THE ISOLATION AND ANALYSIS OF THE HEMICELLULOSES OF RICE HULLS, ORYZA SATIVA, L.

by

John Edward Pickering

A Thesis
submitted to the faculty of the
Department of Chemistry
in partial fulfillment of
the requirements for the degree of

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THE ISOLATION AND ANALYSIS OF THE ELECTROLYTE-FREE

OF BLOOD SERUM, UREIC ACIDITY, B.

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INTRODUCTION

It has been known for many years that certain substances similar to cellulose are present in the plant cell wall. Upon hydrolysis they give sugars other than d-glucose.(1) They are insoluble in water but are dissolved by alkaline solutions and are reprecipitated by alcohol when the solution is acidified. Schulze called these substances hemicelluloses.(2) The hemicelluloses are more easily hydrolyzed by dilute acids than is cellulose, but they are believed to be in some way related to cellulose. They yield a mixture of pentose and hexose sugars on hydrolysis. For many years the hemicelluloses were believed to be true pentosans or hexosans, or probably hexopentosans.(3) Recently, however, the presence of uronic acid has been established, the most common of these being d-glucuronic acid and d-galacturonic acid. O'Dwyer, in investigating the hemicellulose from American white oak, isolated d-xylose, l-arabinose, d-mannose, and d-galactose. Later in another investigation on beech wood, she unquestionably demonstrated the presence of uronic acid units in the hemicellulose.(4) From these results, it is evident that hemicelluloses are not true carbohydrates as originally believed, but compounds containing acid groups in the form of hexuronic

acids. These results have been confirmed by the work of other investigators, particularly Preece and Norris who worked on wheat bran.(5) They obtained d-xylose, l-arabinose, d-glucose, and a hexuronic acid.(6) It might be pointed out here, however, that not all of the fractions yielded a uronic acid. At the present time, two general types of hemicellulose are recognized.(7) The first of these are polysaccharoses which yield upon hydrolysis only monosaccharoses. The second type consists of the acid hemicelluloses or polyuronides. This type on hydrolysis yields both a uronic acid and monosaccharoses. Norman,(8) has suggested the classification of the hemicelluloses given on page 4.

