Carbohydrate Research, 215 (1991) 25–38
Elsevier Science Publishers B.V., Amsterdam

High-performance reversed-phase chromatographic mapping of 2-pyridylamino derivatives of xyloglucan oligosaccharides

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(Received July 2nd, 1990; accepted for publication, October 16th, 1990)

ABSTRACT

Xyloglucan oligosaccharides from cotton cell walls and tamarind seeds were derivatized with 2-aminopyridine and subsequently separated by reversed-phase chromatography (r.p.c.) using an octadecyl-silyl silica stationary phase and aqueous–organic eluents with 0.01% (v/v) trifluoroacetic acid. The chromatographic behavior of the 2-pyridylamino derivatives of xyloglucan oligosaccharides was examined under a wide range of elution conditions, including gradient steepness and shape, initial acetonitrile concentration in the eluent, and pore size of the r.p.c. packings. Relatively steep acetonitrile gradients resulted in poor resolution of the different xyloglucan fragments, which is believed to be the result of acetonitrile-induced conformational changes. Under these circumstances the elution order of the derivatized xyloglucan oligosaccharides was such that the smaller fragments eluted from the column before the larger ones. R.p.c. packing with a 70-Å pore size necessitated relatively high acetonitrile concentration in the eluent when compared with 300-Å stationary phase. The r.p.c. mapping of 2-pyridylamino derivatives of xyloglucan oligosaccharides was best achieved when both a wide-pore octadecyl-silyl silica stationary phase and a shallow gradient with consecutive linear segments of increasing acetonitrile concentration in the eluent were employed. This combination yielded rapid r.p.c. maps of the xyloglucan fragments from different sources with high separation efficiencies and concomitantly high resolution. The effects of the nature of the sugar residues in the xyloglucan oligomers and their degree of branching on r.p.c. retention and selectivity are also highlighted.

INTRODUCTION

Over the last fifteen years, numerous high-performance liquid chromatography (h.p.l.c.) techniques have been applied for the separation of carbohydrates. Aminopropyl-silica stationary phases[1,2] and their variants, *i.e.*, naked silica[3] or hydrocarbonaceous silica-based stationary phases[4] with dynamically coated amines, cation exchanger columns in metal forms[5,6], and anion exchange stationary phases[7] have traditionally been employed in the analysis and purification of carbohydrates. Recently, a significant development in the field of carbohydrate h.p.l.c. has been the application of resinous

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0008-6215/91/$03.50

micropellicular anion-exchange columns at high pH for the separation of mono- and oligo-saccharides[8,9]. In the last five years, reversed-phase chromatography (r.p.c.), which originally was developed for other purposes, has also found use in the separation and purification of both derivatized and underivatized sugars[10-12]. R.p.c. separations of underivatized carbohydrates are carried out with plain water as the mobile phase, whereas r.p.c. of derivatized saccharides is performed with aqueous–organic eluents. A drawback in r.p.c. of sugars is that the presence of anomers in carbohydrate structure leads to broad peaks containing poorly resolved anomers for each component of the sample[12,13]. This heterogeneity can be overcome by reducing the terminal aldehyde group to the alcohol with sodium borohydride[12] or by attaching to the reducing end an amino compound by reductive amination.

