Table IX. Breakdown of Total Environmental Variation in Oil Content

(Into that due to association with rainfall and mean maximum temperature during maturation period, No. 3, expressed as $R^2(sy^2)$, and that due to all other enviromental factors)

Source	D.F.	sy^2	$R_{y.x_1x_2}$[a]	$R^2(sy^2)$	$(1 - R^2)(sy^2)$
Locations	9	101.97	0.3960	15.99	85.98
Years-in-locations	20	130.52	0.6814[b]	60.60	69.92
Total (location-years)	29	232.49			

[a] y = % oil in kernels; x_1 = inches of rainfall (period 3); x_2 = mean maximum temperature, ° F. (period 3).
[b] Highly significant, 1% level.

gression equations for these relationships are shown graphically in Figure 1. Since the standard errors of estimate are rather high, the regression equations are not suitable for prediction purposes. However, the plot of the equations illustrates those varieties which average highest, lowest, or intermediate in oil content (Table I).

The results of this investigation suggest that it may be possible to increase oil content of cottonseed kernels as cotton varieties are improved, provided this character is considered in selection. However, rainfall and mean maximum temperature during the maturation period seem to play important roles in elaboration of oil in the kernel, as do other environmental factors.

Acknowledgment

This paper is one of several reporting the results of a Regional Cottonseed Study, 1947–49. Acknowledgment is made to H. D. Barker and his associates of the Section of Cotton and Other Fibers of the Field Crops Research Branch. Barker assisted with the over-all plans and developed coordination among the field locations. W. H. Tharp assisted in planning the influence of environment. Thanks are also due individuals responsible for growing the cotton varieties at the field locations, and to Julian F. Jurgens for some of the analyses.

Literature Cited

(1) Am. Oil Chemists' Soc., Chicago, "Official and Tentative Methods of Analysis," 2nd ed., rev. to 1952, Methods Ba 2-38, Ba 3-38, 1946–1952.
(2) Barrow, E. R., and Agee, G. W., *Cotton Oil Press*, **2,** No. 10, 44–5 (1918).
(3) Gallup, W. D., *J. Agr. Research*, **36,** 471–80 (1928).
(4) Garner, W. W., Allard, H. A., and Foubert, C. O., *Ibid.*, **3,** 227–49 (1914).
(5) Grindley, D. N., *J. Sci. Food Agr.*, **1,** 147–51 (1950).
(6) Hancock, N. I., Tenn. Agr. Expt. Sta., *Circ.* **79** (April 1942).
(7) Harrington, M. T., Texas Agr. Expt. Sta., *Bull.* **374** (1928).
(8) Law, T. C., *Cotton Oil Press*, **3,** No. 8, 37–8 (1919).
(9) Meloy, G. S., *Cotton and Cotton Oil Press*, **42,** No. 9, A-7, A-18 (April 26, 1941).
(10) Pons, W. A., Jr., Murray, M. D., O'Connor, R. T., and Guthrie, J. D., *J. Am. Oil Chemists' Soc.*, **25,** 308–13 (1948).
(11) Pope, O. A., and Ware, J. O., U. S. Dept. Agr., *Tech. Bull.* **903** (1945).
(12) Seale, C. C., *Trop. Agr.* (*Trinidad*), **19,** 210–14 (1942).
(13) Sievers, A. F., and Lowman, M. S., "Study of Cottonseed with Reference to Varietal Characteristics and Sources of Production," U. S. Dept. Agr., Bur. Plant Ind., January 1932.
(14) Stansbury, M. F., and Guthrie, J. D., *J. Agr. Research*, **75,** 49–61 (1947).
(15) Sturkie, D. G., Ala. Agr. Expt. Sta, *Bull.* **263** (1947).
(16) Tharp, W. H., Skinner, J. J., Turner, J. H., Jr., Bledsoe, R. P., and Brown, H. B., U. S. Dept. Agr., *Tech. Bull.* **974** (1949).

Received for review March 27, 1954. Accepted June 8, 1954.

FORAGE CROP CONSTITUENTS

The Isolation and Analysis of Hemicelluloses From Orchard Grass

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THE HEMICELLULOSES OF NONWOODY PLANTS have been only sporadically investigated. These structural polysaccharides often amount to 30% of the dry weight of forage plants and supply a major portion of the caloric value of these plants to ruminant animals. Elucidation of their chemical composition would be valuable in determining their nutritive value and their role in plant physiology.

