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INVESTIGATIONS ON THE FIBERS OF PINEAPPLE [*Ananas comosus* (L). MERR.] LEAVES

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ABSTRACT

Hemicelluloses were isolated from pineapple-leaf fibers under different conditions. Study of the properties of these hemicelluloses gave direct evidence of some ester linkages between the hemicellulose and the lignin in this fiber. An aldobiouronic acid was isolated from this fiber hemicellulose, and characterized as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. This indicates that the general nature of the hemicellulose is similar to those of jute and other fiber hemicelluloses.

INTRODUCTION

The hemicelluloses of a number of bast and leaf fibers have been studied[1–10]. Most of these hemicelluloses have been shown to contain polymers of (1→4)-linked β-D-xylopyranosyl groups to which branch units of 4-*O*-α-D-glucopyranosyluronic acid and D-xylopyranosyl groups are attached. It has been suggested by Sarkar *et al.*[11,12] that some of these 4-*O*-methylglucuronic acid residues are linked to the lignin moiety through an ester bond, as they had shown that the acid value of these lignin–hemicellulose complexes increases linearly when such complexes are progressively delignified with sodium chlorite. This reasoning seems to be untenable, as the hemicelluloses, isolated from chlorite-treated jute fibers[13] and wood[14] with dimethyl sulfoxide, have been found to retain the *O*-acetyl groups. Whether the increased acidity found by Sarkar and co-workers is due to the release of these uronic acid groups, or to production of carboxylic acid groups by oxidation of lignin itself[15] when treated with chlorite, is not clear. Moreover, it has been shown[16] that, in some plants, there are ester bonds in the lignin portion itself. In the present communication, direct evidence for an ester linkage between hemicellulose and lignin has been established. The pineapple [*Ananas comosus* (L.) Merr.[17]] leaf fiber, from which good paper can be made[18], and attempts at making spinnable fibers are being made, was chosen for study.

TABLE I

PRELIMINARY ANALYSIS OF THE DIFFERENT HEMICELLULOSE FRACTIONS FROM PINEAPPLE FIBER AND REDUCED PINEAPPLE FIBER

Fractions	*Yield (%)*	*Sugar present*							
	on dry holo-cellulose	*Xylose*	*4-O-Methyl-glucuronic acid*	*Arabinose*	*Rhamnose*	*Mannose*	*Galactose*	*Glucose*	*4-O-Methylglucose*
Original pineapple fiber									
(1) Hot-water extract	0.8	55.2	11.2	7.0	4.3	3.1	11.0	8.2	—
(2) 4% NaOH extract	24.5	74	20.1	3.5	trace	trace	2.5	trace	—
(3) 10% NaOH extract	14.8	72	18	5	trace	trace	3	2	—
Reduced pineapple fiber									
(1) Hot-water extract	1.2	56.5	9.2	4.1	3.0	4.1	12.2	9.0	2
(2) 4% NaOH extract	28.2	73.8	14.5	3.1	trace	trace	3	trace	5.1
(3) 10% NaOH extract	12.5	72	13.3	4.2	trace	trace	3.3	2.5	4.2

RESULTS AND DISCUSSION

The pineapple-fibers, containing 2.5% of waxy matter, 13.1% of lignin, and 3.8% of *O*-acetyl group, were cut into small pieces, and then freed of wax by extraction with 1:2 (v/v) ethanol–benzene. Lignin was removed from the resulting material by two treatments with sodium chlorite. The product was then extracted successively with hot water, and 4 and 10% sodium hydroxide solutions at room temperature, under nitrogen. The three hemicellulose fractions obtained, and the residual, cellulose-rich material respectively accounted for 0.8, 24.5, 14.8, and 64.5%, on the basis of the dry holocellulose. The results of preliminary analysis of these materials are shown in Table I.

All of the hemicellulose fractions contained mainly D-xylose and 4-*O*-methyl-D-glucuronic acid, and various proportions of arabinose, glucose, mannose, and galactose (see Table I). Partial hydrolysis of the hemicellulose fraction II with 0.5M sulfuric acid for 6 h at 100° yielded several oligosaccharides, from which an acidic oligosaccharide was isolated in good yield by resolution on paper. On hydrolysis, this oligosaccharide, having $[\alpha]_D^{23}$ +100.5° and an equivalent weight of 335, gave products which, in paper chromatography, showed the same mobilities as the original oligosaccharide, 4-*O*-methylglucuronic acid, and xylose, indicating that the oligosaccharide was an aldobiouronic acid of xylose and 4-*O*-methylglucuronic acid.

The aldobiouronic acid was then converted into its methyl ester methyl glycoside, which was reduced with lithium aluminum hydride. The carboxyl-reduced product and the original aldobiouronic acid were separately methylated by the Kuhn method[19,20]. The methylated aldobiouronic acid was then reduced with lithium aluminum hydride. These two products were now separately hydrolyzed, and the *O*-methyl sugars obtained were studied both by g.l.c., and by preparing derivatives after resolution on paper. The results are shown in Table II. Both compounds yielded 3,4-di-*O*-methyl-D-xylose, but the one that was reduced before methylation afforded

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**	52		+81.5	anilide	134
2,3,4-Glc	2.23		53	+67.5	anilide	145
3,4-Xyl	1.2	48	47	+20	anilide	115

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

2,3,4,6-tetra-*O*-methyl-D-glucose, and the one that was reduced after methylation yielded 2,3,4-tri-*O*-methyl-D-glucose. Characterization of 3,4-di-*O*-methyl-D-xylose clearly showed that the uronic acid was linked to the D-xylose residue at O-2. These results, together with the data mentioned earlier, proved that the aldobiouronic acid is 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, a component found in fibers of jute[4], roselle[6], mesta[7], agave[8], sisal[9], and sansevieria[21].