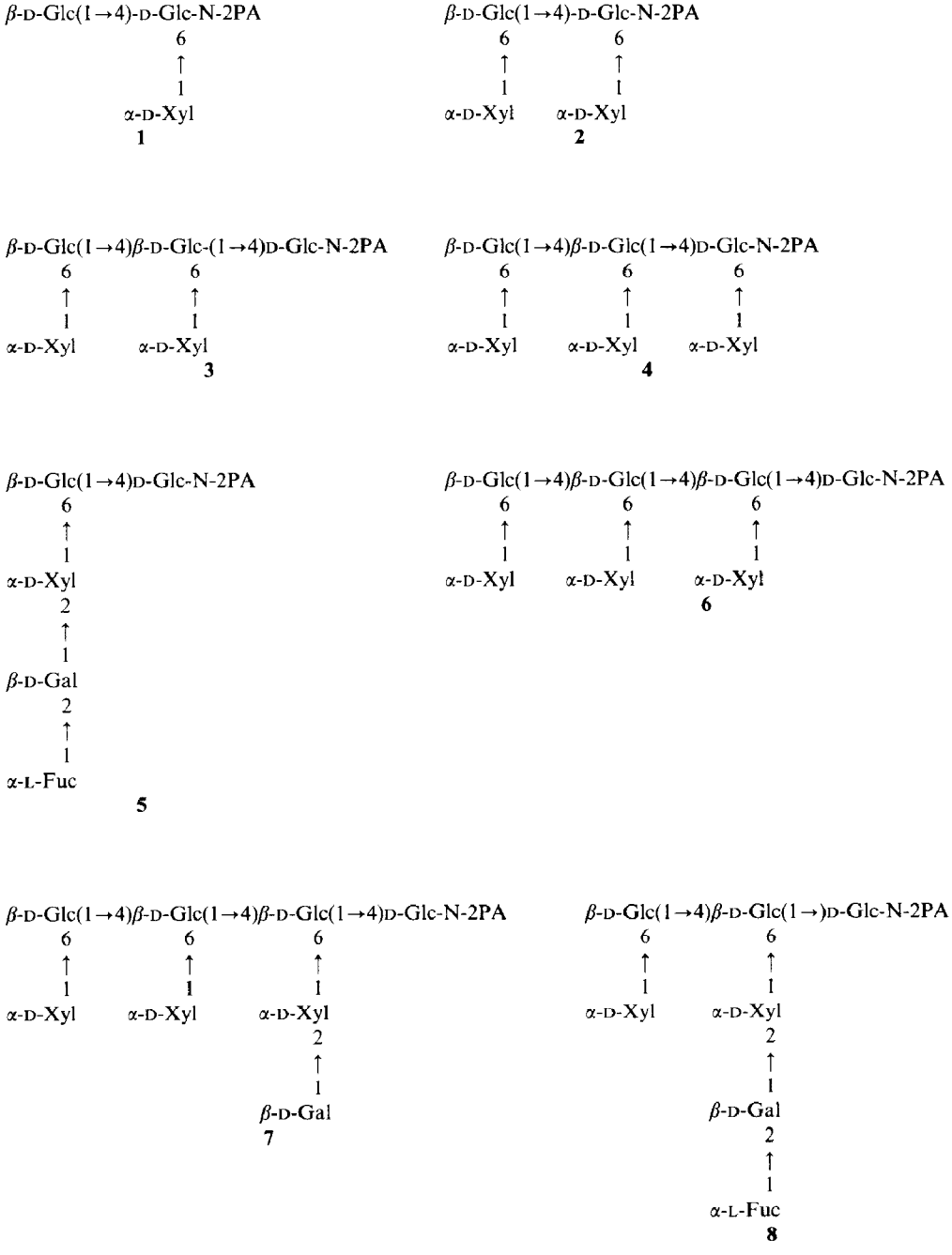
A major difficulty in carbohydrate analysis by h.p.l.c. has been the lack of appropriate chromophores in their structure to allow for their sensitive detection. Although pulsed amperometric detection (p.a.d.) permits carbohydrate analysis to be carried out at the picomole level[14], the use of relatively high pH that is required for the sensitive detection by p.a.d. imposes several limitations on the chromatographic system as far as the choice of mobile phase additives and stationary phase types are concerned. In such circumstances, other detection techniques should be used. Several pre-column derivatization schemes have been developed for the sensitive detection of carbohydrates by u.v. absorption[15] or by fluorescence[16] (for reviews see refs. 17 and 18).

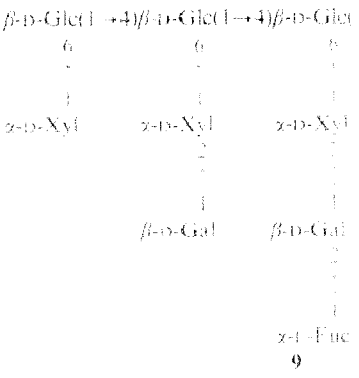
In this study, we report the separation and mapping of 2-pyridylamino (PA) derivatives of xyloglucan oligosaccharides from cotton cell walls and tamarind seeds using reversed-phase chromatography with octadecyl-silyl silica stationary phases and organic-lean eluents. The derivatization of oligosaccharides with 2-aminopyridine provides a center for sensitive u.v. detection, leads to an enhanced retentivity on reversed-phase columns, and eliminates the problems associated with anomerization.

In an ongoing project to determine the structure of the pectins of cotton suspension culture cell walls, xyloglucans (XGs) were unexpectedly extracted in association with pectins. It, therefore, became of interest to characterize the XGs of cotton. The importance of XGs in the plant kingdom and their interactions with cellulose are well documented. XGs have been found in dicots to be a major component of the primary cell walls and to be present to a lesser extent in monocots[19]. The basic backbone of xyloglucans is identical to that of cellulose, a (1→4)-β linked D-glucan. Variations in xyloglucans are caused by the differences in the nature and distribution of xylose, galactosyl-xylose, fucosyl-galactosyl-xylose and in some cases arabinosyl-xylose side chains on the glucan. A key enzyme for the characterization of xyloglucans has been the cellulase from *Trichoderma viridis*[20]. This complex of enzymes is able to digest the backbone of xyloglucans after any glucosyl residue which does not subtend a side chain. Very limited digestion takes place at branched (1→4)-β-D-glucosyl residues. This selectivity allows fragments of the polymer reflective of its branching patterns to be isolated after cellulase digestion. Proportions of the various fragments vary among species. Table I shows the 2-pyridylamino derivative of the most frequently observed fragments obtained after cellulase digestion of xyloglucans.

