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INVESTIGATION ON THE STRUCTURE OF THE HEMICELLULOSE OBTAINED FROM THE FIBER OF *Sansevieria trifasciata* LEAVES

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ABSTRACT

On purification, the hemicellulose released from the extractive-free, delignified fiber of *Sansevieria trifasciata* leaves by 4% alkali yielded a product containing D-xylose and 4-*O*-methyl-D-glucuronic acid in the molar ratio of ~5:1. An aldobiouronic acid from this hemicellulose was characterized. The investigation revealed that the hemicellulose consists of a polymer of (1→4)-linked D-xylopyranosyl residues having branches of D-xylopyranosyl and 4-*O*-methyl-α-D-glucopyranosyluronic acid groups on the O-2 atoms of the main chain.

INTRODUCTION

Natural, carbohydrate fibers other than cotton include those of bast and leaves, and these are of importance in the textile industry. The hemicelluloses of several such fibers have been studied[1–9]. We now report the results of investigations conducted on one of the hemicellulose fractions isolated from the fibers of the leaves of *Sansevieria trifasciata* (family, Liliaceae). The principal uses of these leaf fibers include cordage, fishnet, and coarse fabrics for making bags[10]. These plants grow wild, but are also cultivated, and are grown as decorative plants.

RESULTS AND DISCUSSION

The extractive-free fibers contained 9.6% of lignin, which was removed by two successive treatments of the material with sodium chlorite. The product was successively extracted with hot water, and 4 and 10% sodium hydroxide solutions. The three hemicellulose fractions, and the residual, cellulose-rich material, were obtained in yields of 0.5, 20.4, 11.5, and 68%, respectively, on the basis of the dry

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weight of the holocellulose. The results of analysis of these fractions are shown in Table I.

TABLE I

RESULTS OF PRELIMINARY ANALYSIS OF THE DIFFERENT FRACTIONS OF HEMICELLULOSES FROM *Sansevieria trifasciata*

Fraction	*Yield*[a] *(g)*	$[\alpha]_D^{23}$ *in* M *NaOH (degrees)*	*Approximate mole%*[b]					
			Xylose	*Uronic acid*	*Arabinose*	*Mannose*	*Galactose*	*Glucose*
1	0.23	−20	58.1	10.8	5.2	7.3	8.4	10.2
2	9.18	−64	78.4	15.4	4.2	trace	trace	trace
3	5.12	−61	72.2	12.4	4.4	2.8	3.5	5.0
Purified fraction 2		−72	82.0	16.8	trace	—	—	—

[a]On dry-weight basis of the hollocellulose (45 g). [b]The carboxyl-reduced hemicelluloses were analyzed, after hydrolysis, by g.l.c. using column (*a*).

Fraction II, containing xylose, 4-*O*-methylglucuronic acid, arabinose, and traces of galactose, glucose, and mannose, was studied in detail. It was purified through formation of its copper complex[11,12]. On hydrolysis, the purified material yielded xylose as the only neutral sugar, and 4-*O*-methylglucuronic acid and an aldobiouronic acid as acidic sugars. The uronic acid in the purified hemicellulose was reduced completely, and, on hydrolysis, the product yielded xylose and 4-*O*-methylglucose in the molar ratio of 5:1. These two products were isolated from a large batch of reduced, hemicellulose hydrolyzate, and study of their specific rotation (+20° and +59° in water, respectively) indicated that both had the D configuration.

Partial hydrolysis of the pure hemicellulose with 0.5M sulfuric acid for 6 h at 100° yielded several oligosaccharides, from which an acidic oligosaccharide could be isolated in good yield by resolution on paper. On hydrolysis, the oligosaccharide ($[\alpha]_D^{23}$ +101°, and equivalent weight 332) yielded three products that, in paper chromatography, had the same mobility as those of the unreacted oligosaccharide, 4-*O*-methylglucuronic acid, and xylose. The values of the equivalent weight and specific rotation indicated that the oligosaccharide might be 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. To check this, the aldobiouronic acid was converted into its methyl ester methyl glycoside[9], and this was reduced with lithium aluminum hydride in oxolane. This carboxyl-reduced aldobiouronic acid (now neutral), and the original aldobiouronic acid, were methylated by Kuhn's procedure[13,14]. The methylated aldobiouronic acid was reduced with lithium aluminum hydride. These two products were then separately hydrolyzed, and the hydrolyzates studied both by g.l.c. and by isolating the materials by resolution on paper. The results, shown in Table II, together with those discussed earlier, proved that the oligosaccharide was, indeed, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

TABLE II

METHYL ETHERS OF SUGARS FROM (*A*) THE HYDROLYZATE OF REDUCED, METHYLATED ALDOBIOURONIC ACID, AND (*B*) THE HYDROLYZATE OF METHYLATED, REDUCED ALDOBIOURONIC ACID

Sugars[a]	*T*[b]	*Mole%*		*Properties of isolated sugars*		
		A	*B*	$[\alpha]_D^{23}$ *in water (degrees)*	*Derivative*	*M.p. of derivative (degrees)*
2,3,4,6-Glc	1.00	53		+82	anilide	135
2,3,4-Glc	2.23		51	+68.4	anilide	144
3,4-Xyl	1.2	47	49	+19	anilide	116

[a]2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methylglucose, etc. [b]Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucose as unity, in column (*b*).