In modern usage hemicelluloses are designated as those cell-wall polysaccharides which contain, in addition to pentoses and hexoses, some uronic acids, and which are soluble in alkali. They are also referred to as polyuronide hemicelluloses (*23*). The nomenclature used in this report for hemicelluloses and for related polysaccharides, follows that adopted by Norman (*23*) and Wise and Ratliffe (*29*). Natural cellulose is considered as being composed of a mixture of true cellulose, a polymer of D-glucose, and the cellulosans (xylans, galactans, arabans, etc.). Holocellulose is the mixture of lignin-free, structural components of the plant cell. This report is concerned principally with the polyuronide hemicelluloses which are designated only as hemicelluloses.

Recent interest in possible commercial utilization of many soft plant materials—e.g., sugar cane bagasse, wheat straw, and corn cobs and stalks—has given impetus to investigations of the hemicelluloses from these sources. Since the early investigations of Schulze (*26*) and Shryver and his associates (*11, 12*) modern methods have been developed, such as chlorite delignification to produce holocellulose from which hemicelluloses may be obtained more readily (*28*), the use of mild extracting agents to avoid solu-

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Polyuronide hemicelluloses, isolated from orchard grass (*Dactylis glomerata* L.), comprise up to 20% of the dry weight. Holocellulose was prepared by treatment of the fat- and pectin-free grass with sodium chlorite and this was extracted successively with hot water, 0.5% potassium hydroxide, and 1.5% potassium hydroxide. Hemicelluloses were obtained from these extracts as precipitates upon acidification, addition of alcohol, and finally by evaporation of the alcoholic solution. These precipitates were hydrolyzed to yield reducing sugars which were separated by paper chromatography and estimated by electron reflection densitometry. Xylose, glucose, arabinose, galactose, and uronic acids were found in almost all hemicellulose fractions, with the first two sugars comprising the major portion of the carbohydrates. Over 70% of all hemicelluloses obtained were from the water extraction of the holocellulose, and these contained lower percentages of pentose than those which were alkali-extracted.

bilizing substances other than hemicelluloses (*2, 6, 22*), and the application of paper chromatography to the polysaccharide hydrolyzates (*7, 9*). The scheme carried out here including these features is presented in Figure 1, which also identifies the fraction numbers referred to in the test.

Methods

Aboveground portions of orchard grass (*Dactylis glomerata* L.) were collected by clipping, at 4 inches from the ground, spaced plants of a single clone at the flowering stage—i.e., when over half the plants were in bloom. They were dried in a forced-draft oven at 80° C. and ground in a Wiley mill to pass a 40-mesh screen. This air-dried grass substance (fraction 1) was treated according to the procedure, indicated in Figure 1, to produce the holocellulose, which, as a white residue, was washed 6 times with ice water, and the washings were discarded, and the residue (fraction 2) dried, first over calcium chloride and finally at 105° C.

The hemicelluloses were isolated from the holocellulose by extraction and precipitation. About 60-gram portions of the holocellulose were extracted with 1.5 liters of water at 100° C. for 72 hours. The mixture was stirred during the whole period, and at the end was filtered, and washed with hot water. The combined filtrate and washings constituted extract A. The wet residue was extracted with about 1.5 liters of 0.5% potassium hydroxide at 25° C. for 72 hours. The filtrate and washings from this batch were extract B. A third extraction with 1.5% potassium hydroxide under similar conditions yielded extract C. Stirring was continuous during these extractions. The final cellulosic residue constituted fraction 12.

The three extracts, A, B, and C, each amounting to several liters, were treated nearly alike. They were acidified to pH 3.0 with acetic acid whereupon precipitates formed from extracts B and C, but not from extract A. These precipitates were removed by centrifuging or filtering, and dried, and constituted the acid-precipitated hemicellulose fractions 6 and 9. Extract A and the filtrates from B and C were brought to pH 7.0 with potassium hydroxide and poured, with stirring, into excess ethyl alcohol. White precipitates formed and were allowed to settle for 48 hours. These precipitates were also removed by centrifuging, and filtering, and dried; they constituted the alcohol-precipitated fractions 4, 7, and 10. The filtrates were evaporated to a sirup under reduced pressure, dialyzed against running water, and evaporated to dryness. These residues constituted the hemicellulose fractions 5, 8, and 11.

