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STRUCTURE OF HEMICELLULOSES ISOLATED FROM *CANAVALIA ENSIFORMIS* AND *TRITICUM AESTIVUM* STRAWS

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Abstract—Hemicellulose was extracted from horse bean and wheat straws in a yield of 5 and 9% respectively. The whole hemicellulose was hydrolysed and the molar ratio of the component monosaccharides was determined. Uronic acid, galactose, glucose, arabinose and xylose were found in both hemicelluloses. The molar ratio of the monosaccharides was determined in each of 4 fractions derived from the saccharide. The main fractions (B and C) were partially hydrolysed and an oligosaccharide containing arabinose and xylose (1:1) was isolated from both hemicelluloses. Another oligosaccharide containing xylose and glucose (2:1) was also isolated from wheat straw hemicellulose. Periodate oxidation was carried out on fractions B and C. The formic acid and the consumed periodate were determined. Each hemicellulose was subjected to Smith's degradation. Glycerol, erythrytol and compounds containing xylose and glycerol (1:1), and xylose and erythrytol (1:1) were isolated.

INTRODUCTION

IT IS believed that groups of hemicellulose differ in structure and are characterized according to their origin and method of isolation. The nature of the sugar residues which form the building units of the hemicellulose varies according to the source of the polysaccharides[1].

The hemicellulose is generally a mixture of different polysaccharides; however, in many cases the separation into a single polysaccharide is difficult. The most important of these polymers is xylan which generally consists of a main linear chain of 1–4 β-linked xylopyranose units. In some cases, the xylan chain is branched[2,3]. Branches were formed of L-arabinofuranose units in some hemicelluloses,[4,5] while in other hemicelluloses D-glucuronic acid and its 4 *O*-methyl derivative are directly attached to the xylan chain.[1,6,7] Details about the chemical structure of wheat straw hemicellulose have been reported.[8–13]

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In the present work the structure of horse bean and wheat straw hemicelluloses have been studied using graded hydrolysis, periodate oxidation and Smith's degradation techniques.

RESULTS AND DISCUSSION

Prior to the extraction of hemicellulose, the samples were extracted with ethanol:benzene to remove the lipids. Soluble pectic substances were removed by exhaustive extraction with ammonium oxalate solution followed by washing with warm water (50°). After delignification, the hemicellulose was extracted with sodium hydroxide solution (4% at 20–25°) under nitrogen to prevent depolymerization due to the alkali.

The yield of hemicellulose obtained from wheat and horse bean straws was 9 and 5·1%. This yield is relatively low as compared with that reported in literature for wheat straw (20–25%).[8,10]

The hemicelluloses of wheat and horse bean straws were hydrolysed, the molar ratio of the component sugars determined and their relative percentage calculated. The results are reported in Table 1. Horse bean straw hemicellulose contains a low percentage of arabinose (8·0%) as compared with that of wheat straw (17·1%). On the other hand the percentage of uronic acids was higher in the horse bean straw (14·5%) and relatively lower in wheat straw (8·3%). The percentage of the other monosaccharides as glucose, galactose and xylose seems to be quite close in both hemicelluloses.

TABLE 1. MOLAR RATIO (MR) AND RELATIVE PERCENTAGE OF THE MONOSACCHARIDES IN THE HYDROLYSATE OF HEMICELLULOSES

Source of hemicellulose	Uronic acid MR	Galactose MR	Glucose MR	Arabinose MR	Xylose MR
Wheat	1·4	1	2·2	3·7	12·4
Horse bean	3·2	1	2·2	2·3	18·5
	%	%	%	%	%
Wheat	8·3	5·6	12·1	17·1	56·9
Horse bean	14·5	4·1	9·6	8·0	63·5