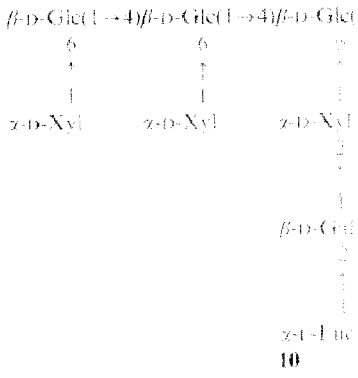
TABLE I

Structures of PA-XG fragments of cellulase digestion[a]

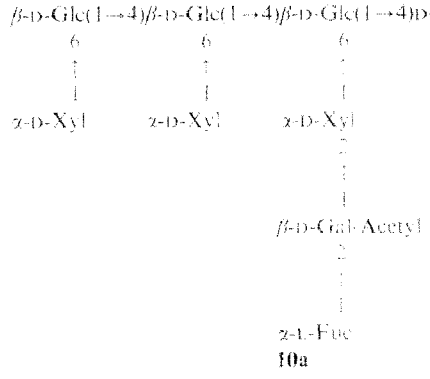



β-D-Glc(1→4)β-D-Glc(1→4)β-D-Glc(1→4)D-Glc-N-2PA
6
6
6
α-D-Xyl
α-D-Xyl
α-D-Xyl
β-D-Gal
β-D-Gal
α-L-Fuc

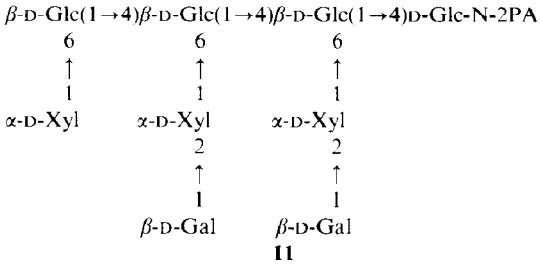
9


β-D-Glc(1→4)β-D-Glc(1→4)β-D-Glc(1→4)D-Glc-N-2PA
6
6
α-D-Xyl
α-D-Xyl
α-D-Xyl
β-D-Gal
α-L-Fuc

10


β-D-Glc(1→4)β-D-Glc(1→4)β-D-Glc(1→4)D-Glc-N-2PA
6
6
6
α-D-Xyl
α-D-Xyl
α-D-Xyl
β-D-Gal-Acetyl
α-L-Fuc

10a



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[a] Compounds **1–10** are those generated from cotton cell walls by Method 1, and **1–10a** are those obtained by Method 2. The PA-XG fragments from tamarind seeds are: **1–4, 6, 7,** and **11**. (Note: 2PA refers to 2-pyridylamino.)

Thus far, no high-resolution chromatographic method has been developed for separating and quantitating the fragments that may be generated after cellulase digestion of xyloglucans. Previous separations have relied mostly on gel filtration[21] on Bio-Gel P-2 or on paper chromatography[19]. The high resolving power of r.p.c. was exploited in the present study which is aimed at the characterization of xyloglucan fragments obtained by cellulase digestion. The resolving power and selectivity of r.p.c. systems are particularly good when gradient elution can be used. For this method to be practical, a detector insensitive to changes in solvent composition needed to be used. We therefore, prelabeled the oligosaccharides with a u.v.-absorbing (also fluorescent) tag prior to chromatography[16].

EXPERIMENTAL

H.p.l.c. instrumentation. — The chromatograph was assembled from an LDC–Milton Roy (Riviera Beach, FL, U.S.A.) Model CM4000 solvent delivery pump with a dual-beam variable-wavelength detector, SpectroMonitor 3100. A Rheodyne (Cotati, CA, U.S.A.) model 7125 sampling valve with a 100-μL sample loop was used for injection. Chromatograms were recorded with a Shimadzu (Columbia, MD, U.S.A.) model C-R6A integrator interfaced with a single floppy disk drive and a CRT monitor.

Materials. — Cell walls from Acala 44 cotton suspension cultures were prepared as previously described[22], as was the purified endopolygalacturonase from an *E. coli* clone containing its gene from *Erwinia carotovora*[23]. A cellulase preparation from Worthington ("chromatographically purified") was used as received. Reagent-grade trifluoroacetic acid (TFA) and h.p.l.c.-grade acetonitrile were obtained from Fisher Scientific Co. (Pittsburgh, PA, U.S.A.).

R.p.c. columns. — A Zorbax ODS-silica column (250 × 4.6 mm), having mean particle and pore diameters of 5 μm and 70 Å was obtained from Du Pont (Wilmington, DE, U.S.A.). A Bakerbond wide-pore octadecyl-silyl silica column (250 × 4.6 mm), having mean particle and pore diameters of 5 μm and 300 Å, was a gift from J. T. Baker, Inc. (Phillipsburg, NJ, U.S.A.). The Zorbax ODS-silica has a specific surface area (340 m^2/g) which is greater than that of the Bakerbond wide-pore octadecyl-silyl silica (76 m^2/g).

Gradients for r.p.c. — In all cases, solvents A and B were water and 40% (v/v) acetonitrile in water, respectively, both at 0.01% (v/v) trifluoroacetic acid (TFA). The following gradients were used: Gradient I, 15 min at linearly increasing acetonitrile concentration from 0 to 40% (v/v) in water; Gradient II, 30 min at linearly increasing acetonitrile concentration from 0 to 40% (v/v) in water; Gradient III, 30 min linear gradient from 4.8 to 40% (v/v) acetonitrile in water; Gradient IV, consecutive linear segments of increasing acetonitrile concentration in water from 4.8 to 6% (v/v) in 5 min, from 6 to 10% (v/v) in 20 min, from 10 to 20% (v/v) in 10 min, and from 20 to 40% (v/v) in 5 min; and Gradient V, consecutive linear segments of increasing acetonitrile concentration from 0 to 3.2% (v/v) in 15 min, from 3.2 to 6.8% (v/v) in 10 min, from 6.8 to 13.2% (v/v) in 15 min, followed by isocratic elution at 13.2% acetonitrile for 5 min.