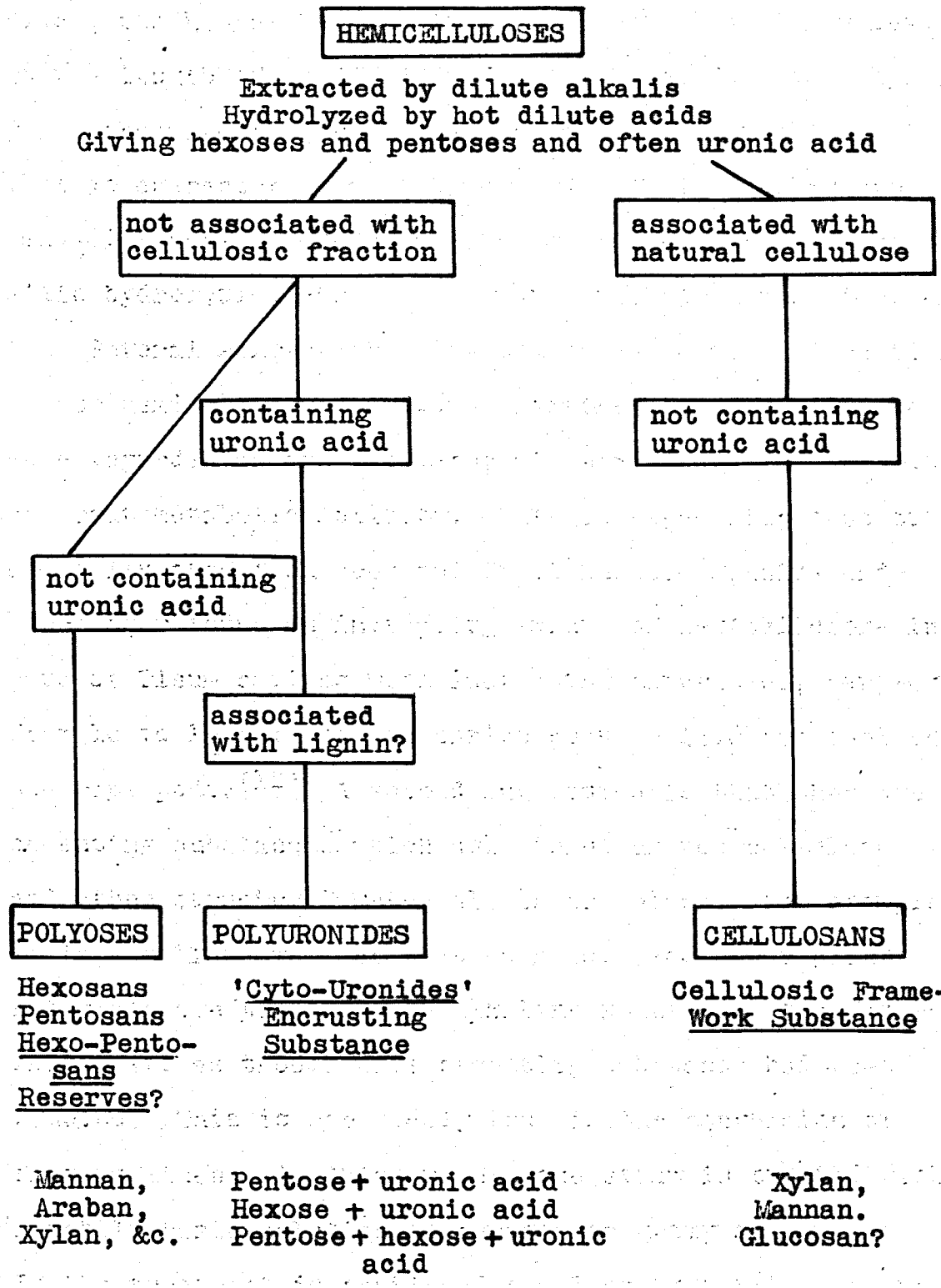
The work of Anderson, O'Dwyer, Preece, Norris and others on the composition of hemicellulose from various materials indicates that they may differ enormously in their composition and structure. The composition of the hemicellulose even in the same type of material may be quite different. Starch, pectin, and cellulose appear to be fairly uniform in their composition wherever found but this is not the case with hemicellulose. Anderson, Kesselman, and Bennet(9) have shown that in certain hardwoods, the sapwood and compression wood vary in their composition. This variation in composition may be a result of the manner in which the hemicellulose is formed. A series of transformations such as the following would, to a certain extent account for the variation in composition

of the various hemicelluloses:[10]

Glucose-----Glucuronic acid
Glucose-----Glucuronic acid-----xylose
Glucose-----Galactose
Glucose-----Galactose-----Galacturonic acid
Glucose-----Galactose-----Galacturonic acid-----Arabinose
Glucose-----Mannose

Table I

The Classification of the Hemicelluloses



There is some evidence for the presence of a beta glycosidic linkage in the hemicelluloses. In the first place, the hemicelluloses in general rotate strongly levo. As the length of the chain is gradually decreased by hydrolysis, the material shows a decrease in levo rotation. This fact is characteristic of beta glycosides. Furthermore, taka-diastase[11] and malt diastase, which are alpha glycosidic hydrolyzing enzymes, do not hydrolyze the hemicellulose.