Fractionation of hemicellulose

In very few cases, it was possible to obtain a pure hemicellulose fraction by a single extraction. However, in most cases the product was a mixture of different polysaccharides. The fractionation could be carried out by several procedures.[14–16] In this investigation, the fractionation was carried out by fractional precipitation from ethanol. The results given in Table 2 are a rough indication that the hemicellulose is a mixture of different polysaccharides which may have different molecular weights and shapes. This assumption has been roughly proved by determination of the ferricyanide number of the hemicellulose fractions, as reported in Table 3. The hemicellulose of wheat straw has a relatively low MW as compared with that of the horse bean straw, as indicated by the ferricyanide number. Each hemicellulose fraction, was hydrolysed and the molar ratio of the com-

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ponent sugars was determined. The results given in Table 4 show that the galactose disappeared from some fractions. This is proof that the galactose is not incorporated in all the polysaccharide fractions. It arises probably from a galactan which is mixed with the other polysaccharides. All the other hemicellulose fractions contain uronic acid, glucose, arabinose and xylose in different percentages. All these monosaccharides may be incorporated in a single heterogeneous polysaccharide or may arise from a polysaccharide mixture.

TABLE 2. WEIGHT OF THE HEMICELLULOSE FRACTIONS OBTAINED FROM 5 g OF HEMICELLULOSE

Fraction	Wheat (wt)	Wheat (%)	Bean (wt)	Bean (%)	EtOH–H_2O ratio
A	0·17	3·4	0·31	6·2	1:5
B	2·0	40·0	3·0	60·0	2·5:5
C	2·4	48·0	1·0	20·0	3·5:5
D	0·2	4·2	0·41	8·2	4·5:5

TABLE 3. FERRICYANIDE NUMBER OF THE DIFFERENT HEMICELLULOSE FRACTIONS

Plant	Fractions A	B	C	D	Plant	Fractions A	B	C	D
Horse bean straw	0·82	1·0	1·06	1·14	Wheat straw	1·10	1·65	1·85	1·95

TABLE 4. MOLAR RATIO (MR) AND RELATIVE PERCENTAGE OF THE MONOSACCHARIDES IN THE HYDROLYSATE OF DIFFERENT HEMICELLULOSE FRACTIONS

Hemicellulose	Uronic acids MR	Galactose MR	Glucose MR	Arabinose MR	Xylose MR
Horse bean fractions					
A	2·5	1·0	2·8	1·6	25·0
B	1·8	0·0	1·0	1·0	6·0
C	2·0	1·0	1·8	2·3	12·0
D	2·8	0·0	1·0	1·8	12·2
	%	%	%	%	%
A	9·5	3·5	9·8	4·6	72·6
B	21·3	—	10·9	9·0	58·8
C	12·7	5·9	10·6	11·4	59·4
D	19·4	—	6·4	9·5	64·7
Wheat straw fractions					
A	1·0	0·0	1·5	2·8	7·8
B	1·0	0·0	1·0	1·5	18·5
C	2·4	0·0	1·0	2·5	31·3
D	3·0	1·0	2·0	5·0	8·0
	%	%	%	%	%
A	9·5	—	12·2	20·4	57·9
B	6·0	—	5·3	6·3	82·4
C	8·1	—	3·3	6·6	82·0
D	19·1	5·9	11·8	24·2	39·0

Partial hydrolysis of the hemicellulose

Fractions B and C of both hemicellulose fractions were first hydrolysed in 0·02 N oxalic acid. In all cases the hydrolysate was found to contain arabinose and an oligosaccharide with R_g 0·64. The presence of arabinose in the mild acid hydrolysate suggests that this sugar is in the furanose form. The oligosaccharide (R_g 0·64) gave only one spot on developing with two different chromotographic solvents, which indicates that this oligosaccharide is a single compound and not a mixture of different sugars. Hydrolysis of this oligosaccharide gave xylose and arabinose in the molar ratio 1:1. The isolation of such an oligosaccharide is proof that the arabinose is linked to the xylose. The presence of such xylose-arabinose linkage in wheat straw hemicellulose is in agreement with that reported by other investigators.[9,12]