All hemicellulose fractions which had been precipitated from solution were redissolved in some of the original extracting solutions and reprecipitated. When dry, they were powdered in a mortar. The alcohol-precipitated fractions were almost white, the acid-precipitated fractions tan, and the alcohol-soluble residues brown.

Moisture, ash, and protein were determined on all fractions by standard procedures (*3*). On some fractions lignin was determined by the method of Ellis, Matrone, and Maynard (*14*), natural cellulose according to Matrone, Ellis, and Maynard (*20*), and true cellulose by the method of Crampton and Maynard (*13*).

Each hemicellulose fraction was analyzed for reducing sugars obtained on hydrolysis. Samples of 0.1 gram were refluxed for 6 hours in a boiling water bath with 5 ml. of 1.0*N* sulfuric acid. The insoluble residues were removed and the solutions neutralized with barium carbonate, and centrifuged. The resulting solutions were chromatographed and also analyzed for total reducing power as glucose. The residues remaining from the hydrolysis were washed, dried, and weighed. They amounted to only 4 to 7% of fractions 4, 5, 7, 10, and 11, but were 34 to 86% of fractions 1, 2, 6, 8, 9, and 12, which were low in hydrolyzable carbohydrates.

The procedure employed for the separation of the sugars by chromatography has been described by Adams and Castagne (*1*), Bennett (*7*), Jermyn and Isherwood (*18*), McFarren *et al.* (*19*), Partridge (*24, 25*), and others (*4, 9*).

Table I. Analysis of Samples

Fraction	Description	Air-Dry Weights, G.	Moisture, %	Ash, %	Protein (Ash-and moisture-free), %
1	Grass	464.5	6.1	8.0	7.1
2	Holocellulose	288.0	5.1	4.3	4.0
	Hemicelluloses H_2O extd.				
3	Acid pptd.	...	...	...	...
4	Alc. pptd.	5.1	9.0	13.0	1.0
5	Alc. sol.	39.6	9.7	6.1	8.2
	0.5% KOH extd.				
6	Acid pptd.	1.8	8.2	14.1	47.5
7	Alc. pptd.	3.3	10.3	6.6	3.5
8	Alc. sol.	7.9	5.0	20.9	22.7
	1.5% KOH extd.				
9	Acid pptd.	0.5	9.2	5.8	20.4
10	Alc. pptd.	4.4	8.8	8.9	1.0
11	Alc. sol.	2.0	9.6	19.6	7.2
12	Cellulosic residue	166.0	6.8	2.2	0.2

Table II. Analysis of Hemicelluloses in Different Fractions

Fraction	Wt. of Fraction, G.	% of Original Grass	% of Holocellulose	% of Total Hemicellulose
4	3.8	1.0	1.5	8.0
5	30.6	8.2	12.2	64.6
6	0.7	0.2	0.3	1.4
7	2.6	0.7	1.0	5.5
8	4.5	1.2	1.8	9.5
9	0.3	0.1	0.1	0.7
10	3.6	1.0	1.4	7.6
11	1.3	0.4	0.5	2.8
Total	47.4	12.8	18.8	100.0

The solvent system was ethyl acetate–pyridine-water, found by Jermyn and Isherwood to be suitable for the resolution of a mixture of glucose, galactose, xylose, arabinose, and uronic acids with the descending technique.

For the quantitative determination of the resolved individual sugars, elution from the paper and measurement of the reducing power was not feasible (*17*). For accurate determinations the concentrations of sugar should be greater than 20 γ per ml. of solution. In these experiments this concentration was attained only with xylose. All the hydrolyzates were low in uronic acids and galactose, and some were low in arabinose and glucose. It was necessary, therefore, to resort to a colorimetric method for the determination of the individual sugars on the chromatographic paper. The paper, after the pyridine had evaporated, was sprayed uniformly with a solution of aniline hydrogen phthalate as recommended by Partridge (*24*). Quantitative measurements of the color intensity were made by densitometry.