Several suggestions have been made as to the function of the hemicellulose in plant materials. At one time they were regarded as reserve materials stored against periods of great metabolic activity. This is especially true of hemicelluloses from seed and fruit husks. Schulze and Pfenninger found an increasing amount of hemicellulose in pods of Pisum sativum with increasing maturation, varying from 16 to 19 per cent in unripe pods to 48.6 per cent in the ripe pods.[12] A second suggestion is that they are cementing substances which hold together the cellulose and other structural materials in the plant. For example, it is a well known fact that when wood and other plant materials are treated with alkaline solutions, the fibers fall apart as though some cementing substance had been removed. This is especially true in the conversion of flax to linen. In this process the straw is treated with slightly basic solution to remove the gummy substances. If the treatment is continued too long, the fibers of the

linen are weakened. This is probably caused by the removal of too much of the hemicellulose. Another suggestion is that the hemicelluloses are the polysaccharides that bridge the gap between the insoluble celluloses and the clearly recognized reserve carbohydrates such as starch.[13] Finally, it is known that hemicelluloses exhibit the properties of hydrophilic colloids and hold large amounts of water which can be utilized during long periods of drought.

A more complete review of the literature on hemicellulose will be found in the theses of Kinsman,[14] Nutter,[15] Seeley,[16] Fruin,[17] and in the publications of Butler and Cretcher,[18] Sands and Klass,[19] Anderson and Sands,[20] Anderson and Otis,[21] Anderson and Kinsman,[22] Link and Dickson,[23] and Schoeffel and Link.[24]

EXPERIMENTAL

PRELIMINARY TREATMENT OF THE RICE HULLS

The purpose of the present investigation was to study the hemicelluloses isolated from rice hulls, Oryza Sativa, L.[25] Table II gives the results obtained on analyses of the original rice hulls.[26]

Table II

Analysis of Rice Hulls

*Ash	24.10
Ash after H_2F_2 treatment	2.91
Moisture	4.77
Ether extract	0.022
Crude fiber	41.10
Proteins	1.88
Nitrogen	0.30
**Carbohydrates	69.23

*Contains 88 per cent silica
**Nitrogen Free Extract (by difference) and crude fiber[27]

ISOLATION OF THE HEMICELLULOSES

1. Preparation of Hemicellulose A. Five 400 gram samples of rice hulls were placed separately in six-liter flasks and covered with a 0.5 per cent solution of ammonium oxalate and heated in a boiling water bath for two hours. After cooling, the rice hulls were filtered off on a Buchner funnel and washed with cold water. This extraction with hot ammonium oxalate was repeated twice to insure the complete removal of pectic and other soluble materials. The residual hulls were then mixed with seven times their weight of a 5 per cent solution of sodium hydroxide and heated for four hours in a bath of boiling water. After cooling, the hulls were filtered on a Buchner funnel and washed first with a 4 per cent solution of sodium hydroxide, and then with water. The hulls were set aside for further extraction.

The filtrate, from the first extraction was dark and syrupy in appearance. It was filtered again to remove any foreign particles, and acidified to a pH of 4 with 6N. hydrochloric acid. The dark brown filtrate became lighter in color and a large amount of precipitate formed. An equal volume of 85 per cent ethanol was added to insure complete precipitation and to make possible the filtration. After standing overnight, the clear supernatant liquid was siphoned from the precipitate. The latter was placed on a Buchner funnel and washed with 85 per cent ethanol

until free of chloride. It was then washed three times with 95 per cent ethanol and dried rapidly on porous plates and in vacuo. The yield was 974 grams or 42.35 per cent of the hulls used.

2. Preparation of Hemicellulose B. The rice hulls from which hemicellulose A had been extracted were again extracted with a 4 per cent solution of sodium hydroxide as described above. Since no precipitation formed on acidification, two volumes of 85 per cent ethanol were added and the resulting precipitate was allowed to settle overnight. The clear liquid was tested for complete precipitation and then siphoned off. The precipitate was filtered, washed, and dried as described above. The yield was 100 grams or 5.00 per cent of the original hulls used.