Isolation of the xyloglucan–rhamnogalacturonan complex. — Cell walls were exhaustively digested with endopolygalacturonase (EPG) in 50mM ammonium acetate buffer, pH 5.2, to remove all homogalacturonan. Approximately 80% of the initial galacturonic acid in the walls was solubilized by this treatment. A subsequent extraction of the water-washed residue with alkali (24% KOH), containing 0.1% $NaBH_4$ to prevent the peeling reaction, extracted all of the sugars expected to be in xyloglucan along with *those* expected in what has been called rhamnogalacturonan I (RG I). The solubilized material was neutralized with acetic acid and dialyzed against distilled water. The xyloglucan-RG I complex was further fractionated by DEAE-Sephadex chromatography (the complex was not bound), and a portion was then subjected to chromatography on a TSK 400 (from Bio-Rad, Richmond, CA, U.S.A.) gel-filtration column and found to elute predominantly in the void volume. About 25% of the material consisting predominantly of arabinose eluted much later and is not further considered here. Experiments to determine if there is a covalent attachment between the xyloglucan and the RGI are underway in the laboratory of A.M.

Generation of xyloglucan fragments (Method 1). — The xyloglucan-RG I complex was found to be digested completely by 0.64 units of cellulase per mg of complex in 24 h at room temperature in 50mM ammonium acetate buffer at pH 5.2. Less enzyme led to only partial digestion. More enzyme could have led to overdigestion[20] or to the action of trace contaminating activities. Some other cellulase preparations have been found to contain activities which degrade xyloglucans into smaller less informative fragments than those generated by the Worthington enzyme[19]. After the digestion, the enzyme and undigested polymer (RGI) were removed from the xyloglucan fragments by chromatography on the TSK 400 column mentioned above.

Generation of xyloglucan fragments (Method 2). — Direct digestion of the residue from EPG-treated cell walls with cellulase-solubilized xyloglucan fragments along with ca. 50% of the RGI. The enzyme and RGI could be separated from the xyloglucan fragments by gel filtration on a 50 × 1 cm Toyopearl HW 40(S) column from Suppelco, Inc. (Bellefonte, PA, U.S.A.) equilibrated with 50mM ammonium acetate buffer, pH 5.2. The enzyme and RGI eluted in the void volume, and the cellobiose and glucose eluted after the major xyloglucan fragments.

Labeling of oligosaccharides. — The xyloglucan fragments were labeled with

2-aminopyridine at their reducing termini by reductive amination as described by Hase *et al.*[16] The excess 2-aminopyridine was removed by one of two alternate methods: (*i*) The reaction mixture was passed through the Toyopearl HW 40(S) gel-filtration column mentioned above, or (*ii*) The reaction mixture was passed through a cation exchange cartridge (Extract-Clean from Alltech Associates, Deerfield, IL, U.S.A.) in the ammonium form and eluted with water[24].

Liquid secondary ion mass spectrometry. — The 2-pyridylamino derivatives of xyloglucan (PA-XG) oligosaccharides separated by r.p.c. were tentatively identified by liquid secondary ion mass spectrometry (l.s.i.m.s.). The pooled fractions were evaporated to dryness using a SpeedVac Concentrator (from Savant Instrument Co., Farmingdale, NY, U.S.A.). The dried materials were then dissolved in 2 to 5 μL of either water or methanol, and 1 μL of the solution was mixed with 1 μL of 1-thioglycerol on a stainless steel target. Spectra were obtained on a ZAB 2SE mass spectrometer (from VG Instruments, Manchester, UK). Using cesium ions at 35 Kv for the ionization, spectra were collected in the positive-ion mode. Both $[M + H]^+$ and $[M + Na]^+$ ions were observed. Since only molecular weight information was obtained from the l.s.i.m.s., structures were inferred from previously proven structures of xyloglucan fragments obtained from both sycamore and tamarind using *Trichoderma* endoglucanase[25]. A number of minor peaks in the r.p.c. map gave spectra which we were not able to interpret and therefore were not identified.

Sugar composition. — Sugar compositions were determined by methanolysis and trimethylsilylation as described by Komalavilas and Mort[22].

RESULTS AND DISCUSSION

The chromatographic behavior of 2-pyridylamino derivatives of xyloglucan oligosaccharides (PA-XG) was examined in reversed-phase chromatography by varying gradient steepness and shape, initial acetonitrile concentration in the eluent, and pore size of the r.p.c. packings. In addition, the effects of the nature of the sugar residues in the PA-XG oligomers and their degree of branching on r.p.c. retention and selectivity are highlighted.