Graded hydrolysis of another portion of fractions B and C of both hemicelluloses with oxalic acid (1%), gave xylose, arabinose, glucose, galactose and different oligosaccharides. One oligosaccharide with R_g 0·15 was isolated from both fractions of hemicelluloses. After purification this was found to contain only xylose. Another oligosaccharide (R_g 0·13) isolated from the hydrolysate of wheat straw hemicellulose (fraction B and C), was found to contain xylose and glucose in the molar ratio 2:1. The identification of this oligosaccharide proved that the glucose is linked to the xylose in wheat straw hemicellulose samples. This oligosaccharide gave only one spot by paper chromatography in several solvents. Smith's degradation and detection of xylosyl-erythritol is also a proof for the presence of xylose-glucose linkages in the hemicellulose sample.

Periodate oxidation and Smith's degradation of the hemicellulose

Periodate oxidation was carried out on fraction B and C of both hemicelluloses, and the molar ratio of APU (anhydropentose units) to formic acid, and the molar ratio of periodate consumed per mol of APU were calculated (Table 5). The results reveal that the hemicellulose isolated from horse bean straw has generally a longer chain than that of wheat straw. It is also observed that about 1 mol of periodate is consumed per mol of APU. The periodate consumption suggests the presence of unbranched chains of xylan, glucan or xyloglucan.

TABLE 5. MOL OF APU CORRESPONDING TO 1 MOL FORMIC ACID AND 1 MOL OF PERIODATE CONSUMED PER MOL APU

	Mol APU/mol formic acid		Mol $NaIO_4$/mol APU	
Fraction	B	C	B	C
Horse bean straw	10·17	8·9	0·92	0·94
Wheat straw	9·2	6·3	0·94	1·06

Smith's degradation technique was also applied to study the structure of fractions B and C of both hemicelluloses. The oxidized hemicellulose, after being reduced, was partially hydrolysed. Glycerol, erythritol and a compound with R_g 0·95 were detected in the hydrolysate of both hemicellulose fractions. Another compound (R_g 0·90) was detected in fraction B of wheat and horse bean straw hemicelluloses. Erythritol originates from a 1–4 linked glucose units. Glycerol derives mainly from the pentose units linked through 1–4

in the main xylan chain. Smaller amounts of glycerol may also be derived from terminal arabofuranose residues or from terminal hexopyranose units. The compound with R_g 0·95 gave xylose and glycerol in the molar ratio of 1:1 after purification and hydrolysis. The presence of immune xylose linked to the glycerol residue is proof of the presence of a main chain of 1–4 linked xylose units with some branching at C_2 or C_3.

The compound with R_g 0·90 was separated and purified and found to contain xylose and erythritol in the molar ratio of 1:1. It originates most probably from a xylose unit immune to periodate and linked to a glucose unit. The isolation of the xylosyl erythritol is further proof for the presence of glucose linked to xylose in the hemicellulose samples.

EXPERIMENTAL

Source of the samples. The samples of wheat and horse bean straws were obtained from the experimental station of the Faculty of Agriculture, Cairo University.

Extraction of hemicellulose. A finely ground and dried sample (20 g) was extracted with EtOH–C_6H_6 (1:2) in a Soxhlet apparatus for 6 hr. The pectic substances were removed by exhaustive extraction with ammonium oxalate (1 l. 0·5%) at 85° and the residue was thoroughly washed with dist. H_2O. The lignin was removed by treatment with sodium chlorite–HOAc soln.[17] The hemicellulose was extracted from the residual material by shaking with NaOH soln (1 l., 4%) at 25° under N_2 for 20 hr. The mixture was filtered through muslin cloth and the residue re-extracted for 48 hr with 4% NaOH (1 l.) as described. The combined solns were acidified with HOAc (pH 5) and the polyuronide precipitated with EtOH (2 vol.). The hemicellulose was separated, washed with dist. H_2O and dialysed in cellophane for 24 hr at 5°. The residue was separated and then successively washed with EtOH, Et_2O and dried *in vacuo* at 60°.

