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A new undecasaccharide subunit of xyloglucans with two α-L-fucosyl residues*

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ABSTRACT

A new oligosaccharide subunit of xyloglucan was isolated from the β-(1→4)-endoglucanase digestion products of the xyloglucan in what is referred to as "sycamore extracellular polysaccharides" and found to be an undecasaccharide having two terminal α-L-fucopyranosyl residues. The undecasaccharide was structurally characterized by ^{1}H-n.m.r. spectroscopy, fast-atom bombardment mass spectrometry (f.a.b.-m.s.), and glycosyl-residue and glycosyl-linkage composition analyses. The structure of the undecasaccharide was confirmed by digesting it with a hydrolase that releases α-D-Xyl*p*-(1→6)-D-Glc from the non-reducing end of xyloglucan oligosaccharides.

INTRODUCTION

Xyloglucans (XGs) are an important class of hemicellulosic polysaccharides found in the primary cell walls of all higher plants[1–11]. XGs adhere strongly to cellulose and appear to coat the surface of cellulose microfibrils in the walls of growing plant cells[1,11]. It has been suggested that XGs may thus act as cell-wall stabilizing molecules. Furthermore, there is evidence suggesting that oligosaccharide fragments of XG may play a role in regulating plant cell growth[12–15].

XGs are highly branched polymers consisting of a β-(1→4)-glucan chain in which ~75% of the β-D-glucosyl residues are substituted at C-6 with α-D-xylosyl residues. Some of the α-D-xylosyl residues are also substituted at C-2 with β-D-galactosyl, or α-L-fucosyl-(1→2)-β-D-galactosyl moieties[2,4,9]. Most of the 2-linked galactosyl residues of XG fragments isolated from either sycamore cell walls or sycamore extracellular polysaccharides have one or two *O*-acetyl substituents[5,16].

Treatment of XGs with a β-(1→4)-endoglucanase isolated from *Tricoderma reesei* cleaves the β-(1→4)-glucan backbone at unbranched β-D-glucosyl residues, generating oligosaccharide subunits[2] of the polymer. Products obtained when XGs from sycamore cell walls or from sycamore extracellular polysaccharides[2] are treated with the β-(1→4)-endoglucanase include the reducing oligosaccharides **1–4**. This paper describes

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the use of h.p.l.c. to rexamine the primary oligosaccharide subunits released from sycamore extracellular XG by β-(1→4)-endoglucanase. This analysis resulted in the discovery of a new XG oligosaccharide subunit, an undecasaccharide, that contains two fucosyl residues. Treatment of either sycamore cell walls or alkali-soluble xyloglucan from rapeseed hulls with β-(1→4)-endoglucanase released the same undecasaccharide, identified by ^{1}H-n.m.r. spectroscopy after purification and borohydride reduction.

MATERIALS AND METHODS

Nomenclature for XG oligosaccharides and oligoglycosyl alditols. — The letters a, b, and c are used as superscript identifiers (Glc^a, Xyl^a, etc.) in the text to indicate the location of residues *vis-a-vis* the reducing Glc or Glcol residues[9]. Oligoglycosyl alditols (**6–10**) were prepared by the reduction of the corresponding oligosaccharides (**1–5**) with $NaBH_4$.

Enzymes. — An endoglucanase [(1→4)-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4], that cleaves only the unbranched 4-linked glucosyl residues of XGs, was isolated from cultures of *Trichoderma reesei*, as described[2]. An oligoxyloglucan hydrolase that cleaves α-D-Xyl*p*-(1→6)-D-Glc moieties from the non-reducing end of xyloglucan oligosaccharides was prepared from Sanzyme 1000 (Sankyo), as described[9]. Endo-α-(1→4)-polygalacturonase (EC 3.2.1.15, see below) was purified[17] from the culture filtrate of *Aspergillus niger* by the procedure of Cervone *et al.*

