Phytochemistry, Vol. 22, No. 9, pp. 1961–1963, 1983.
Printed in Great Britain.

0031-9422/83 $3.00 + 0.00

STRUCTURAL FEATURES OF A POLYSACCHARIDE FROM THE MUCIN OF WATER HYACINTH

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(*Received* 17 *January* 1983)

Key Word Index—*Eichhornia crassipes*; Pontederiaceae; water hyacinth; mucin; polysaccharide structure; D-xylo-L-galacto-L-arabinan.

Abstract—The mucin found in the nodal region of the weed, water hyacinth (*Eichhornia crassipes*), is a heteropolysaccharide composed of D-xylose, L-galactose and L-arabinose in the mol ratio of 1.3:1.2:1.0. Partial hydrolysis with acid gave four oligosaccharides which were characterized as: D-Xylp-(1 → 3)-L-Ara, L-Galp-(1 → 2)-L-Ara, D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Ara, and D-Xylp-(1 → 2)-D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Ara. These, together with the results of methylation analysis using GC and GC/MS and periodate oxidation, indicated that the trisaccharide repeating unit, → 4)-D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Araf-(1 →, constitutes the backbone of the polysaccharide. Further, all the D-xylopyranosyl residues of the backbone are substituted at O-2 and, in addition, one out of seven such residues is also substituted at O-3; the substituents being L-Araf-(1 →, D-Xylp-(1 →, L-Galp-(1 →, D-Xylp-(1 → 3)-L-Araf-(1 →, residues.

INTRODUCTION

Eichhornia crassipes, commonly known as water hyacinth, is a water-borne, perennial, tropical weed [1]. The weed, in addition to lowering the availability of inland waters, is a menace to navigation, generation of hydroelectric power, fish culture, and a health hazard as it harbours pests and insects [2]. It is estimated that, in India alone, *ca* 4×10^6 ha of water surface is covered with this weed [2]. Attempts to eradicate the weed or control its growth have been unsuccessful so far. However, attempts to utilize the weed, in India and elsewhere, for the manufacture of paper and production of biogas are being made [2]. Locally it is used as a cattle-feed.

We noted that the weed secretes a mucilaginous material found deposited in the nodal region of the plant. Hitherto no studies seem to have been made either on the chemical nature or on the biological significance of this mucin. The mucin was found to be polysaccharide in nature. The present report deals with the main structural features of the polysaccharide.

RESULTS AND DISCUSSION

The mucin collected from the nodal region of the weed, water hyacinth, was found to be essentially a 1.5% aq. solution of a polysaccharide. The polysaccharide was isolated by dissolution of the mucin in aq. sodium hydroxide followed by precipitation with ethanol. It was purified by extensive dialysis of the aq. solution, and ethanol precipitation. The polysaccharide was completely and sharply precipitated from its alkaline solution, by cupric acetate [3] and barium hydroxide [4]. On free-boundary electrophoresis [5] in 0.1 M sodium tetraborate, the polysaccharide moved as a single symmetrical peak, indicating it to be homogeneous. The purified polysaccharide had an intrinsic viscosity [6] of 66 dl/g.

Complete acid hydrolysis and identification of the constituent sugars by PC, and GC as their alditol acetates [7], indicated that the polysaccharide is composed of xylose, arabinose and galactose in the mol ratio of 1.3:1.2:1.0. Isolation of the sugars by prep. PC and determination of their specific rotations indicated that xylose has D- while arabinose and galactose have L-configurations. It is interesting to note the presence of L-galactose, which is rather a rare constituent of plant polysaccharides [8].

Partial acid hydrolysis of the polysaccharide gave, in addition to D-xylose, L-arabinose and L-galactose, four oligosaccharides (**1**–**4**) in yields of 2.0, 6.7, 11.7 and 2.1%, respectively. These were characterized by their sugar composition, before and after sodium borohydride reduction; methylation analysis; and MW determination as: (**1**) D-Xylp-(1 → 3)-L-Ara, (**2**) L-Galp-(1 → 2)-L-Ara, (**3**) D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Ara, and (**4**) D-Xylp-(1 → 2)-D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Ara.

