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STRUCTURAL FEATURES OF AN ARABINOXYLAN AND A RHAMNOGALACTURONAN DERIVED FROM LINSEED MUCILAGE

GUDIPATI MURALIKRISHNA, PARAMAHANS V. SALIMATH, AND RUDRAPATNAM N. THARANATHAN*
Biochemistry Section, Department of Food Chemistry, Central Food Technological Research Institute, Mysore – 570 013 (India)

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ABSTRACT

The mucilage, obtained (6%) by aqueous extraction of linseed, on fractionation with Cetavlon gave precipitable (CeP, 58%) and non-precipitable (CeNP, 28%) fractions. Further fractionation on DEAE-cellulose furnished a series of fractions of which the water-eluted neutral fraction of CeNP and the 0.2M ammonium carbonate-eluted acidic fraction of CeP were homogeneous. The neutral fraction contained L-arabinose, D-xylose, and D-galactose (3.5:6.2:1), and the acidic fraction contained L-rhamnose, L-fucose, L-galactose, and D-galacturonic acid (2.6:1:1.4:1.7). The neutral fraction was an arabinoxylan having a (1→4)-β-D-xylan backbone to which arabinose and galactose side-chains were attached at positions 2 and/or 3. The acidic fraction had a backbone of (1→2)-linked α-L-rhamnopyranosyl and (1→4)-linked D-galactopyranosyluronic acid residues, with side-chains of fucose and galactose residues, the former essentially at the non-reducing end.

INTRODUCTION

Linseed (flax, *Linum usitatissimum*), which belongs to the family *Linaceae*, is a commercial crop grown chiefly for the production of fibre, from which linen is made, and linseed oil, which is used in varnishes and paints[1]. Except for a few reports on the chemical nature of the so-called homogeneous fractions of linseed mucilage[2–6], no information on the mucilage from Indian varieties of linseed is available. We now describe the fractionation of linseed mucilage and the structural features of an acidic and a neutral polysaccharide.

EXPERIMENTAL

The various analytical and structural methods were as reported earlier[7,8]. Periodate consumption was determined by the TPTZ method[9]. H.p.l.c. was per-

*To whom correspondence should be addressed.

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formed using a Waters Associates liquid chromatograph fitted with a refractive index detector, a carbohydrate column, and elution with acetonitrile–water (70:30).

Isolation and fractionation of the mucilage. — The mucilage was extracted by soaking the seeds in water (1:6, w/v) for 24 h with occasional stirring. After decantation, the mucilage was precipitated with alcohol and dried. An aqueous 0.5% solution of the mucilage was fractionated with Cetavlon into precipitable (CeP, 58%) and non-precipitable (CeNP, 28%) fractions. The latter was not completely soluble in water and was separated into water-soluble (65%) and water-insoluble (35%) portions. Elution of the water-soluble portion from a column of DEAE-cellulose (CO_3^{2-} form) with water and 0.1M ammonium carbonate yielded two fractions, whereas the CeP gave two fractions eluted with 0.2M ammonium carbonate and 0.3M NaOH.

Partial acid hydrolysis. — The acidic polysaccharide was treated with 0.25M CF_3COOH (100°, 8 h), and the neutral polysaccharide with 2M CH_3COOH (100°, 10 h). Excess of acid was removed and each hydrolysate subjected to chromatography.

Determination of the reducing end and the d.p. of the oligosaccharide. — The difference in the total sugar content of the oligosaccharide was determined[10] before and after borohydride reduction. The reduced oligosaccharide was hydrolysed with acid and the products were analysed by p.c. (nitromethane–acetic acid–ethanol–water saturated with boric acid, 1:1:1:1) in order to identify the glycitol[11].