The pure hemicellulose was now permethylated; that it was fully methylated[15-17] was shown by the absence of OH bands from the i.r. spectrum. The product was first hydrolyzed with 85% formic acid, and, after removing the formic acid, with 0.5M sulfuric acid; this acid was neutralized with $BaCO_3$. Part of the hydrolyzate was converted into alditol acetates, and the mixture analyzed by g.l.c. The rest of the hydrolyzate was passed through Dowex-50W X-8 (H^+) and then through Dowex-1 X-4 (HCO_3^-) resin. The eluate and washings were combined, and evaporated to a syrup. The mixture was resolved on paper, and the three partially methylated sugars were characterized (see Table III). The acidic fraction was eluted from the column of Dowex-1 X-4 with 0.5M sulfuric acid, and the eluate was made neutral, decationized, the product converted into its methyl ester methyl glycoside, and this reduced with lithium aluminum hydride. The product was then hydrolyzed, and individual sugars characterized after isolation from paper. The results are given in Table III.

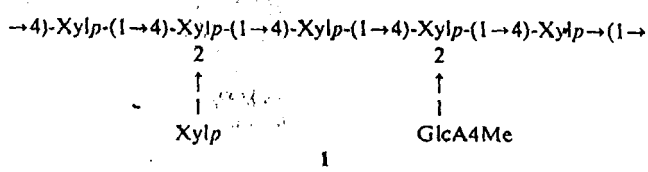
TABLE III

METHYL ETHERS OF SUGARS FROM THE HYDROLYZATE OF METHYLATED HEMICELLULOSE FROM *Sansevieria trifasciata*

Sugars[a]	*T*[b]	*Approximate mole%*	*Properties of isolated sugars*		
			$[\alpha]_D^{23}$ *in water (degrees)*	*Derivative*	*M.p. of derivative (degrees)*
2,3,4-Xyl	0.55	4	+20	anilide	96
2,3-Xyl	1.20	74	+25	anilide	121
3-Xyl	2.17	3	+15.5	anilide	135
Me-Aldobiouronic acid	—	19	+145	—	—
2,3,4-Glc	2.23		+68	anilide	145
3-Xyl	2.17		+14.5	anilide	133

[a]2,3,4-Xyl = 2,3,4-tri-*O*-methylxylose, etc. Me-Aldobiouronic acid = 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. [b]Retention times calculated as stated in Table II.

From these results, and those discussed earlier, it was possible to assign the structure of the average repeating unit of the fiber hemicellulose as **1**. That is, it



1

proved to be a polymer of (1→4)-linked D-xylopyranosyl residues having branches at O-2 consisting of 4-*O*-methyl-α-D-glucopyranosyluronic acid and D-xylopyranosyl groups. For every five D-xylopyranosyl residues in the main chain, there is one uronic acid group and, for ~22 such D-xylopyranosyl residues, there is one D-xylopyranosyl group.

EXPERIMENTAL

General. — All values of specific rotation reported are equilibrium values, and were measured with a Perkin–Elmer Model 241 MC spectropolarimeter at 23 ±1° and 589.6 nm. All evaporations were performed under diminished pressure at bath temperatures below 40°. Small volumes of aqueous solutions were lyophilized. Paper partition-chromatography was performed on Whatman No. 1 and, for preparative purposes, 3 MM, papers, with the following solvent systems: (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 2-butanone–water azeotrope, (*C*) 9:2:2 ethyl acetate–acetic acid–water, and (*D*) 40:11:19:11 1-butanol–ethanol–water. Sugars were detected with (*1*) alkaline silver nitrate and (*2*) aniline hydrogenoxalate. For gas–liquid chromatography, a Hewlett–Packard 5730A gas chromatograph with flame-ionization detector was used. Resolutions were performed on glass columns (1.83 m × 6 mm) containing (*a*) 3% of ECNSS-M on Gas-Chrom Q (100–120 mesh) at 190° (for alditol acetates of sugars), and (*b*) 1% of OV-225 on Gas Chrom Q (80–100 mesh) at 170° (for alditol acetates of partially methylated sugars). Alditol acetates were prepared as follows: to a solution of the sugar(s) (~5 mg) in water (10 mL) was added sodium borohydride (~40 mg), and the solution was kept for 5 h at room temperature, and then decationized with Dowex 50-W X-8 (H^+) ion-exchange resin to pH 4, the suspension filtered, and the filtrate evaporated to dryness. After removal of boric acid as methyl borate, the resulting alditol was acetylated with acetic anhydride (2 mL) in pyridine (2 mL) by heating on a boiling-water bath for 1 h. The excess of the reagents was removed by co-evaporation with toluene, and then the product was dried. Chloroform solutions of the alditol acetates were injected into the g.l.c. apparatus.

