STRUCTURAL INVESTIGATION OF SODIUM HYDROXIDE-SOLUBLE TOBACCO *(NICOTIANA TABACUM)* POLYSACCHARIDES: A XYLAN[1]

By IQBAL R. SIDDIQUI[2] and NESTOR ROSA[3]

The sodium hydroxide-soluble polysaccharides extracted from bright tobacco (*Nicotiana tabacum*, Delhi 76) were fractionated successfully by chromatography on DEAE-cellulose (CO_3^{2-}), (acetate), and PO_4^{3-}) columns and finally by copper complexing to yield a xylan. Acid hydrolysis of the xylan produced arabinose, galactose, glucose, mannose, and xylose in molar proportions of 1 : 1.3 : 3.8 : 0.2 : 17.3. Sedimentation data indicated that the xylan was ~70% pure. Metylation studies showed that it was composed of a linear chain of β-(1-4)-linked D-xylopyransosyl units.

INTRODUCTION

In previous papers (6,7) we have described the isolation and characterization of an arabinoxyloglucan and a galactoxylogulucan from the sodium hydroxide-soluble fraction of cured leaf laminae of tobacco, and we now describe further work on the sodium hydroxide-soluble polysaccharide fraction. Previous to this xylans have been reported from the midrib and the stalks of tobacco (3). This is the first report on the isolation and characterization of a xylan from the leaf laminae of tobacco.

EXPERIMENTAL

The general methods have been reported previously (6,8)

Isolation of tobacco xylan

The carbonate-eluted fraction A_2 (3g) from the fractionation of hemicellulase B (6) on the DEAE-cellulose (CO_3^{2-}), was dissolved by stirring in water (90ml) for 17h. The solution was added to a column (4 x 52 cm) of DEAE-cellulose (acetate form). Stepwise elution with water and 0→.5M potassium acetate, dialysis and freeze drying yielded fractions **1**, 0.41g (+29.4°); **2**, 0.13g (+16°); **3**, 0.28g (+17.9°); **4**, 0.03g (+8.9°); **5**, 0.63g (+45.8°); **6**, 0.08g (+50.1°); **7**, 0.06g (+26.1°); **8**, 0.74g (+10.6°); **9**, 0.08g (0±0.3°); **10**, 0.23g 10±0.3°)[a].

Acid hydrolyses (MH_2SO_4, 100°C, 3h) of fractions 1-10, reduction ($NaBH_4$), acetylation and GLC (6,8) of the alditol acetates gave derivatives of arabinose - rhamnose - xylose - galactose - glucose in the molar proportions of 1 : 0.1 : 0.2 : 1.2 : 0.5 in **1**, 1 : 0.2 : 2.6 : 2.3 : 3.3 in **2**, 1: 0.7 : 1.3 : 3.2 : 1.0 in **3**, 1 : 0.7 : 0.7 : 3.5 : 1.5 in **4**, 1 : 1.1 : 0.6 : 1.7 : 0.2 in **5**, 1 : 1.5 : 0.9 : 2.9 : 1.2 : in **6**, 1 : 0.8 : 1.6 : 2.6 : 0.7 in **7**, 1 : 1 : 1.7 : 2.9 : 2.2 in **8**, 1 : 0.6 : 1.6 : 2.6 : 1.6 in **9**, 1 : 0.7 : 3.8 : 2.7 : 1.9 in **10**.

Fractions 2 and 3 (0.40g), 5 and 6 (0.64g) and 10 (0.22g) from the acetate column were separately fractionated on (2 × 34 cm) column of DEAE-cellulose (PO_4^{3-}). Elution with water, followed by gradient elution with 0 → 0.05M sodium dihydrogen phosphate buffer at pH 5.5, and finally 0.5M sodium hydroxide yielded fractions summarized in **Table 1.**

The sodium hydroxide-eluted fractions from acetate fractions 2 and 3 and 5 and 6 (90mg) in **Table 1** were dissolved in 0.5M sodium hydroxide (7ml), insoluble material (18mg) was centrifuged off and the clear supernatant was mixed with freshly prepared Fehling's solution (6ml). The solution (13ml) was acidified with acetic acid, mixed with ethanol (4 vol) and the precipitated material (20 mg) was recovered by centrifugatiion, dialysis and freeze drying.

[a] $[\alpha]^{27}{}_D$ values in parenthesis.

The fraction (20 mg) was combined with the sodium hydroxide-eluted fraction from phosphate fractionation [fraction 10 (40 mg) table 1]. The combined fraction (60 mg) was dissolved in 0.5M sodium hydroxide (5 ml), insoluble material was centrifuged off, and 7% cupric acetate (0.75ml) was added to the supernatant solution. The precipitate was removed, suspended in ice-cold water and acidified with M hydrochloric acid. The greenish solution was mixed with ethanol (4 vol) and centrifuged, and the slightly green precipitate was reprecipitated from an acidic solution. The final precipitate was suspended in water, dialysed for 24 h against running tap-water and 4 h against distilled water, concentrated and freezed dried to yield a xylan (11.3 mg).