The residual hulls were then washed with water, covered with 0.05N hydrochloric acid and heated in a bath of boiling water for two hours. The liquid was filtered and mixed with three volumes of 85 per cent ethanol to precipitate any pectin. No appreciable precipitate formed. The hulls were next extracted for 24 hours in the cold with a 5 per cent solution of ammonium hydroxide. The filtrate was acidified in the same way and three volumes of 85 per cent ethanol were added. Since no precipitate formed it appears that pectic materials are absent.

3. Chlorination of the Hulls and the Preparation of Hemicellulose C. The rice hulls from which hemicellulose A

and B had been extracted were washed and made neutral with dilute hydrochloric acid and then covered with water. The water solution was made slightly acid with hydrochloric acid and chlorine gas was bubbled through the solution with constant shaking until the hulls became deep red in color. The time required for this color change was one hour, but the chlorination was continued for another hour as a margin of safety. After chlorination, the hulls were thoroughly washed first with water and then with 85 per cent ethanol to remove any of the lignon chloride. To insure complete removal of all of the material rendered soluble by the chlorination process, the hulls were extracted under reflux for one-half hour with 85 per cent ethanol. Since no pectic material was found before chlorination, the test for this was omitted at this point. The hulls were then covered with a 4 per cent solution of sodium hydroxide and hemicellulose C was isolated as described for hemicelluloses A and B. The yield of hemicellulose C was 11 grams or 0.55 per cent of the original hulls used.

4. Purification of the Hemicelluloses. Forty gram samples of hemicelluloses A and B and a ten gram sample of hemicellulose C were dissolved in 4 per cent solutions of sodium hydroxide and the solutions were filtered to remove any insoluble material. The filtrates were brought to a pH of 5 with hydrochloric acid and liquid bromine was added to the solutions until they were saturated. As the

bromine was used up, additional amounts were added so that a slight excess was present during the 24 hour period. At the close of 24 hours, the solutions were mixed with three volumes of 85 per cent ethanol to destroy the slight excess of bromine and to precipitate the hemicelluloses. The precipitates were filtered off, washed free of chloride ion, and dried by suction, porous plates, and in vacuo. The resulting hemicelluloses were white in color.

5. Fractionation of the Hemicelluloses. The purified hemicelluloses were again dissolved in a 2 per cent solution of sodium hydroxide and filtered free of any insoluble material. They were then acidified with hydrochloric acid to a pH of 6. A gelatinous precipitate formed in all of the solutions. The precipitates were separated by centrifuging and washed with 95 per cent ethanol and dried in the usual manner. These precipitates were called fractions A_1, B_1, and C_1 respectively. To the clear filtrates from A_1, B_1, and C_1 was added one quarter of a volume of 95 per cent ethanol. This caused the formation of a second precipitate which was isolated as described above. These were termed fractions A_2, B_2, and C_2. An equal volume of 95 per cent ethanol was added to the filtrate from which the previous fractions had been isolated. This precipitated fractions A_3 and B_3. All of the fractions were thoroughly washed with 85 per cent ethanol and then once with 95 per cent ethanol. When the filtrates from A_3 and B_3 were mixed

with 3 volumes of ethanol only a slight turbidity appeared. Three fractions of hemicelluloses A and B were thus obtained and two fractions of hemicellulose C.

Table III

Summary on the Preparation of the Hemicelluloses

Name	Method of Preparation	Weight
Hemicellulose A	Precipitate obtained by the addition of hydrochloric acid to the first sodium extract.	947.0 g
Hemicellulose B	Precipitate obtained by acidifying the second sodium hydroxide extract.	100.0 g.
Hemicellulose C	Precipitate obtained by adding two volumes of ethanol after chlorination to the acidified sodium hydroxide extract.	10.0 g.
Total weight of Hemicellulose	.....	1057.0 g.
Total weight of hulls used	.....	2000.0 g.
Percentage yield of Hemicellulose	.....	52.9%

ANALYSES OF THE HEMICELLULOSES

All fractions of the hemicelluloses were ground to a powder, passed through a one hundred mesh screen, and analyzed.