The ring size of the monosaccharides and positions of the various glycosidic linkages were determined by methylation analysis. Thus, Hakomori methylation [9], acid hydrolysis, and analysis of the resulting partially methylated sugars as their alditol acetates by GC and GC/MS [10] gave the results shown in Table 1. The formation of 2,3,5-tri-*O*-methyl, 3,5-di-*O*-methyl and 2,5-di-*O*-methyl derivatives of arabinose, indicates its exclusive presence in the furanosidic form; both as non-reducing terminal and non-terminal residues. The identification of 2,3,5-tri-*O*-methylxylose and 2,3,4,6-tetra-*O*-methylgalactose reveals that *ca* 20% of the xylose and *ca* 13% of the galactose are present as non-reducing terminal pyranosyl residues; the remainder as non-terminal residues. 3,5-Di-*O*-methylarabinose, 2,4,6,-tri-*O*-methylgalactose and 3-*O*-methylxylose, along with xylose, found in the highest and nearly equimolar proportions indicates that these residues are possibly linked to one another. Furthermore, the

Table 1. Methylation analysis data for the polysaccharide from water hyacinth

Sugar*	Approximate mol ratio
2,3,5-Tri-*O*-methylarabinose	3.1
2,3,4-Tri-*O*-methylxylose	2.0
3,5-Di-*O*-methylarabinose	5.8
2,5,Di-*O*-methylarabinose	2.1
2,3,4,6-Tetra-*O*-methylgalactose	1.0
2,4,6-Tri-*O*-methylgalactose	6.5
3-*O*-Methylxylose	6.2
Xylose	1.2

*Identified as alditol acetates by GC and GC/MS.

formation of D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Ara as the major oligosaccharide on partial acid hydrolysis suggests that the polysaccharide contains the trisaccharide repeating unit, ---- → 4)-D-Xylp-(1 → 3)-L-Galp-(1 → 2)-L-Araf-(1 → ----, as its backbone. Further, all the D-xylopyranosyl residues of the backbone are substituted at O-2, and one out of seven, on average, also at O-3. L-Arabinofuranosyl, L-galactopyranosyl and D-xylopyranosyl, as shown by the formation of the oligosaccharide **4**, and *O*-D-xylopyranosyl-(1 → 3)-L-arabinofuranosyl, as indicated by the formation of the oligosaccharide **1**, units constitute the side-chain substituents.

On periodate oxidation [11], the polysaccharide reduced 0.44 mol of the oxidant and liberated 0.1 mol formic acid per pentosyl residue. The oxidized polysaccharide on borohydride reduction, acid hydrolysis and GC of the sugars as alditol acetates gave xylose, arabinose and galactose in equimolar proportion. These results lend support to the suggested structural features of the polysaccharide.

Based on the results of partial hydrolysis, methylation analysis and periodate oxidation, the average structural features of the polysaccharide can be summarized as shown in Fig. 1.

EXPERIMENTAL

General methods. Unless stated otherwise, all hydrolyses were carried out with 0.25 M H_2SO_4 at 100° for 4–10 hr. The hydrolysates were neutralized with $BaCO_3$, filtered and the clear filtrates deionized on columns of Amberlite IR-120(H^+) and Amberlite IRA-400(CO_3^-) resins. The effluents were evaporated under red. pres. below 45° and examined by PC and GC for neutral sugars. The Amberlite IRA-400 column was eluted with 2 M HCOOH, evaporated as above and examined by PC for acidic sugars.

Descending PC was performed on Whatman No. 1 and 3 MM papers with the solvent systems: (a) *n*-BuOH–C_6H_6–C_5H_5N–H_2O (5:1:3:3, upper layer); (b) *n*-BuOH–C_5H_5N–H_2O (6:4:3); (c) *n*-BuOH–HOAc–H_2O (4:1:5, upper layer); and (d) *n*-BuOH–EtOH–H_2O (10:1:2). Sugars were detected with *p*-anisidine hydrochloride [12] and alkaline $AgNO_3$ [13].

GC was performed on: (a) a Packard 428 gas chromatograph (FID), using a glass column, 200 × 0.4 cm, packed with 3% OV-225 on Gas Chrom Q, 80–100 mesh, at 180° for partially methylated alditol acetates, and at 195° for alditol acetates; and (b) a Hewlett Packard 5830 A gas chromatograph using an SP-1000 capillary column, 25 m × 0.25 mm, with N_2 as carrier gas.

GC/MS was carried out on: (a) a Varian 3700 gas chromatograph coupled to a Varian MAT 44S mass spectrometer and a Varian Spectro Spin MAT 200 data processing system; and (b) a Varian MAT 311 gas chromatograph/mass spectrometer coupled to Spectro System SS-100. In both cases OV-225 was the column material. MS were recorded at an ionization potential of 70 eV.