The densitometric apparatus was assembled according to the recommendations of Block (*8*), and included a sensitive galvanometer, a light source (automobile headlight tungsten filament lamp and a focusing lens), and a photosensitive cell (barrier layer type). The measurement of color intensity by the light transmitted through the paper was not made successfully because of the random matting of the paper fibers, so the apparatus was rearranged so as to serve as a reflection densitometer (*21*). The photocell received light reflected from the paper at an angle of 45°. The spots from the hemicellulose hydrolyzates were compared with those of known concentrations of sugars appearing on the same chromatographs and bracketing the unknowns. A straight-line relationship existed be-

Figure 1. Flow sheet for hemicellulose extraction and precipitation

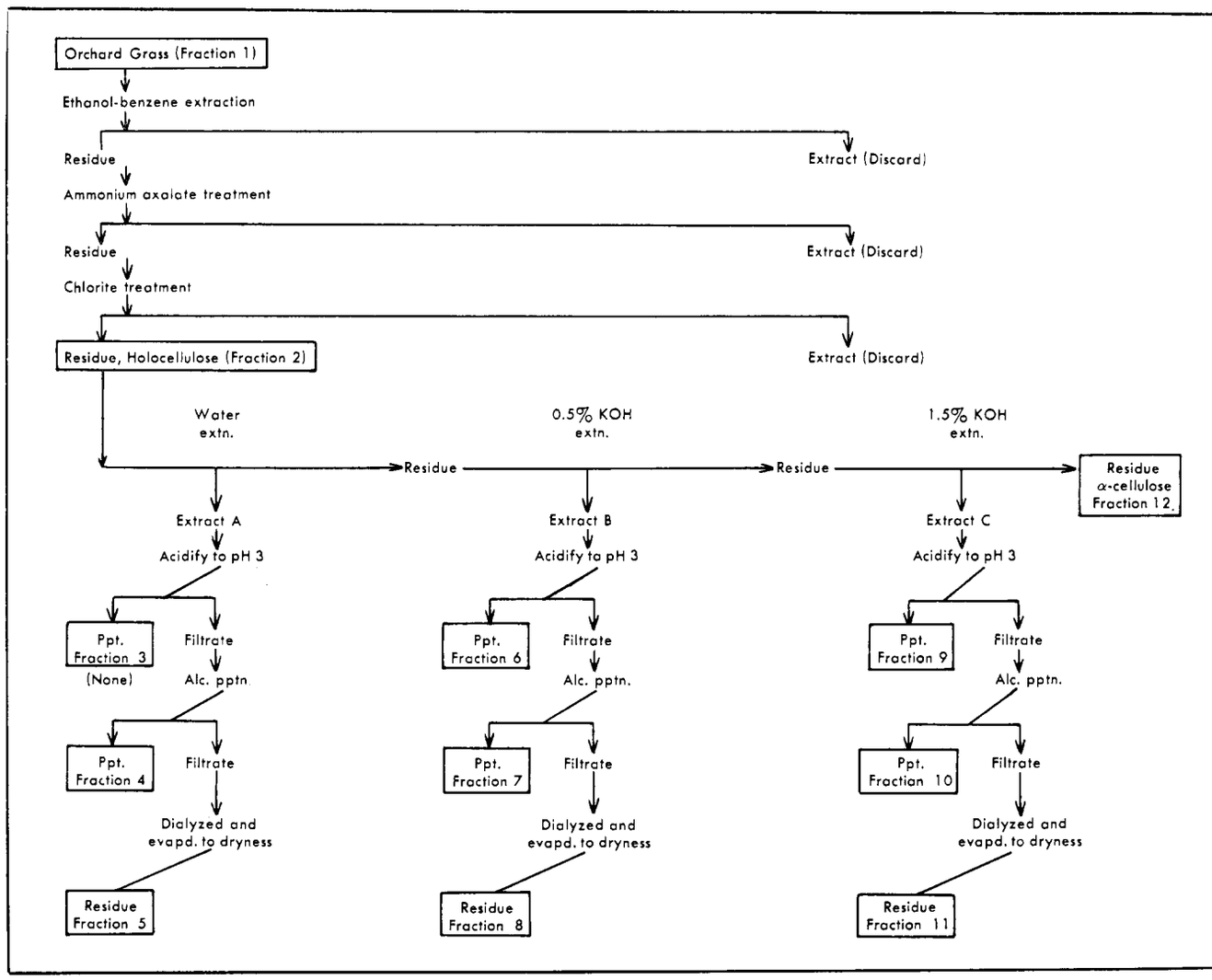

Table III. Analysis of Celluloses, Lignin, and Unhydrolyzable Residues

Fraction		Natural Cellulose %	Natural Cellulose G.	True Cellulose %	True Cellulose G.	Lignin %	Lignin G.	Unhydrolyzable Residue, G.
1	Grass	50.0	185.7	37.5	139.3	8.0	29.7	148.1
2	Holocellulose	71.0	177.8	55.8	139.9	2.5	6.2	130.9
12	Cellulosic residue	98.0	147.6	90.6	136.3	2.2	3.3	130.1

tween the maximum density of the colored spots and the concentrations for each sugar. Blank readings were constant.