An attempt to ascertain whether the 4-*O*-methylglucuronic acid groups, present as branches on the xylan chain, are involved in an ester linkage between the lignin and hemicellulose was made by reducing the dewaxed fibers with aqueous sodium borohydride at room temperature. The extent of reduction was monitored by the decrease in the *O*-acetyl content of the pulp. The product obtained after the first reduction contained 0.6% of *O*-acetyl group. A second reduction by the same procedure yielded a product containing no *O*-acetyl groups. The washed and dried, reduced fiber contained 10.8% of lignin, and there was an overall loss of ~10% of the pulp. After dialysis and lyophilization, the material obtained from the filtrate of the foregoing reaction-mixture gave tests for both carbohydrate and lignin.

The original, dewaxed pineapple-fiber and the reduced fiber were hydrolyzed, and the monosaccharides obtained were characterized. Both contained rhamnose, arabinose, xylose, mannose, galactose, glucose and 4-*O*-methylglucuronic acid in common, but the reduced fiber also afforded an extra sugar, characterized as 4-*O*-methylglucose, indicating reduction of some of the 4-*O*-methylglucopyranosyluronic residues during the reduction with sodium borohydride.

Hemicelluloses were then extracted from the reduced pineapple-fiber (after delignification) as for the original fiber, with hot water, and 4 and 10% sodium hydroxide solutions. The results of preliminary analysis of these fractions are shown in Table I. The hemicelluloses obtained with 4% alkali in the two experiments were compared. The carbazole–sulfuric acid estimation of uronic acid indicated that, whereas the hemicellulose from the original fiber contained 20.1% of uronic acid, that obtained after reduction had only 14.5% of uronic acid. Hydrolysis, and estimation of neutral sugars by g.l.c. (results shown in Table I), indicated that the extra sugar, *viz.*, 4-*O*-methylglucose, was present in the hemicellulose of reduced fiber to the extent of 5.1%, which is of the same order as the loss of uronic acid (5.6%).

If the existence of an ester bond between hemicellulose and lignin is accepted, the loss of uronic acid of ~28% (20.1 to 14.5%) should correspond to the loss of lignin, which accounted for only 17.5% (13.1 to 10.8%). Moreover, loss of uronic acid, which is presumably due to reduction of ester bonds, was only 28% when all of the *O*-acetyl groups (3.6%) were reduced. This demonstrates that the uronic acid groups are only partially esterified (~28%), and that there may be other types of linkage between the hemicellulose and the lignin. The facts that the hemicellulose from the original pineapple-fiber contained 20.1% of uronic acid, and that that from borohydride-reduced fiber had only 14.5% of uronic acid (the rest being found as 4-*O*-methylglucose), indicate that this ~28% of the acid function is linked as ester bonds. Previous reports differ in the extent of esterification found in jute fiber, *viz.*, 50% (by a different method[22]) and 34% by this method[23].

EXPERIMENTAL

General. — All reported values of specific rotation 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 3 MM (for preparative purposes) papers, with the following solvent systems (v/v): (*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 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 50W X-8 (H^+) ion-exchange resin to pH 4, the suspension filtered, and the filtrate evaporated to dryness. After removing boric acid as methyl borate, the resulting alditol was acetylated with acetic anhydride (2 mL) in pyridine (2 mL) by heating for 1 h on a boiling-water bath. The excess of the reagents was removed by codistillation with toluene, and then the product was dried. Chloroform solutions of alditol acetates were injected into the g.l.c. apparatus.

Isolation of the hemicelluloses. — 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 (~3.5 ft). 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 sodium acetate–acetic acid buffer, pH 4.0 (fiber:liquor = 1:150) for 5 h at 75°. The fibrous material was removed by filtration, and dried. The process was repeated once. The resulting holocellulose (43·1 g) was extracted successively with hot water for 4 h at a bath temperature of 100°, and 4% sodium hydroxide, and 10% sodium hydroxide for 6 h at room temperature, under nitrogen, in each case. The alkali in the solutions was neutralized with acetic acid, and the hemicellulose in each fraction was precipitated with ethanol, and the suspension centrifuged. Fraction II, which was obtained in relatively large amounts, was used for further study. Isolation of the hemicelluloses from the borohydride-reduced fiber was performed in the same way.

Reduction of the pineapple-fiber. — The extractive-free fiber (10 g) was kept under M aqueous sodium borohydride solution (150 mL) for 24 h at room temperature. The mixture was filtered, and the clear filtrate was made neutral, dialyzed, and lyophilized. The fibers were washed several times with water, once with 0.1M oxalic acid, and finally with water until free from acid. The whole process of reduction was repeated once.

Hydrolysis. — For isolation of the aldobiouronic acid, the hemicellulose was partially hydrolyzed with 0.5M sulfuric acid for 6 h at 100°. For complete hydrolysis, the material was heated with 2M sulfuric acid for 20 h at 100°. Methylated oligosaccharides were hydrolyzed with M 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, they were characterized by isolating them from paper. The xylose, crystallized from ethanol had m.p. 145° and $[\alpha]_D^{23}$ +20° (equil., 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 of oligosaccharides by the Kuhn method[19,20]. — The aldobiouronic acid (20 mg) was dissolved in *N,N*-dimethylformamide (10 mL), silver oxide (6 g) and Drierite (4 g) were added, and the mixture was stirred for 30 min. Methyl iodide (5 mL) was added, and stirring was continued for 40 h. Chloroform (50 mL) was added, and the mixture was filtered through a bed of Celite. The filtrate was washed with water, dried (sodium sulfate), and evaporated to dryness. The neutral oligosaccharide was methylated by the same procedure. A few such batches were made, for isolation of the *O*-methyl sugars for derivatization.

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