Gradient steepness and shape. — The effects of gradient steepness and shape on the separation of PA-XG oligosaccharides generated from cotton cell walls by Method 1 were examined with the Zorbax ODS column. The gradient duration, which is a convenient definition of gradient steepness, was varied in order to study the effect of volume percent acetonitrile on retention and to determine the optimum elution conditions of the PA-XG oligosaccharides. Using a 15-min linear gradient from 0 to 40% (v/v) acetonitrile in water (Gradient I), so that the gradient was 2.67% acetonitrile/min, yielded poor resolution of the PA-XG oligosaccharides. The derivatives eluted as two major peaks with adjusted retention volumes of 9.36 mL and 9.98 mL, respectively. The adjusted retention volume, V'_R, of a solute as measured in both isocratic and gradient elution is the difference between the retention volume, V_R, of that solute and the void volume of the column, V_M. With a 30-min linear gradient from 0 to 40% (v/v)

acetonitrile in the eluent (Gradient II), *i.e.*, a gradient of 1.33% (v/v) acetonitrile/min, the resolution between the two major peaks improved with concomitant increase in adjusted retention volumes; the two major peaks eluted with adjusted retention volumes of 12.79 mL and 14.0 mL, respectively. In addition, two minor components of the sample appeared slightly before the first major peak. Despite the difference in branching and in the polar character of the PA-XG derivatives, the relatively high rate of change of acetonitrile composition, when using Gradients I and II, apparently resulted in the concentration of acetonitrile being high enough to cause conformational changes of the oligosaccharides so that the difference in molecular surface areas of the various sugar chains became negligible before the oligomers had eluted from the column, and, as a result, low selectivity was obtained.

With a 30-min linear gradient from 4.8 to 40% (v/v) acetonitrile in water (Gradient III), so that the gradient was 1.17% acetonitrile/min, all the PA-XG oligosaccharides were less retained than with Gradient II, and the resolution between the sample components improved. The corresponding chromatogram is portrayed in Fig. 1, with tentative structural identification (see Table I) of the separated oligosaccharides as inferred from a combination of f.a.b.m.s. molecular weight information, sugar composition, and previously confirmed structures[5].

Starting at 4.8% (v/v) acetonitrile in the eluent but using a gradient with consecutive linear segments (Gradient IV) of even lower steepness than in the preceding experiment improved further the separation of PA-XG oligosaccharides, as can be seen in Fig. 2.

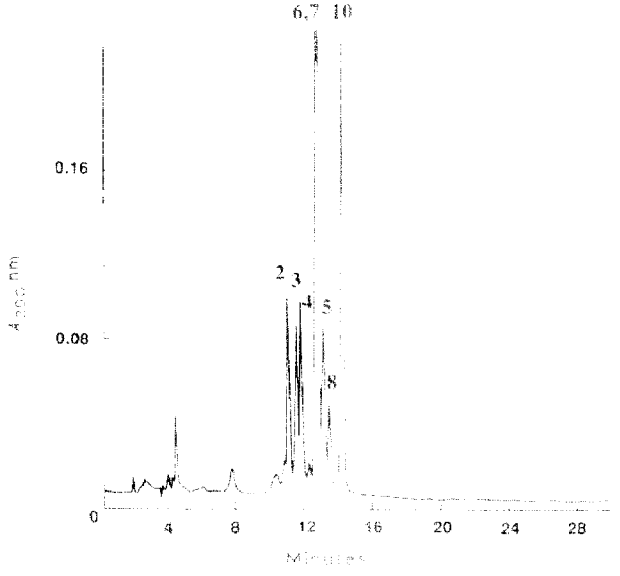

Fig. 1. Separation of 2-pyridylamino derivatives of xyloglucan oligosaccharide fragments obtained by cellulase digestion of xyloglucan from cotton cell walls. Column, Zorbax-ODS, 250 × 4.6 mm; flow-rate, 1 mL/min; temp., 25°. Gradient III (see Experimental for details).

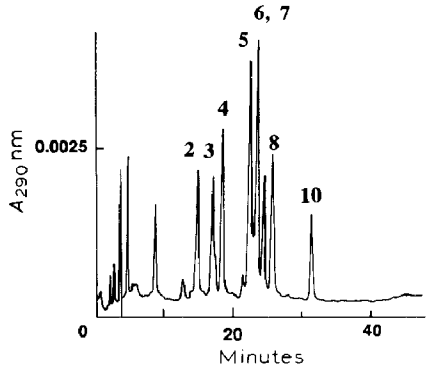

Fig. 2. Reversed-phase chromatography of the xyloglucan mixture reconstituted by mixing aliquots from fractions collected during chromatographic runs under elution conditions as in Fig. 1. Column, Zorbax-ODS, 250 × 4.6 mm; flow-rate, 1 mL/min; temp., 25°. Gradient IV. Other conditions are as in Fig. 1.

Returning to Fig. 1, it can be seen that fragment **5**, which is smaller than fragments **6** and **7**, eluted later in the gradient than expected. This irregular behavior was caused by organic solvent-induced conformational changes, since it did not persist when Gradient IV was used, and fragments **5**, **6**, and **7** eluted from the column in the expected order; compare Figs. 2 and 3 to Fig. 1. It must be noted that the chromatograms in Figs. 2 and 3 were obtained by chromatographing a mixture of aliquots from the fractions collected under elution conditions of the chromatogram illustrated in Fig. 1, and the identity of each peak in Figs. 2 and 3 was determined by individual injections into the chromatographic column of diluted aliquots from those fractions.