Results and Discussion

Table I lists the different fractions obtained and their moisture, ash, and protein contents. In most cases the acid-precipitated and the alcohol-soluble hemicellulose fractions had very high ash and high protein contents. On the other hand, the alcohol-precipitated hemicelluloses (fractions 4, 7, and 10) had low ash and low protein contents and therefore the highest carbohydrate contents. The relative purity of the final cellulosic residue (fraction 12) is indicated by its low ash and protein.

On an ash-, moisture-, and protein-free basis, the yield of hemicelluloses (fractions 4 through 11) from orchard grass was 12.8% (Table II). This percentage is lower than that usually noted for grasses, partly because of losses, and also because more severe extraction procedures were used by others (*27*). Buston (*10*), who early used mild extraction procedures, reported a yield of only 9% as compared to an anticipated yield of 18% from orchard grass.

About 73% of all the hemicellulose obtained resulted from the water extraction of the holocellulose. These water-soluble hemicelluloses amounted to 13.7% of the holocellulose, as compared to 14.7% of wheat straw holocellulose (58% of all the hemicellulose obtained) which has been reported by Adams and Castagne (*1*). These figures are illustrative of results which have necessitated some alteration in the definition of hemicelluloses. Prior to the present decade hemicelluloses were believed to be polysaccharides, which were not water-soluble (*23*), and therefore required alkaline reagents for extraction. The development of the chloriting techniques, coupled with the fact that highly alkaline extracting reagents are nonspecific, has led to the incorporation of a water-extraction step in the procedures currently employed for the isolation of hemicellulose material. These hemicelluloses are rendered water-soluble only after strenuous pretreatment of the whole plant material. Therefore, hemicelluloses are those substances which may be extracted by water from chlorite holocellulose.

Analyses for celluloses, lignin, and unhydrolyzable residues were made in order to determine the procedures necessary for extracting the hemicelluloses from holocellulose. The results are given in Table III. They show that mild alkaline treatments removed a portion of cellulosan material from the holocellulose, because the weight of natural cellulose in fraction 12 is 30 grams less than that originally present in the holocellulose (fraction 2).

The natural cellulose, determined by the modified Norman-Jenkins method (*20*), is usually considered to contain most of the cellulosan material. It is not known, however, how much hemicellulose is also determined along with cellulosans and cellulose. Similarly, in the determination of true cellulose by the method of Crampton and Maynard (*13*), it is not known to what extent cellulosan material interferes. If true cellulose contains no cellulosan material, the weight of the unhydrolyzable residue should be equal to the weight of true cellulose. However, this does not prove to be the case for fractions 2 and 12 (Table III). This comparison is not possible for fraction 1 because of the complex mixture of substances in the original grass, and because of its greater resistance to hydrolysis as compared to the chlorited material. The close agreement of the weights of the unhydrolyzable residues of fractions 2 and 12 with one another indicates that a more exact value for true cellulose would be about 130.5 grams, and that the difference, 6 to 9 grams, between this weight and that of true cellulose determined by the method of Crampton and Maynard, is probably cellulosan. In fraction 12, the difference between the weight of natural cellulose and that of the unhydrolyzable residue is 17.5 grams, which accounts for over 80% of the sugars found in that fraction after hydrolysis. The amounts of hemicellulose and cellulosans contained in the 17.5 grams were not determined.

The steady decrease in the weight of natural cellulose—8 grams from fraction 1 to fraction 2, and 30 grams from fraction 2 to fraction 12, a total of 20% from starting material to final residue—indicates the complexity of the material called natural cellulose. The percentages of hemicellulose and cellulosan were not determined in the 30 grams of natural cellulose removed during the hemicellulose extraction procedures.

The recovery of hemicelluloses was not complete. If 71.0% of the holocellulose was natural cellulose (Table III) the remainder or 29.0% should be hemicellulose. Only 18.8% of the holocellulose was recovered as hemicellulose (Table II) or two thirds of the possible amount. Losses may have occurred during the solution and reprecipitation of hemicellulose fractions and during the dialysis of the final filtrate residues.