1. Moisture. The per cent moisture was determined by drying the samples to constant weight in an oven at 100 C.

2. Ash. The samples were ignited in weighed crucibles at a low temperature until all the volatile matter had been removed. They were then heated to constant weight with a blast burner. The ash was white. In fractions A_1, A_2, A_3, the ash content was approximately 65 per cent of the sample. The presence of silica in fractions A_1, A_2, and A_3, was indicated by the sodium metaphosphate bead test.(28) Treatment of the ash with hydrofluoric acid established the presence of large amounts of silica. The silicon tetraflouride fumes given off formed a gelatinous precipitate with water. A quantitative determination of the per cent silica in the ash from fractions A_1, A_2, and A_3, was carried out with hydrofluoric acid. The ash in the hemicelluloses B and C was so small that no tests for silica were made on these fractions. In all cases, the ash, after removal of silica, had a very faint pink color.

3. Hexose Uronic Acid. This determination is based upon the fact that each molecule of uronic acid present in

the hemicelluloses will give quantitatively one molecule of carbon dioxide when the sample is heated in a boiling 12 per cent solution of hydrochloric acid. This determination is carried out by the method of Lefevre and Tollens[29] with modifications by Dickson, Otterson, and Link.[30] A complete description of the method is given in the thesis of Krznarich.[31] The determination showed that fractions A and C of the hemicellulose did not contain any uronic acid while fraction B did contain a small amount of uronic acid.

4. Pentosan. The per cent pentosan in the hemicelluloses was determined by the phloroglucinol method described in the A. O. A. C. [32] The results were calculated as xylan, from Krober's tables,[33] since d-xylose was found during the investigation and the absence of l-arabinose and methyl pentoses was established.

5. Methoxyl. The uronic acid unit in hemicelluloses often has a methoxyl substituent, however, a quantitative methoxyl determination as described in the thesis of Bennet[34] showed the absence of this group in the hemicelluloses.

6. Optical Activity. 0.1 gram samples of the hemicellulose fractions were dissolved in a 2 per cent solution of sodium hydroxide and the readings were made with sodium light. In all cases except fraction A_1 the solutions were sufficiently clear to be read.

7. Discussion of the Analyses. The analyses indicate that fractions A_1 and A_2 consist of a series of d-xylose units joined to several molecules of d-mannose. The two sugars occur in the ratio of one molecule of d-mannose to seven molecules of d-xylose. On the other hand, fraction A_3 contains the two sugars in the ratio of one molecule of d-mannose to one molecule of d-xylose. Since all of these fractions contained approximately 65 per cent silica, the possibility of silicic acid being in the molecule is suggested. The silicic acid may be joined to the hydroxyl groups by the loss of a molecule of water, or it may be present as free silicic acid. Fractions B_1, B_2, and B_3 contain a small amount of uronic acid which indicates a rather large molecule. These fractions are of the polyuronide type. The equivalent weights and the number of xylose units were calculated from the uronic acid determination and it was found that the molecule varied in length from 27 to 42 xylose units, while the equivalent weights varied from 3789 to 5752. All this seems to indicate that hemicellulose B is a polyuronide made up of from 27 to 42 xylose units joined to one glucuronic acid. If the equivalent weight is calculated from these facts with the assumption of one hexuronic acid per hemicellulose molecule, there is found a decided agreement with the molecular weight calculated from the carbon dioxide basis.

It thus appears that two types of hemicelluloses are

present. Hemicelluloses A and C appear to be similar and are polysaccharoses, while hemicellulose B is a polyuronide.

Tables IV and V give the results obtained on analyzing these hemicelluloses.