Analysis of xylan

The tobacco leaf xylan had $[\alpha]^{28}{}_D$ 16° (C 0.2, 0.5M NaOH). Sedimentation analysis (9) using a synthetic boundry-cell and a 0.5% solution in 0.02M sodium acetate pH 5.0 at 34,000 rev./min. showed a major, symmetrical peak with a minor shoulder. The areas under the peaks showed that the major peak amounted to ~70% of the total area under the peaks. The xylan (2 mg) was hydrolyzed with M sulphuric acid at 100°C for 3h. The hydrolysate was neutralized ($BaCO_3$), filtered and the solution treated with sodium borohydride (5 mg). The resulting alditol mixture was then acetylated and examined by GLC (6,8).

Methylation analysis of xylan

The dried xylan (8 mg) in dry methyl sulphoxide (1 ml) was methylated under nitrogen by the Hakomori method (4), using methyl sulphinyl carbanion (1 ml), reaction time (7 h) and methyliodide (2 ml) reaction time 2h. The sample was recovered by dialysis and continuous extraction with chloroform. The methylated product (3 mg) was methanolyzed (methanolic 2% hydrogen chloride 4 ml, 17 h, reflux; neutralization with Ag_2CO_3) and hydrolyzed (MH_2SO_4 2 ml, 100°C, 30 h; neutralization with

[1] *Contribution No. 661 from the Food Research Institute*
[2] *Food Research Institute, Agriculture Canada, Ottawa, K1A 0C6 (Canada).*
[3] *Research Station, Delhi, Ontario N4B 2W9 (Canada).*
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Table 1. Fractionation of acetate column fractions on DEAE-cellulose (PO_4^{3-}).

Fraction (g)	Eluant 0→0.5M phosphate buffer pH 5.5	Eluant 0.5M NaOH	Yield (g)	$[\alpha]_D$ (degrees) (0.5M NaOH)	Hydrolysis results					
					Gal	Glc	Man	Ara	Xyl	Rha
2 and 3 (0.40)	1		0.07		major	minor	-ve	major	major	minor
	2		0.10		major	minor	-ve	major	minor	major
	3		-		major	major	-ve	trace	trace	trace
		1	0.04	-14.1	major	major	trace	minor	major	trace
5 and 6 (0.64)	1		0.01		major	major	-ve	trace	trace	trace
	2		0.26		major	minor	-ve	major	trace	major
		1	0.05	- 6.5	major	major	-ve	minor	major	trace
10 (0.22)	1		0.08		-	-	-	-	-	-
		1	0.04	- 3.2	trace	trace	-ve	-ve	major	-ve

$BaCO_3$) to yield the methylated sugars.

The mixture of methyl sugars was reduced ($NaBH_4$), then acetylated and examined by GLC. From the GLC-MS results (1) the major component, was identified as 2,3-di-0-methyl-D-xylitol triacetate (fragment ions M/Z 43, 87, 101, 117, 129, 189. A number of other components detected appeared to be characteristic of the amyloids and pectin contaminants.

RESULTS AND DISCUSSION

Rigorous purification of certain components from hemicellulose fraction B (6) (see experimental) using a combination of separations on DEAE-cellulose (acetate) and phosphate columns and further purifications using Fehling and cupric acetate solutions yielded, *inter alia,* minor amounts of a xylan which could not be purified further due to lack of the material.

The xylan showed $[\alpha]_D^{24}$ - 16° and an acid hydrolysis produced, arabinose, galactose, glucose, mannose and xylose in the molar ratios 1 : 1.3 : 3.8: 0.2 : 17.3 and traces of galacturonic acid. The xylan showed a major, symmetrical peak and a minor peak on sedimentation analysis. The major peak was 70% of the total. The minor peak is suspected to be due to the contaminating pectic polysaccharide and amyloids. The total of the ratios of glucose, galactose, arabinose to xylose (6.2:17) showed that the xylan was contaminated to the extend of 27% which is in good agreement with the value determined by sedimentation analysis. The nature of contaminating sugars (glucose, galactose, arabinose and galacturonic acid) and the $[\alpha]_D$ value (- 16°) for the xylan further substantiated the suggestion that the contaminants were pectic polysaccharide ($[\alpha]_D$ + 136°) and amyloids ($[\alpha]_D$ + 29°, + 94°). Their absence would have resulted in a higher $[\alpha]_D$ value for the xylan such as that (-65°) reported for tobacco stalk xylan, (3).

The xylan was methylated (4) and the methylated product was subjected to methanololysis and hydrolysis. GLC-MS (1) of the derived glycitol acetate revealed 2,3-di-0-methylxylitol triacetate. The methylation analysis showed that the xylan was composed of a linear chain of β-(1-4)-linked D-xylopyranose units.

The xylans of angiosperms generally consist of a backbone of β-(1-4)-linked D-xylopyranosyl units with branching through L-arabinofuranosyl and/or D-glucuronopyranosyl (or its 4-O-methyl ether). Xylans containing a single branching point formed by a 1→3 linkage have been reported from Esparto grass (2) and Tararind seed (5). The tobacco leaf laminae xylan reported here appears to be unbranched and is similar to that reported from tobacco stalks (3).

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