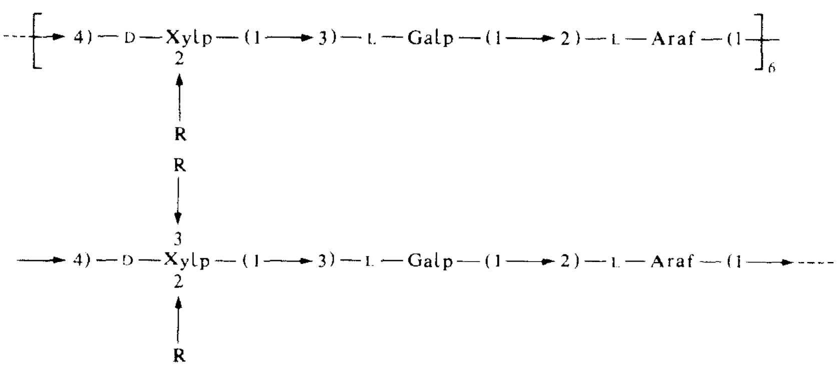
Isolation of the polysaccharide. From the locally available weed, water hyacinth, the mucin (40 g) was collected from the nodal region of the plant and was stirred with 5% aq. NaOH (300 ml) for 4 hr. The soln was squeezed through linen cloth and centrifuged. EtOH (four vols.) was added to the clear soln. The resultant ppt was collected, dissolved in H_2O, dialysed extensively against H_2O and poured into EtOH (six vols.) with stirring. The polysaccharide was collected, washed with EtOH, Me_2CO, Et_2O and dried over P_2O_5: yield 600 mg.

Purification. The polysaccharide was purified by pptn from 0.5% soln in 1.25 M NaOH with aq. $Cu(OAc)_2$ [3] and $Ba(OH)_2$ [4].

Electrophoresis [5]. Free boundary electrophoresis of a 1% soln of the polysaccharide in 0.1 M $Na_2B_4O_7$ (pH 9.28) was performed under an applied voltage of 22 V and a current of 5 mA. The movement of the boundary was followed by Schlieren optics.

Viscosity. The viscosity of aq. solns (0.004–0.04%) was determined with H_2O as a reference at 30° using an Ubelohde-type viscometer [6].

Sugar composition. The polysaccharide was hydrolysed with



R = L—Araf—(1→, L—Galp—(1→, D—Xylp—(1→, and D—Xylp—(1→3)—L—Araf—(1→.

Fig. 1.

0.25 M H_2SO_4 at 100° for 8 hr. The neutral sugars were examined by PC using solvents (a) and (b), and the acidic sugars in (c). The sugars were converted into their alditol acetates and analysed by GC. Optical rotations of the sugars were measured after isolation by prep. PC.

Isolation and characterization of oligosaccharides. The polysaccharide (600 mg) was hydrolysed with 0.125 M H_2SO_4 (250 ml) at 80° for 3 hr. The hydrolysate was subjected to prep. PC in solvent (a). Four oligosaccharide fractions [**1**–**4**, R_{Glc} 0.92, 0.68, 0.53 and 0.13, respectively, in solvent (a)] were isolated, and purified using solvent (d). Oligosaccharides, before and after $NaBH_4$ reduction were hydrolysed with acid, the sugars were analysed by PC and their alditol acetates by GC. The glycosidic linkages of the oligosaccharides were determined by methylation analysis [9, 10] and the MWs by Bertrand's method [14].

Methylation analysis. The polysaccharide (10 mg) was methylated twice according to the Hakomori procedure [9]. The permethylated product was hydrolysed with HCOOH for 1 hr at 100° and, after evaporation of HCOOH, with 0.25 M H_2SO_4 for 10 hr. The product after reduction with $NaBH_4$ and acetylation using pyridine–Ac_2O, was analysed by GC and GC/MS.

Periodate oxidation [11]. The polysaccharide (100 mg) was oxidized with 45 mM $NaIO_4$ (100 ml) in darkness at 6°. The IO_4^- consumption and HCOOH liberation were monitored by titration of aliquots against 0.1 N $Na_2S_2O_3$ and 0.01 N NaOH, respectively. After 48 hr the excess IO_4^- was destroyed with ethylene glycol (0.5 ml), dialysed and reduced with $NaBH_4$. Excess $NaBH_4$ was destroyed with 50% HOAc, dialysed and evaporated. The resulting polyalcohol was hydrolysed with acid, the sugars were identified by PC, and by GC as their alditol acetates.

Acknowledgements—We thank Professor R. Schauer and Dr. G. Reuter, University of Kiel, West Germany, and Professor B. Lindberg, University of Stockholm, Sweden, for GC and GC/MS analysis, Professor S. Hjerten, University of Uppsala, Sweden, for free-boundary electrophoresis. One of us (B.N.) thanks the UGC (Government of India) for an FIP fellowship, and the Government of Karnataka for study leave.

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