Table IV

Summary of Results of the Analyses of the Hemicelluloses

Hemicellulose	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2
Ash %	67.54	64.59	69.80	0.707	0.364	6.41	1.17	1.43
Ash % after treatment with H_2F_2	3.07	2.42	8.80	- - -	- - -	- - -	- - -	- - -
Xylan %*	85.30	84.50	41.52	95.50	95.40	96.20	98.00	86.11
Uronic Anhydride %*	- - -	- - -	- - -	3.06	3.34	4.64	- - -	- - -
Methoxyl %	This determination proved the absence of Methoxyl units in the molecule							
Hexosan %**	14.70	15.50	58.48	- - -	- - -	- - -	- - -	13.89
Total percentage	100.00	100.00	100.00	98.56	98.74	100.66	98.00	100.00

*Calculated on the Ash and Moisture Free Basis
**Calculated by difference

Table V

Optical Rotation and Equivalent Weights of the Hemicelluloses

Hemicellulose	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2
$[\alpha]_D^{25°}$ *	- - -	-52.0°	-65.0°	-62.1°	-64.6°	-68.5°	-75.7°	-81.5°
Equivalent weight from Uronic Acid**	- - -	- - -	- - -	5752	5269	3789	- - -	- - -
Mols of Xylan per mol of Uronic Acid	- - -	- - -	- - -	42	39	27	- - -	- - -
Calculated Equivalent Weight	- - -	- - -	- - -	5720	5324	3740	- - -	- - -

* Absorbed all the light
**No Uronic Acid present

HYDROLYSIS OF THE HEMICELLULOSES AND THE IDENTIFICATION OF THE PRODUCTS OF HYDROLYSIS

1. Method of Hydrolysis. The hemicelluloses were mixed with 15 times their weight of a 4 per cent solution of sulfuric acid and heated for 14 hours in a bath of boiling water. The solutions were then filtered from the insoluble material. In the case of hemicellulose A this residue amounted to 62 per cent of the hemicellulose used. It was identified as silica as previously described. The insoluble material left on hydrolysis of fraction B was very small. It was not examined.

After removal of the insoluble residues the filtrates were neutralized with barium carbonate and the barium sulfate was filtered from the solutions containing the sugars and the barium salts of the sugar acids. The solutions were concentrated in vacuo to a small volume and the salts of the acids were precipitated by addition of warm 95 per cent ethanol. Since there was no uronic acid in fraction A of the hemicellulose, there was no barium salt obtained from this fraction. Fraction B of the hemicellulose gave some barium salt. This salt was heated under a reflux with 95 per cent ethanol to increase the particle size and to aid in its isolation. It was colored a faint yellow. The filtrate from this salt contained the sugars.

2. Analysis of the Barium Salt of the Uronic Acid Complex. The barium salt was purified by solution in

water and treatment with decolorizing carbon. The filtrate was concentrated and the salt was isolated as described above. It was dried on a porous plate and *in vacuo*.

The per cent moisture was determined by drying the salt to constant weight in an Abderhalden dryer at the temperature of boiling water.

The presence of a uronic acid was established by the Napthoresorcinol test[35] on the above barium salt. Since the fraction was known to contain no hexoses, the uronic acid present in the barium salt was identified by conversion to potassium acid saccharate as described by Morrow and Sandstrom.[36] Further identification was established by converting the potassium salt to silver saccharate. The crystals were dissolved in water, neutralized with ammonium hydroxide, and mised with 1.5 times their weight of silver nitrate in a little water. After standing overnight, the silver saccharate was filtered on ashless filter paper, washed, and dried over sodium hydroxide and calcium chloride *in vacuo*. On ignition it gave 52 per cent silver, while the theory for silver saccharate is 50.91 per cent silver.

3. Qualitative Tests for the Sugars. The original hemicelluloses were tested qualitatively for specific sugars as follows.

Orcinol Test. A small sample of the hemicellulose from each fraction was heated with hydrochloric acid and Orcinol.