RESULTS AND DISCUSSION

Aqueous extraction of the seeds followed by precipitation with alcohol furnished the mucilage in ~6% yield (dry-weight basis). Acid hydrolysis of the mucilage, followed by g.l.c. analysis of the derived alditol acetates and determination of the $[\alpha]_D$ values of the isolated sugars, revealed L-rhamnose, L-fucose, L-arabinose, D-xylose, D- and L-galactose, and D-glucose in the ratios 24:8:9:17:21:4. The uronic acid (17%) was identified (p.c.) as D-galacturonic acid. The overall composition of the mucilage accorded with the earlier reports[3,4].

Preliminary fractionation by the addition of aqueous 3% Cetavlon to an aqueous 0.5% solution of the mucilage gave precipitable (CeP, 58%) and non-precipitable (CeNP, 28%) fractions. CeP was easily soluble in water, whereas only 65% of CeNP was water-soluble. Both CeP and the water-soluble portion of CeNP, on further fractionation on DEAE-cellulose, were resolved into neutral and acidic polysaccharides of differing sugar composition (Table I). The fractionation scheme as well as the sugar composition of the derived fractions were different from those in the earlier reports[3,4]. Of these, CePA (0.2M ammonium carbonate-eluted fraction of CeP) and CeNPN (water-eluted fraction of CeNP) were homogeneous by cellulose acetate membrane electrophoresis, gel filtration, and ultracentrifugation.

Hydrolysis of CeNPN gave L-arabinose, D-xylose, and D-galactose in the ratios

TABLE I

SUGAR COMPOSITION (%) OF THE FRACTIONS DERIVED FROM LINSEED MUCILAGE

Fraction	*Yield (%)*	*Neutral sugars*[a]						*GalA*[a]
		Rha	*Fuc*	*Ara*	*Xyl*	*Gal*	*Glc*	
Cetavlon-precipitable	58	37	12	6	—	22	2	21
DEAE-cellulose, eluted with 0.2M $(NH_4)_2CO_3$ (CePA)	62	37	14	—	—	21	—	28
DEAE-cellulose, eluted with 0.3M NaOH	30	36	9	30	—	17	8	N.d.[b]
Cetavlon-non-precipitable	28	—	—	26	53	15	3	3
Non-precipitable, water-soluble	65	—	—	28	52	16	2	2
DEAE-cellulose, eluted with water (CeNPN)	75	—	—	32	57	11	—	—
DEAE-cellulose, eluted with 0.1M $(NH_4)_2CO_3$	21	—	—	54	26	9	7	4
Cetavlon-non-precipitable water-insoluble	35	—	—	25	50	20	—	5

[a]Quantified by integration of the peak area in g.l.c. of the alditol acetates and GalA by the carbazole method. [b]Not determined.

3.5:6.2:1, and the molecular weight (g.p.c. on Sephacryl S-400) was 500,000. Methylation of the polysaccharide followed by subsequent hydrolysis, derivatisation (→alditol-1-$^2H_1$ acetates), and g.l.c.–m.s.[12] revealed the main glycosidic linkages (Table II). The 2,3,4-tri-*O*-methyl derivatives of arabinose and xylose could not be separated on OV-225, OV-101, and OV-275 columns, but were resolved on 3% of ECNSS-M and were formed in equal amounts. Arabinose residues were present both in pyranoid and furanoid forms with the former preponderating. The presence of significant amounts of free arabinose and xylose suggested extensive branching in the parent molecule. This inference was also supported by the quantitative recovery of the terminal glucose residues. The arabinose was mainly (1→3)-linked, and not (1→5)-linked as reported earlier[4].

