluloses in solubility.

Acknowledgment

The authors wish to thank G. A. Adams for several authentic samples of aldobiuronic acids.

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Received for review August 17, 1959. Accepted November 6, 1959. Contribution No. 172, 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. 245 of the New Hampshire Agricultural Experiment Station, Durham, N. H.