The above chromatographic behavior, which revealed irregularities concerning the dependence of retention of oligosaccharides on eluent composition, has also been observed in r.p.c. of other polar compounds such as homo- and hetero-oligonucleotides[26,27], whereby a reversal in the elution order occurred, *i.e.*, small oligonucleotides were more retarded than larger ones, and non-linear dependence of retention on volume

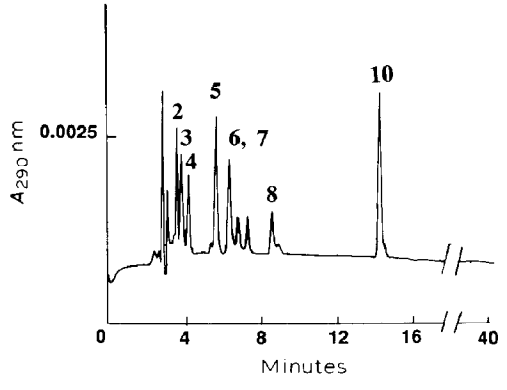

Fig. 3. Same as Fig. 2, except column is Bakerbond Wide-pore ODS, 250 × 4.6 mm.

percent organic solvent was observed as the acetonitrile concentration in the hydro-organic eluent increased.

Pore size of the stationary phases. — Fig. 3 illustrates the separation obtained on a Bakerbond wide-pore ODS-silica column under the same elution conditions as in Fig. 2 (Gradient IV). As expected, due to the smaller phase ratio of wide-pore silica stationary phase, the retention times of the components of the PA-XG mixture decreased substantially when going from 70-Å to 300-Å silica under otherwise identical elution conditions.

The term phase ratio, φ, refers to the ratio of the volume of the stationary phase, V_S, to that of the mobile phase, V_M, in the column. Since the Bakerbond wide-pore ODS-silica has a specific surface area of 76 m^2/g, which is smaller than that of Zorbax ODS-silica (340 m^2/g), the amount of the non-polar bonded stationary phase per column, and, in turn, φ, is decreased with the former when compared with the latter.

As seen in Fig. 3, the PA-XG oligosaccharides eluted from the wide-pore ODS-silica column at below 10% (v/v) acetonitrile in water. This represents an advantage in carbohydrate h.p.l.c. whereby mobile phases of low organic content are well suited for avoiding solute precipitation during the chromatographic run.

R.p.c. mapping of PA-XG oligosaccharide fragments with wide-pore ODS silica. — Based on the above results, Bakerbond wide-pore ODS-silica was chosen for the rest of

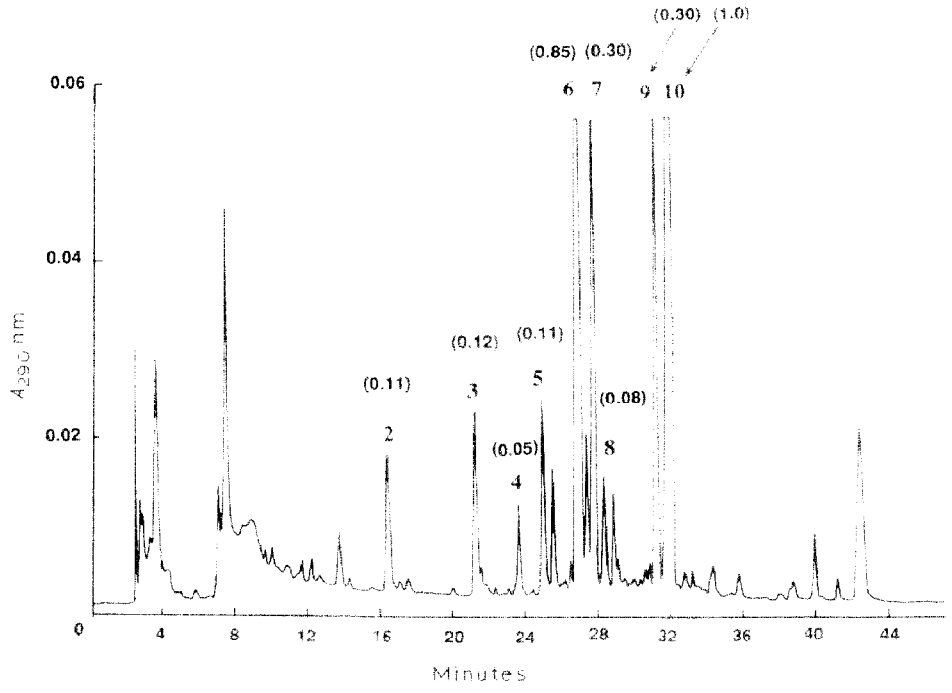

Fig. 4. Reversed-phase chromatography map of 2-pyridylamino derivatives of xyloglucan oligosaccharide fragments from cotton cell walls obtained by cellulase digestion. Column, Bakerbond wide-pore ODS, 250 × 4.6 mm; flow-rate, 1 mL/min; temp., 25°. Gradient V. The numbers in parentheses indicate the relative peak areas as normalized to that of the nonasaccharide **10**.

the studies since acetonitrile-lean eluent could bring about full separation of the sample components in a shallow gradient. Gradient V was used, which consisted of consecutive linear segments of shallow gradient changes (see Experimental). The r.p.c. map of the PA-XG oligosaccharides from cotton cell walls is depicted in Fig. 4. The different fragments were collected and analyzed by mass spectrometry. The tentative structures of the different fragments are given in Table I. As can be seen in Fig. 4, the complete mapping is obtained in less than 45 min with baseline resolution between most of the fragments. The numbers displayed in parentheses on each peak (see Fig. 4) represent the relative peak areas as normalized to that of the nonasaccharide (structure **10**), and should be considered as the relative mole amounts of each oligomer[23]. Nona- and hepta-saccharides (structure **6**) are the most abundant fragments in the mixture, followed by octa- and deca-saccharides. This is in agreement with reported proportions for cotton fibers[28] and similar to that for most dicotyledonous cell wall xyloglucans[19], and provides support to the method established in this study.