Hydrolysis of the hemicellulose. The hemicellulose (0·5 g) was mixed with H_2SO_4 soln (3·4 ml, 72%) and the temp. kept at 0° for 20 hr. The suspension was mixed with dist. H_2O to give a final conc of 1 N H_2SO_4. The mixture was then heated at 100° under a reflux condenser for 6 hr.[18]

Determination of the molar ratio of the monosaccharides in the hydrolysate. The hydrolysate was neutralized with $BaCO_3$ filtered, then evaporated under red. pres. to 5 ml. A portion of the soln was subjected to quantitative PC on Whatman No. 1 paper with pyridine–*n*-BuOH–H_2O (3:10:3) as solvent.[19] The molar ratio of the component sugars was determined according to the method described by Smith *et al.*[20]

Fractionation of the hemicellulose. The whole hemicellulose (5 g) was dissolved in KOH soln (50 ml, 4%) at 25°. Any insoluble residue, was separated by centrifugation and the soln was neutralized with HOAc to pH 6. The hemicellulose was then fractionated by adding increasing amounts of EtOH.

Graded hydrolysis of the hemicellulose fractions. Fractions B and C of the hemicellulose (0·5 g) were partially hydrolysed with oxalic acid soln (100 ml, 0·02 N) at 100° for 3·5 hr.[21] Another portion was also hydrolysed with oxalic acid (100 ml, 1%) at 100°, 1 hr.[22]

Chromatographic isolation of some oligosaccharides. The partially hydrolysed hemicellulose was neutralized with $BaCO_3$, filtered, then evaporated under red. pres. to a small vol. (2 ml). The oligosaccharides were separated chromatographically on a Whatman No. 3 thick paper by using pyridine–*n*-BuOH–H_2O (3:10:3) as solvent. Each oligosaccharide was separately eluted from the paper then conc. under red. pres. and re-chromatographed on Whatman No. 1 paper with pyridine–EtOAc–H_2O (1:2·5:5) as solvent.[23]

Hydrolysis of the oligosaccharides. Each oligosaccharide was hydrolysed with H_2SO_4 (3 ml, 0·5 N in a sealed tube). The hydrolysate was analysed by PC as described above and the monosaccharides quantitatively determined with the phenol H_2SO_4 procedure.

Periodate oxidation of the hemicellulose. Hemicellulose (0·2 g) of fractions B and C was oxidized with an excess of 0·1 N sodium metaperiodate in the dark at 5°. Aliquots were titrated periodically for the determination of HCO_2H[24] and periodate consumption.[25]

Smith's degradation of hemicellulose.[26] Hemicellulose (0·5 g) of fractions B and C was oxidized with periodate.

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The polyaldehyde in soln was treated with slight excess of $Ba(OAc)_2$ to ppt the iodate and periodate. After filtration, the polyaldehyde was reduced with sodium borohydride.[27] The excess of reducing agent was destroyed by acidification with HCl. The polyalcohol was partially hydrolysed by maintaining the soln at pH 0·5 at 25° for 6 hr. The soln was deionized by passing through cation exchange resin (Amberlite IR 120), an anion exchange resin (Amberlite IRA 400). The deionized solution was evaporated to dryness under red. pres. at 60°. The residue was treated 3 × with MeOH (20 ml) and evaporated to dryness to remove the borate as methyl borate. PC analysis of the borate-free soln was carried out with *n*-BuOH–EtOH–H_2O (3:2:1) as solvent and ammonical silver nitrate as spray reagent.

Two of the components (R_g 0·95 and 0·90) were separated by thick PC and purified as described above. Each compound was hydrolysed with H_2SO_4 solution (0·5 N) by heating for 3 hr at 100°. The hydrolysate was subjected to PC analysis using *n*-BuOH–EtOH–H_2O (3:2:1) as solvent. The sugars were determined quantitatively using the phenol–H_2SO_4 procedure.[20] The alditols were oxidized with periodate and the formaldehyde was determined by the chromotropic acid method.[28] The molar ratio of the monosaccharides and alditols was determined.

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