In all cases a blue precipitate and a green solution formed immediately. This is a positive test for pentoses and negative test for methyl pentoses.(37)

Hydrochloric acid spectral analysis.(38) Small fractions of the hemicelluloses were heated in boiling hydrochloric acid and the solutions were observed through the spectroscope. In all cases there was no absorption of the light in the region of the blue line. This is positive for pentoses and negative for methyl pentoses.(39)

Rosenthaler's Test.(40) Samples of the hemicelluloses were mixed with 2ml. of hydrochloric acid and 1ml. of acetone and heated to boiling. A red color appeared. This indicates the presence of pentoses and the absence of methyl pentoses.(41)

Napthoresorcinol Test.(42) Hemicellulose samples were heated in hydrochloric acid and two drops of napthoresorcinol added. A red color appeared instantly. This also indicates the presence of pentoses and the absence of methyl pentoses.

Alpha Benzyl Phenyl Hydrazone.(43) The pure sugar crystals were dissolved in a small quantity of a 70 per cent solution of ethanol and mixed with alpha benzyl phenyl hydrazine and allowed to stand overnight. No precipitate of the hydrazone was obtained. This proves the absence of l-arabinose.

4. Isolation and Identification of the Sugars from

Hydrolysis. After the removal of the barium salts as described above, the alcohol solutions containing the sugars were concentrated in vacuo to a thick syrup. The resulting syrups were mixed with glacial acetic acid. The syrups from fractions A and B began to crystalize after standing in the refrigerator. The crystals were filtered off and washed with glacial acetic acid and alcohol, and dried in the air. These sugars were identified as d-xylose by their $[\alpha]_D^{25}$, melting point, and by the Bertrand test.(44) They gave the characteristic boat shaped crystals of the cadmium bromide-cadmium xylonate double salt. The results obtained in the qualitative tests are given in Table VI.

5. Isolation and Identification of d-Mannose. Quantitative analyses of fractions A_1, A_2, A_3, and C_2 indicate the presence of a hexose in these fractions. The Skatole(45) test also indicated the presence of a hexose. The phenyl hydrazone test for d-mannose was positive.(46) When this test was applied a precipitate formed immediately. The crystals were similar to those of d-mannose phenyl hydrazone, prismatic when crystalized from a water solution and needle-like when recrystalized from an ethanol solution.(47) The melting point was 171° C. which is lower than the melting point of pure d-mannose phenyl hydrazone. However, it is possible that some d-mannosazone was formed and that the crystals were not perfectly pure.

Table VI

Summary of Analyses of Barium Salts and Sugars

Name	Method of Identification	Weight
Barium Salts from Fraction B	Napthoresorcinol color test Potassium Hydrogen Saccharate Crystals Silver Saccharate Salt The weight of the Silver obtained was 53% of the Silver Salt	0.60 g.
Sugar A	$[\alpha]_D^{25°}$ .....+16.4 Melting Point.....145.0 Cadmium bromide cadmium xylonate double salt Mannose phenyl hydrazone Crystals Melting Point.....171.4	13.02 g.
Sugar B	$[\alpha]_D^{25°}$ .....+19.5 Melting Point.....145.1 Cadmium bromide cadmium xylonate double salt were observed	19.50 g.

SUMMARY

Rice hulls gave a mixture of hemicelluloses amounting to approximately 53 per cent of their weight. This mixture was separated into eight different fractions. Two types of hemicelluloses were present. One of these belongs to the polysaccharose type of hemicellulose and the other to the polyuronide type.

One of the fractions among the polysaccharoses consisted of a pure xylan. The other fractions consisted of manno-xylan complexes, varying in composition from one to seven molecules of d-xylose, combined with one molecule of d-mannose.

The polyuronide hemicelluloses that were obtained contained from 27 to 42 molecules of d-xylose, combined with one molecule of d-glucuronic acid.

All of the hemicelluloses that dissolved during the first treatment with sodium hydroxide contained approximately 65 per cent silica. The hemicelluloses obtained by later extractions with sodium hydroxide contained only traces of silica. It is possible that the silicic acid is combined in some way with the hemicellulose.

None of the hemicelluloses gave any test for starch.