The sugars and sugar acids obtained by hydrolysis of the hemicellulose fractions and separated by chromatography were identified as xylose, glucose, arabinose, galactose, and one or more uronic acids. Mannose was not present. For the quantitative determination of the total sugars, the reducing power of the total hydrolyzate was obtained toward copper, and the individual chromatographic spots were measured with the densitometer. The two sets of values were of the same order but in most cases the reduction values were somewhat lower than the sum of the individual sugars, probably owing to the fact that all reducing substances were calculated in terms of glucose (*5*). Those fractions with the highest uronic acid contents showed the greatest percentage difference between the two methods. Fractions 1 and 2, containing only 3 to 4% uronic acids, showed best agreement.

The densitometric method determined

Table IV. Determination of Sugars in Fractions

Fractions	Xylose, %	Glucose, %	Arabinose, %	Galactose, %	Uronic Acids, %	Total, %
1	15.6	10.9	4.1	2.4	3.1	36
2	21.4	4.8	6.1	1.4	4.3	38
3	...	...	...	...	...	...
4	37.0	50.0	5.0	5.0	14.5	111
5	63.0	5.8	5.0	4.7	13.0	92
6	4.0	5.0	2.0	0.3	1.7	13
7	59.4	24.2	5.3	2.0	10.7	102
8	8.5	3.8	1.6	1.1	4.9	20
9	49.0	Trace	2.3	Trace	2.0	53
10	69.2	13.0	5.4	3.5	13.1	105
11	30.1	12.5	6.7	4.6	19.2	73
12	10.6	2.9	0.8	Trace	Trace	14

xylose with the greatest accuracy, and uronic acids and galactose with the least. Reproducibility of galvanometer deflection readings was approximately within 2 galvanometer units and maximum errors were obtained for xylose 4%, glucose 7%, arabinose 10%, galactose 15%, and uronic acids 20%. Xylose and glucose, comprising 60 to 95% of the total carbohydrate of all fractions, were collectively determined with an accuracy of better than 7%.

Each sugar, as well as uronic acid, was present in every fraction (see Table IV). In several fractions some of the sugars occurred in only very small amounts, and the significance of their presence is doubtful.

The recovery of the sugars may be seen from Table V. The xylose and arabinose originally present in the grass are almost completely recovered in the holocellulose, as would be expected because free pentoses are not present in foliar material. Glucose and galactose, on the other hand, show considerable losses, glucose because of its presence in soluble sugars found in grass and galactose because it is a component of pectic substances removed with ammonium oxalate.

A comparison of the pentose and hexose contents of the hemicelluloses isolated from the different fractions demonstrates a trend toward a higher proportion of pentosan material as the alkalinity of the solvent increases, a trend which would, theoretically, culminate in the isolation of pure pentosans (cellulosans). Flanders (*15*, *16*) and others have noted this tendency of increase of pentosan content with increase of the alkalinity of the solvent.

The hemicelluloses and other polysaccharides were formerly believed to fall into configurational groups. Xylose, glucose, and glucuronic acid, because of their configurational similarity, were more likely to be found together, and arabinose, galactose, and galacturonic acid likewise. This phenomenon is mentioned by a number of authors including Norman (*23*) who stated that the appearance of units from both groups in purified and separated preparations is unusual. However, small amounts of sugars may now be isolated and identified with ease, and both configurational groups are found together in orchard grass hemicellulose. The xylose-glucose–glucuronic acid group predominates over the other.

Table V. Recovery of Sugars

(370.7 grams of oven-dry grass)

	Xylose, G.	Glucose, G.	Arabinose, G.	Galactose, G.	Uronic, Acid, G.	Total, G.
Grass	57.8	40.4	15.2	8.9	11.5	133.8
Holocellulose	53.6	12.0	15.3	3.5	10.8	95.2
Total hemicellulose	25.6	5.1	2.2	1.9	5.8	40.7
Cellulosic residue	16.0	4.4	1.2	Trace	Trace	21.6

Conclusions

Hemicellulose fractions, isolated from orchard grass, were present in an approximate amount of 10% of the dry weight of the grass. Seventy per cent of the hemicellulose was obtained by water extraction of holocellulose and the remainder by alkaline extraction following the water extraction. Those fractions obtained by water extraction were relatively higher in glucose and lower in pentoses than those obtained by the subsequent alkaline extractions. The fractions least contaminated with other organic substances were obtained by alcoholic precipitation of the extracts of the holocellulose.