Partial hydrolysis of CeNPN gave a degraded polysaccharide consisting mainly of xylose. Methylation analysis of the degraded polysaccharide indicated (Table II) (1→4)-linked xylosyl residues arising from a linear xylan chain. Thus, all the arabinose and galactose residues originate from the highly branched side-chains attached to positions 2 and 3 of the xylan backbone. The molar equivalence in the

TABLE II

METHYLATION ANALYSIS DATA FOR CeNPN AND CARBOXYL-REDUCED CePA

Methyl ether	*Molar ratio*[a]		*Mode of linkage*
CeNPN	*Native*	*Degraded*	
2,3,5-Me_3-Ara	0.74	—	Ara*f*-(1→
2,3,4-Me_3-Xyl[b]	7.26	1.20	Xyl*p*-(1→
2,3,4-Me_3-Ara[b]	8.02	—	Ara*p*-(1→
2,3,4,6-Me_4-Gal	2.87	—	Gal*p*-(1→
2,4-Me_2-Ara	1.51	—	→3)-Ara*p*-(1→
2,3-Me_2-Xyl	10.58	4.44	→4)-Xyl*p*-(1→
3-Me-Xyl	1.00	1.00	→2,4)-Xyl*p*-(1→
Arabinose	3.52	—	→2,3,4)-Ara*p*-(1→ or →2,3,5)-Ara*f*-(1→
Xylose	2.55	—	→2,3,4)-Xyl*p*-(1→
CePA		*Carboxyl-reduced CePA*	
2,3,4-Me_3-Fuc		2.32	Fuc*p*-(1→
3,4-Me_2-Rha		2.40	→2)-Rha*p*-(1→
2,3,4,6-Me_4-Gal		1.20	Gal*p*-(1→
4-*O*-Me-Rha		1.80	→2,3)-Rha*p*-(1→
2,3,6-Me_3-Gal		3.00	→4)-Gal*p*-(1-
Galactose		1.00	→2,3,4,6)-Gal*p*-(1→

[a]With respect to 3-Me-Xyl for CeNPN and Gal for CePA. [b]Quantified by g.l.c. of the alditol acetate derivatives on ECNSS-M.

terminal (2,3,4-Me_3-Xyl) and branching (3-Me-Xyl) xylose residues is in good agreement and indicates extensive branching.

From the partial hydrolysate, four oligosaccharides were isolated and partially characterised (Table III). The data in Table III indicate that all of the arabinose and galactose and some of the xylose were present in the side chains.

The polysaccharide consumed 0.99 mol of periodate and released 0.43 mol of formic acid per mol of "anhydrosugar". Smith degradation of the resulting oxopolysaccharide yielded glycerol, arabinose, and xylose. The presence of the xylose indicated extensive branching. On oxidation[13] of CeNPN acetate with CrO_3, the xylose was completely destroyed, indicating it to be β, whereas the arabinose and galactose were oxidised to a negligible extent and hence may be α.

Highly branched arabinoxylans containing 35–40% of arabinofuranose residues have been isolated from rye[14] and wheat flour[15]. Unlike most arabinoxylans[16,17], in which the arabinose is mainly furanosidic, the linseed arabinoxylan contains a significant amount of terminal arabinopyranosyl groups. During partial hydrolysis, arabinose was released rather slowly, indicating it to be pyranosidic. The polysaccharides of sapote[18], brea[19], and bromelia[20] gums are also highly branched arabinoxylans.

TABLE III

CHARACTERISTICS OF THE OLIGOSACCHARIDES OBTAINED AFTER PARTIAL HYDROLYSIS OF CeNPN AND CePA

Oligo-saccharides	*Yield (%)*	R_F	*Composition (mole proportion)*		*Reducing end*	$[\alpha]_D$ *water (degrees)*	*D.p.*
CeNPN		R_{Mal}[a]					
I	1.50	0.75	Ara–Gal (1:1)		Ara	+29	2
II	1.00	0.35	Ara–Xyl (1:1)		Xyl	+4	3
III	0.25	0.57	Ara		Ara	+80	3
IV	0.20	0.11	Ara–Xyl (1:1)		Xyl	+40	4
CePA		R_{GalA}[b]		*GalA (%)*			
I	5.04	0.84	Rha–GalA (1:1)	48	Rha	+112	2
II	4.13	0.21	Rha–GalA (2:1)	33	Rha	+104	3
III	1.35	0.14	Rha–GalA (3:1)	24	Rha	+60	4
IV	0.63	0.10	Rha–GalA (3:2)	40	Rha	+175	5

[a]Mobility in p.c. with respect to that of maltose, using 1-propanol–ethanol–water (7:1:2). [b]Mobility in p.c. with respect to that of GalA in ethyl acetate–formic acid–acetic acid–water (18:1:3:4).