BIBLIOGRAPHY

1. Onslow, M. W., The Principle of Plant Biochemistry, II, 70, (1931)
2. Schulze, E., physiol. Chem., 14, 227, (1890)
3. Norman, A. G., The Biochemistry of Cellulose The Polyuronides Lignin, etc., 36, II, (1937)
4. O'Dwyer, M. H., Biochem. J. 20, 656, (1926)
5. Norris, F. W. and Preece, I. A., Biochem. J. 24, 59-66
6. Norris, F. W. and Preece, I. A., op. cit., 67
7. Anderson, E., J. of Biol. Chem., 91, 560, (1931)
8. Norman, A. G., op. cit., 39
9. Bennet, E., Master's Thesis, Univ. of Ariz., (1930)
10. Onslow, M. W., op. cit., 76
11. Onslow, M. W., op. cit., 73
12. Schulze and Pfenninger, Z. physiol. Chem., 68, 93, (1910)
13. Hawley, L. F. and Wise, L. E., The Chemistry of Wood, 40, (1926)
14. Kinsman, S., Master's Thesis, Univ. of Ariz., (1932)
15. Nutter, P., Master's Thesis, Univ. of Ariz., (1934)
16. Gary, W. Y., Master's Thesis, Univ. of Ariz., (1932)
17. Fruin, J. C., Master's Thesis, Univ. of Ariz., (1933)
18. Butler and Cretcher, J. A. C. S., 52, 4509, (1930), and J. A. C. S., 53, 4160, (1931)
19. Sands and Klass, J. A. C. S., 51, 3441, (1929)
20. Anderson and Sands, J. A. C. S., 48, 3172, (1926)

21. Anderson and Otis, J. A. C. S., 52, 4461, (1930)

22. Anderson and Kinsman, J. Biol. Chem., 94, 39, (1931)

23. Link, K. P. and Dickson, A. D., J. Biol. Chem., 86, 491, (1930)

24. Schoeffel, E. and Link, K. P., J. Biol. Chem., 100, 397, (1937)

25. Stuttgart Rice Mills, Stuttgart, Arkansas

26. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Washington, second edition, (1925)

27. First Annual Report of the Arizona Feed Control Office, 338, (1938)

28. Reedy, J. H., Qualitative Analysis, 129, (1932)

29. Van der Haar, A. W., Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsauren, Gebruder Borntraefer, Berlin, 71-76, (1920). Lefevre and Tollens, Ber., 40, 4513, (1907). Lefevre, Untersuchungen uber die Glukuronsaure, Dissertation, Gottingen, (1907)

30. Dickson, A. D., Otterson, H. and Link, K. P., J. A. C. S., 52, 775, (1930)

31. Kznarich, P., Master's Thesis, Univ. of Ariz., (1935)

32. Official and Tentative Methods of Analysis of the Agricultural Chemist, Washington, second edition, 284, (1925)

33. Ibid.

34. Bennet, E., Master's Thesis, Univ. of Ariz., (1940)

35. Browne, C. A., A Handbook of Sugar Analysis, 383, (1912)

36. Morrow, C. A. and Sandstrom, W. M., Biochemical Laboratory Methods, 165, (1935)

37. Browne, C. A., op. cit., 382

38. Browne, C. A., op. cit., 385

39. Browne, C. A., op. cit., 386.

40. Rosenthaler, L., Zum Nachweis von Methylpentosen und Pentosen Z. anal. Chem., 48, 165-171, (1909)

41. Ibid.

42. Browne, C. A., op. cit., 382

43. Van der Haar, A. W., Nachweis, zur Trennung und Bestimmung der reinen und aus Glukosiden usw. erhaltenen Monosaccharide und Aldehydsauren, 167, (1920)

44. Morrow, C. A. and Sandstrom, W. M., op. cit., 163.

45. Dische und Popper, Biochem. Zietschrifte, 175, 371 (1926)

46. Morrow, C. A. and Sandstrom, W. M., op. cit., 166-167

47. Ibid.