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MANNOSE IN THE HOLOCELLULOSE OF *PANICUM COLORATUM*

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Key Word Index—*Panicum coloratum*; Gramineae; kleingrass; holocellulose; hemicellulose; mannose; mannan.

Abstract—Mannose was observed in addition to other neutral sugars in the holocellulose of *Panicum coloratum* leaves. It was present in the hydrolyzates of extracted hemicelluloses and was particularly resistant to extraction from the α-cellulose fraction.

The monosaccharide composition of total cell wall hemicelluloses and purified hemicellulose fractions has been reported for several species of Gramineae. As shown by Buchala [1] for *Panicum maximum* Jacq., the predominant hemicelluloses in tropical grasses are xylans. Buchala [1] isolated an arabino(4-*O*-methylglucurono)xylan and a galactoarabinoxylan from *P. maximum*. Hydrolyses of total hemicelluloses and hemicellulose fractions from other tropical grasses have also indicated the presence of similar xylans [2–4].

Hydrolysis of *Panicum coloratum* (kleingrass) holocellulose from leaf tissue produced mannose in addition to the neutral sugars previously reported in tropical grasses. *O*-Acetyl-galactoglucomannan is a common hemicellulose of higher plants and the major hemicellulose of gymnosperms [5]. Early work on woody species indicated that mannans may be integrally associated with α-cellulose [6, 7]. However, the monosaccharide composition of α-cellulose of tropical grasses has not been investigated to see whether components other than glucose are detectable after extraction of hemicelluloses.

Two samples of *P. coloratum* leaf holocellulose were fractionated by different procedures and the resulting fractions prepared for GLC analysis as alditol-acetates by the procedures of Borchardt and Piper [8]. The first *P. coloratum* holocellulose sample was fractionated by procedures commonly used with wood to separate xylans and mannans from α-cellulose. Total holocellulose neutral sugar composition of this sample was arabinose, xylose, mannose, galactose and glucose in the ratio 1.0:2.1:0.15:0.38:6.4. Holocellulose was first extracted with 5% KOH to remove preferentially xylans. This fraction had a neutral sugar composition of arabinose, xylose, mannose, galactose and glucose in the ratio 1.0:2.3:0.03:0.25:0.45. The remaining holocellulose was extracted three times with 17.5% NaOH plus 4% $Na_2B_4O_7$. This extraction produced a smaller fraction of hemicellulose composed of arabinose, xylose, mannose, galactose, and glucose in the proportions 1.0:4.2:0.06:0.30:1.6. Attempts to remove a mannan from this extract by dissolving it in 10% NaOH and precipitating with saturated $Ba(OH)_2$ [9] were unsuccessful. The 5% KOH extract was separated into fractions through a series of EtOH precipitations to give fractions with the monosaccharide proportions shown in Table 1. The resulting α-cellulose had a neutral sugar composition of 96.7% glucose and small amounts of arabinose, xylose, and galactose with a trace of mannose.

A second sample of *P. coloratum* leaf holocellulose with an arabinose, xylose, mannose, galactose, glucose ratio of 1.0:2.8:0.09:0.26:6.0 was extracted once each with 5% KOH and 17.5% NaOH. The extracts were combined and gave a total hemicellulose neutral sugar composition of arabinose, xylose, mannose, galactose, and glucose in the ratio 1.0:5.0:0.04:0.13:1.5. A water insoluble fraction was removed from the total hemicellulose with arabinose, xylose, mannose, galactose, and glucose in the ratio

Table 1. Proportions of neutral sugars from *Panicum coloratum* leaf hemicellulose fractions separated by precipitation in ethanol

Ethanol concentration	Arabinose	Xylose	Mannose	Galactose	Glucose
20	1.0	0.3	0.5	1.7	15.0
40	1.0	0.8		1.0	1.8
60	1.0	4.1	0.2	1.1	16.0
80	1.0	2.3	0.2	0.5	2.0

1.0:5.1:0.03:0.08:1.5. The remaining water soluble hemicellulose fraction was then fractionated on a DEAE-cellulose acetate column as described by Buchala [1]. Elution of polysaccharides from the column with 0.1 M and 0.5 M KOAc produced two small fractions with arabinose: xylose ratios of 1.0:3.0 and 1.0:5.6, respectively. Two fractions were obtained from elution of the column with 5.0 M KOAc. The fraction obtained by precipitating the eluate in 80% EtOH had an arabinose:xylose ratio of 1.0:0.9. The remaining eluate contained significant amounts of polysaccharides consisting of arabinose, xylose, and glucose in the proportion 1.0:3.0:7.8. An α-cellulose was obtained with glucose accounting for 91.8% of the neutral sugars (arabinose:xylose:mannose:galactose:glucose ratio of 1.0:3.1:1.8:0.42:72). The α-cellulose was further extracted with 24% KOH yielding an extract of arabinose, xylose, mannose, galactose, and glucose in the proportion of 1.0:4.0:0.31:0.45:1.2. The remaining α-cellulose was 94.2% glucose with the neutral sugars arabinose, xylose, mannose, and galactose as minor constituents. Mannose was the most abundant of these, comprising 40% of the neutral sugars other than glucose in the final α-cellulose.

The neutral sugar composition of total hemicellulose and hemicellulose fractions from *P. coloratum* leaves is similar to that reported for other species of tropical grasses. This suggests that the predominant hemicelluloses are also the same. Mannose was consistently present in holocellulose fractions. This monosaccharide has commonly been found in higher plants as a component of galactoglucomannans, mentioned previously, and glucomannans rather than as a minor component of other glycans. However, the failure of $Ba(OH)_2$ to remove a mannan from an extract of *P. coloratum* holocellulose indicates that such a mannan was probably not present in all fractions. The persistence of mannose in α-cellulose through several extractions is indicative of the intricate association of mannans with α-cellulose macromolecules suggested for conifers [10]. Additional investigations are needed to determine the molecular nature of mannose-containing heteroglycans in *P. coloratum* holocellulose.

EXPERIMENTAL

Field plantings of *P. coloratum* were clipped and separated into different morphological components. Only the leaf laminae were used for this analysis. Plant material for the two samples was harvested on different dates. The first holocellulose sample was prepared by extracting ground leaf tissue with EtOH–C_6H_6, EtOH, and cold H_2O followed by refluxing (× 3) in acid chlorite to remove lignin. The second holocellulose sample was prepared by extracting ground leaf tissue with hot EtOH, cold H_2O, hot H_2O and ammonium oxalate followed by refluxing once in acid chlorite.

Sugars were analyzed as their alditol acetates by GLC [8] on an FID equipped chromatograph: 3 m × 2 mm glass column packed with 3% SP 2330 on 100/120 Supelcoport; oven temp. 225°; injector and detector temp. 225° and 250°, respectively; He 50 ml/min.

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