CePA, $[\alpha]_D$ +4° (*c* 0.25, water), contained L-rhamnose, L-fucose, L-galactose, and D-galacturonic acid in the ratios 2.6:1:1.4:1.7; the L-galactose isolated had $[\alpha]_D$ −76° (*c* 0.5, water) (*cf.* the theoretical value of −80°). G.p.c. on Sephacryl S-400 of CePA indicated an average molecular weight of 600,000. Carboxyl-reduction[21] of CePA, when performed twice, furnished a neutral polysaccharide containing <1% of galacturonic acid. G.l.c. of the alditol acetates derived from the resulting neutral polysaccharide revealed L-rhamnose, L-fucose, and D-/L-galactose in the ratios 2.2:1:2.8.

The methylation analysis data (Table II) of carboxyl-reduced CePA indicated the majority of the L-rhamnose residues to be (1→2)-linked and ~46% to be substituted at O-3 (identification of 4-*O*-methyl-L-rhamnose). (1→4)-Linked galacturonic acid was present essentially in the main chain. All of the fucose and ~50% of the galactose constituted the non-reducing end groups. The identification of free galactitol provided further support for extensive branching in the molecule.

CePA consumed 1.17 mol of periodate with the liberation of 0.35 mol of formic acid per mol of "anhydrosugar". Borohydride reduction of the resulting oxopolysaccharide followed by hydrolysis gave mainly glycerol and erythritol together with small proportions of rhamnose and galactose. On oxidation[13] of CePA acetate with CrO_3, none of the neutral sugars were destroyed, indicating them to be α.

Partial hydrolysis of CePA with 0.25M CF_3COOH gave four oligosaccharides and significant quantities of fucose and galactose, indicating their possible presence in the side-chains. The yields, relative mobilities (with respect to galacturonic acid), and some details of the composition of these oligosaccharides are given in Table III. The structures of the oligosaccharides indiated the presence of blocks as well as contiguous residues of rhamnose and galacturonic acid in the L-rhamno-D-galacturonan backbone.

The composition of CePA (Rha:Fuc:Gal:GalA, 2.6:1:1.4:1.7) differed considerably from that reported (4:1:2:2) by Hunt and Jones[4] since the fraction characterised[4] was not pure. Moreover, methylation analysis gave[4] 2,3,4-tri-*O*-methyl-L-fucose, 2,3,4,6-tetra-*O*-methyl-L- and 2,3,6-tri-*O*-methyl-D-galactose, 4-*O*-methyl-L-rhamnose, and L-rhamnose together with two unidentified compounds. These data differed from those in Table II in relation to 3,4-di-*O*-methyl-L-rhamnose, L-galactose, and rhamnose. Hunt and Jones[4] also characterised the cupric acetate-soluble material (Rha:Gal:GalA, 2:1:2), and methylation analysis gave 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 4-*O*-methyl-L-rhamnose, and an unidentified compound. The differences between CePA and these two acidic polysaccharides[4] probably reflect varietal differences and the methodologies employed. Similar structural features have been reported for rhamnogalacturonans of soyabean cotyledon[22], lucerne[23], and okra[8]. Pectic fractions from mustard seed carry highly branched chains of arabinose residues[24]; in tragacanthic acid, single xylosyl groups are attached to the rhamnogalacturonan main-chain[25]. Recently, a revised structure for the rhamnogalacturonan of primary cell walls of sycamore has been proposed[26] which contains a repeating unit comprising (1→4)-linked α-D-galacturonic acid and (1→2)-linked α-L-rhamnose.

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