Isolation and purification of the hemicellulose. — Fresh leaves were collected from plants, and fibers were obtained with a raspador type of machine. The average length of the fibers was 107 cm, and they were thoroughly washed with water. The

fibers were cut into small pieces, and then mashed with a hammer. The product (100 g) was extracted with 1:2 (v/v) ethanol-benzene and dried. The extractive-free material (50 g) was delignified by treatment with aqueous sodium chlorite (0.7%) in 0.1M sodium acetate–acetic acid buffer, pH 4.0 (fiber:liquor = 1:150) for 5 h at 75°, and the fibrous material was recovered by filtration, and dried; the whole process was then repeated once. The resulting holocellulose (45 g) was successively extracted with hot water (4 h, bath temperature 100°), 4% sodium hydroxide, and 10% sodium hydroxide (in each case, for 6 h at room temperature, N_2 atmosphere). The alkaline solutions were made neutral with acetic acid, and the hemicellulose in each fraction was isolated by precipitation with ethanol, and centrifugation. Fraction II (9.2 g), which was obtained in relatively large amounts, was used for further study. This fraction was purified through its copper complex by treatment with Fehling solution, dissociation of the complex with acid, and isolation by repeated precipitation with ethanol (yield, 7.1 g).

Hydrolysis. — For isolation of the aldobiouronic acid, the hemicellulose was partially hydrolyzed with M sulfuric acid for 6 h at 100°. For complete hydrolysis, material was heated with 2M sulfuric acid for 20 h at 100°. Oligosaccharides were hydrolyzed with M sulfuric acid for 6 h. Methylated polysaccharide was hydrolyzed, first by heating with 85% formic acid for 2 h at 100°, the formic acid then being removed by co-evaporation with water, and second, with 0.5M sulfuric acid for 16 h at 100°.

Identification of sugars and their derivatives. — Because chromatography does not reveal the D or L configuration, and as the isomers of *O*-methylxylose derivatives have the same retention times, these were characterized by isolating them from paper chromatograms. Xylose could be crystallized from ethanol: m.p. 145°, $[\alpha]_D^{23}$ +20° (in water). The *O*-methyl sugars were characterized by preparing their "anilides" by the standard method, except for 4-*O*-methylglucose, which was converted into its osazone.

Methylation analysis. — The pure hemicellulose (3 g) was first acetylated with pyridine and acetic anhydride in formamide, with stirring at room temperature. The product (2.95 g) was dissolved in oxolane (60 mL), and then methylated with dimethyl sulfate (50 mL) and solid sodium hydroxide (50 g, powdered) according to the procedures of Hamilton and Kircher[15], and Carson and Maclay[16]. The product was further methylated by the Purdie method[17] (twice), whereupon a fully methylated product, showing no OH absorption band in its i.r. spectrum, was obtained; yield 2.1 g, $[\alpha]_D^{23}$ −61° (*c* 1, chloroform). The browinsh, glassy material (2 g) was dissolved in chloroform (25 mL) and fractionated by gradual addition of petroleum ether, giving three fractions (0.31, 1.4, and 0.2 g). The second fraction was used for subsequent analysis. This methylated hemicellulose was hydrolyzed, first with 85% formic acid for 2 h at 100°, and then (after removal of formic acid by co-evaporation with water) with 0.5M sulfuric acid for 16 h at 100°. Neutral, partially methylated sugars were analyzed as their alditol acetates by g.l.c. using column (*b*). For identification

of different, partially methylated sugars, they were resolved on paper, using solvent *C*, and then by preparing suitable derivatives, as described earlier.

Preparation of carboxyl-reduced hemicelluloses. — 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (1 g) was added to a stirred dispersion of the hemicellulose (50 mg) in water (50 mL), and the pH of the solution was maintained at 4.75 by dropwise addition of 0.01M hydrochloric acid. After 2 h, 2M aqueous sodium borohydride (80 mL) was added dropwise during 45 min, the pH being kept at 7.0 by concurrent addition of 4M hydrochloric acid. After being stirred for 1 h, the solution was dialyzed against distilled water for 24 h, and then lyophilized. The whole process was repeated once.

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