The above general elution scheme was also applied to the xyloglucan fragments from cotton cell walls obtained by Method 2. In Method 2 the xyloglucan is never exposed to alkaline conditions. Therefore, any esters which were present on the native polysaccharide should have been retained on the PA-XG fragments. Acetylated xyloglucan oligosaccharides have been characterized from the XG obtained from the medium around sycamore suspension cultures[29] and by cellulase digestion of cell walls from sycamore suspension cultures[30]. Only the galactose residue has been found to be esterified, predominantly at postion 6, but also to a lesser extent at positions 3 and 4. The cellulase mapping of these PA-XG oligosaccharides is portrayed in Fig. 5. Here again, the combined areas under the peaks of acetylated (structure **10a**) and non-acetylated nonasaccharide (structure **10**) made up the highest number with respect to the other peaks in the map. Acetylated and nonacetylated nonasaccharide and heptasaccharide are the most abundant fragments followed by octa- and deca-saccharides.

The following summarizes some general effects of each sugar residue on the chromatographic behavior of PA-XG oligosaccharides. As expected, due to the non-polar character of the substituent, the presence of an acetyl group on the galactosyl residue in the side chain of the nonasaccharide resulted in a substantial increase in the retention of the oligomer when compared to that of the non-acetylated oligomer (see Fig. 5). The fucosylated isopentaose (structure **5**) is more retarded than the pentaose and hexaose. Also isoheptaose (structure **8**), which is fucosylated, is more retarded than the heptaose and octaose. This is an expected behavior since the fucosyl residue has a methyl group, and therefore is less polar than any of the other sugar moieties in the PA-XG oligosaccharides. This imparts to the fucosylated oligosaccharides a stronger interaction with the r.p.c. column than that exhibited by the unfucosylated XG fragments of similar or slightly larger size. It is also apparent from the comparison of the retention behavior of the different XG fragments that a xylosyl residue imparts to the oligomer a smaller retention increment than that of a glucosyl residue. On the other hand, a galactosyl residue produces a retention increment smaller than that produced by xylose and much smaller than that of glucose. An irregular behavior, however, is

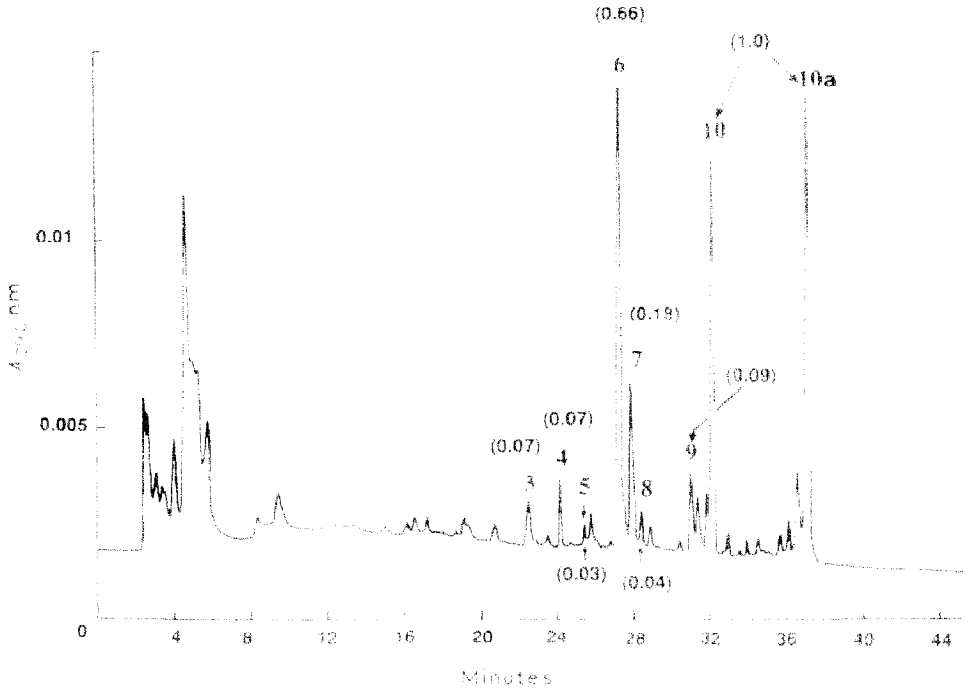

Fig. 5. Reversed-phase chromatography map of 2-pyridylamino derivatives of acetylated xyloglucan oligosaccharide fragments from cotton cell walls obtained by cellulase digestion. The numbers in parentheses indicate the relative peak areas as normalized to the sum of peak areas of the acetylated and deacetylated nonasaccharides. Conditions are as in Fig. 4.

exhibited by the decasaccharide, which is less retained than the nonasaccharide, despite the fact that the former has an additional galactosyl residue with respect to the latter. This may be explained by organic solvent induced conformational changes[20]. A fucosyl residue seemed to impart a retention increment similar to or greater than the sum of retention increments of a glucosyl and a xylosyl residue.