Xylose and glucose comprised 60 to 95%, and arabinose and galactose only 2 to 18% of the carbohydrate portions of all fractions. The hemicelluloses of orchard grass appear then to belong mainly to the xylose-glucose–glucoronic acid configurational group but both of these hypothetical groupings, if they do exist, are found in orchard grass.

The variation in composition among the fractions suggests that a mixture of polyuronide hemicelluloses may occur in grass. Because of the progressive nature of changes that take place in grass during growth and maturation, there may be present a succession of polysaccharide substances which are being incorporated into the structural system of the plant. It is also possible that the different organs and tissues of the plant, at any stage of development, may contain hemicelluloses of different constitution and physical properties.

Acknowledgment

The authors gratefully acknowledge the technical assistance of Ernest Axman of The Pennsylvania State University in assembling the densitometric apparatus. They are indebted to the Corn Products Refining Co. for samples of α-D-galacturonic acid and glucuronolactone.

Literature Cited

(1) Adams, G. A., and Castagne, A. E., *Can. J. Chem.*, **29,** 109 (1951).
(2) *Ibid.*, **30,** 515 (1952).
(3) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 7th ed., 1950.
(4) Balston, J. N., and Talbot, B. E., "A Guide to Filter Paper and Cellulose Powder Chromatography," London, H. Reeve Angel & Co. Ltd., 1952.
(5) Bates, F. J., and Associates, U.S. Dept. of Commerce, Natl. Bur. of Stds., Circ. **C-440** (1942).
(6) Bennett, E., *Arch. Biochem.*, **27,** 99 (1950).
(7) *Ibid.*, *Plant Physiol.*, **26,** 182 (1951).
(8) Block, R. J., *Anal. Chem.*, **22,** 1327 (1950).
(9) Block, R. J., LeStrange, R., and Zweig, G., Academic Press, Inc., "Paper Chromatography," New York, Academic Press, Inc., 1952.
(10) Buston, H. W., *Biochem. J.*, **28,** 1028 (1934).
(11) Candlin, E. J., and Shryver, S. B., *Proc. Roy. Soc.* (*London*), Ser. **B, 103,** 365 (1928).
(12) Clayson, D. H. F., Norris, F. W., Shryver, S. B., *Biochem. J.*, **15,** 643 (1921).
(13) Crampton, E. W., and Maynard, L. A., *J. Nutrition*, **15,** 383 (1938).
(14) Ellis, G. H., Matrone, G., and Maynard, L. A., *J. Animal Sci.*, **5,** 285 (1946).
(15) Flanders, C. A., *Arch. Biochem. and Biophys.*, **36,** 421 (1951).
(16) *Ibid.*, p. 425.
(17) Hawthorne, J. R., *Nature*, **160,** 714 (1947).
(18) Jermyn, M. A., and Isherwood, F. A., *Biochem. J.*, **44,** 402 (1949).
(19) McFarren, E. F., Brand, K., and Rutkowski, H. R., *Anal. Chem.*, **23,** 1146 (1951).
(20) Matrone, G., Ellis, G. H., and Maynard, L. A., *J. Animal Sci.*, **5,** 306 (1946).
(21) Müller, R. H., and Clegg, D. L., *Anal. Chem.*, **23,** 396 (1951).
(22) Norman, A. G., *Biochem. J.*, **31,** 1579 (1937).
(23) Norman, A. G., "The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.," London, *Oxford University Press*, 1937.
(24) Partridge, S. M., *Nature*, **158,** 270 (1946).
(25) *Ibid.*, **164,** 443 (1949).
(26) Schulze, E., *Z. physiol. Chem.*, **16,** 387 (1892).
(27) Weihe, H. E., and Phillips, M., *J. Agr. Research*, **74,** 77 (1947).
(28) Wise, L. E., Murphy, M., and D'Addieco, A. A., *Paper Trade J.*, **122,** 35 (1946).
(29) Wise, L. E., and Ratliffe, E. K., *Anal. Chem.*, **19,** 459 (1947).

Received for review February 1, 1954. Accepted June 11, 1954. Contribution 126, U. S. Regional Pasture Research Laboratory, U. S. Department of Agriculture, in cooperation with the twelve northeastern states, and authorized for publication December 11, 1953, as paper 1847, Journal Series, Pennsylvania Agricultural Experiment Station.