Fig. 6 illustrates the oligosaccharide map of the cellulase digest of xyloglucan from tamarind seed. Due to the lack of L-fucose in the oligosaccharides derived from tamarind, the PA-XG map is less populated with peaks compared to the maps of PA-XG from cotton cell walls. Note the absence of isopentaose, isoheptaose, and the fucosylated nonasaccharide from the map. The abundance of the different fragments as estimated from the areas under the peaks decreased in the following order: nonaose (structure **11**) combined with little of heptaose > octaose > hexaose = tetraose > pentaose. The numbers in parentheses by the peaks in Fig. 6 indicate the relative peak areas as normalized to that of the most intense peak; the nonasaccharide plus a little of heptasaccharide from which it was not well resolved.

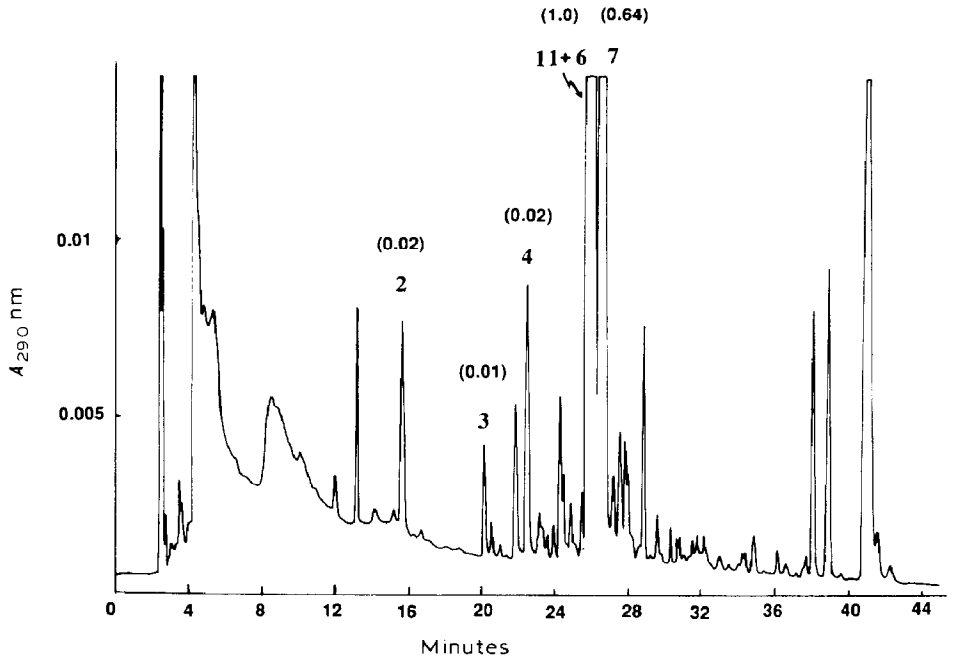

Fig. 6. Reversed-phase chromatography map of 2-pyridylamino derivatives of xyloglucan oligosaccharide fragments from tamarind seeds obtained by cellulase digestion. The numbers in parentheses by the peaks represent their relative areas as normalized to the sum of peak areas of the most intense peak, the nonasaccharide, and a small portion of heptasaccharide from which it was not well resolved. Conditions are as in Fig. 4.

CONCLUSIONS

Reversed-phase chromatography is well suited for the rapid separation of 2-pyridylamino derivatives of xyloglucan oligosaccharides from different sources, especially when wide-pore octadecyl-silyl silica stationary phases and acetonitrile-lean eluents are employed. Under these circumstances high resolution between the different fragments is easily attained and the elution order of the derivatized xyloglucan oligomers can be predicted. As a result, it is possible to draw inferences concerning structural characteristics of the oligomers and, in turn, expedite subsequent details structural analyses. Furthermore, the low-volume-percent acetonitrile in the gradient, which is required to bring about elution and separation, represents an advantage in terms of solute recovery from the chromatographic column, especially when dealing with polar compounds such as oligosaccharides. The method established here is expected to find general use in the field of oligosaccharides.

ACKNOWLEDGMENTS

Z.E.R. acknowledges the financial support in part from The College of Arts and Sciences, Dean's Incentive Grant Program, and in part from Grant No. HN9-004 from

the Oklahoma Center for the Advancement of Science and Technology, Oklahoma Health Research Program. The technical assistance of Carolyn Bowen in some chromatographic experiments is gratefully acknowledged. A.J.M. acknowledges support by DOE (U.S. Department of Energy) Grant DE-FG-05-86 ER13 496 and the Oklahoma Agricultural Experiment Station, Oklahoma State University. Journal Article No. J-5869 of the Oklahoma Agricultural Experiment Station.

The ZAB 2SE mass spectrometer was partially obtained via NSF Grant BSS-8704089. We thank Mr. Paul West and Dr. Paul Geno for obtaining the mass spectra.

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