Treatment of **10** *with immobilized oligoxyloglucan hydrolase.* — Chitin that was 35% *N*-deacetylated was obtained by boiling chitin in 28.6% (w/w) sodium hydroxide for 1 h (ref. 18). The partially deacetylated chitin, which is insoluble in aqueous acid, binds protein when activated with glutaraldehyde. The protein-binding capacity of the glutaraldehyde-activated, partially deacetylated chitin is similar to that of chitosan when the latter is dissolved in water at pH 5 or below[18]. Ten mg of partially deacetylated chitin (−80 mesh) was suspended in a mixture of 0.5 mL of 50 mM sodium acetate buffer (pH 5.0) and 1.0 mL of 25% (v/v) glutaraldehyde. The suspension was gently mixed at room temperature for 1.5 h in an Eppendorf conical centrifugation tube attached to a rotating rod in order to invert the tube ~10 times per min. Excess glutaraldehyde was removed by washing the precipitated residue with acetate buffer. Partially purified oligoxyloglucan hydrolase (0.5 mL containing ~200 μg protein) and acetate buffer (0.5 mL) were added to the washed, partially acetylated chitin, and the suspension was mixed for 1.5 h at 24°. The immobilized enzyme was washed with the acetate buffer containing 0.01% sodium azide to remove the small amount of contaminating anthrone-positive material, and the enzyme was stored at 5°. 1,5-Dideoxy-1,5-imino-L-fucitol (100 μg)[19] was used to inhibit the activity of a contaminating α-L-fucosidase to levels such that no α-fucosidase-generated products were detected by h.p.l.c. Compounds **7–10** (300 μg each) were individually incubated (5 h, 24°) with a suspension of the immobilized enzyme containing the fucosidase inhibitor (100 μg). The digested products, recovered in the supernatant after centrifugation, were treated with Dowex-50 [H^+] to remove sodium ions. The eluate was then mixed with a small amount of

methanol, concentrated, and dissolved in 100 μL of water. Aliquots (10 μL) were analyzed by h.p.l.c. on the NH_2 column.

Purification of deacylated XG. — Sycamore extracellular polysaccharides were prepared as described from the culture filtrate (30 L) of suspension-cultured *Acer pseudoplatanus* cells, deacetylated (2°, pH 12) as described[2], and then treated with endo-α-(1→4)-polygalacturonase at pH 5.2 to digest pectic polymers. The enzyme-digested mixture was dialyzed against 10mM imidazole·HCl buffer (pH 7.0), and then applied on a column of Q-Sepharose (Pharmacia). XG was eluted with 10mM imidazole·HCl buffer and then chromatographed on a column (2.5 × 90 cm) of Bio-Rad agarose A 5m eluted with 50mM NaOAc (pH 5.2). The partially included anthrone-positive[20] fractions were pooled, dialyzed, and lyophilized, yielding 1320 mg of purified xyloglucan (deacetylated form).

Preparation of oligosaccharides of xyloglucan. — Purified XG (620 mg) was incubated (96 h, 25°) in 310 mL of 50mM sodium acetate (pH 5.2) containing 0.02% thimerosal (sodium ethylmercurithiosalicylate) and endo-β-(1→4)-glucanase (20.65 units, one unit being defined as the amount of enzyme required to produce 1 μmol of reducing glucose per min, using carboxymethyl cellulose as substrate). The digestion mixture was then passed through a column (1 × 30 cm) of Dowex-50 × $[H^+]$ (2–200 mesh), and then a column (1 × 30 cm) of Dowex-2 $[CO_2^{2-}]$. The eluate was concentrated and lyophilized, yielding 604 mg of XG oligosaccharides.

Bio-Gel P-2 chromatography. — XG oligosaccharide (10 mg) was applied to a column (1.6 × 96 cm) of Bio-Gel P-2 (−400 mesh) and eluted with water (0.2 mL/min), collecting 1.3-mL fractions. The elution of oligosaccharides (Fig. 1) was monitored by differential refractometry (Waters). The amount of sample pooled in each fraction was estimated by the anthrone assay[20]. For preparative chromatography, 60 mg of XG oligosaccharide was applied on the column.

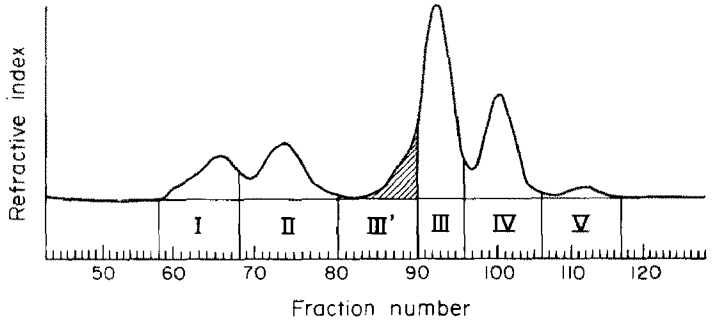


Fig. 1. Bio-Gel P-2 chromatography of oligosaccharides (10 mg) obtained from sycamore extracellular xyloglucan by digestion with the (1→4)-β-D-glucan 4-glucanohydrolase. XG oligosaccharides eluted from the Bio-Gel P-2 column were detected with a differential refractometer. Fractions were pooled as indicated. For preparative purposes, aliquots (60 mg) of the XG oligosaccharides were applied on the column, and fractions were pooled as shown. Pooled fractions from 10 preparative runs (600 mg of digested sycamore extracellular XG) were concentrated and lyophilized. The following yields were obtained: 66 mg in peak I, 102 mg in peak II, 38 mg in peak III', 225 mg in peak III, 144 mg in peak IV, and 25 mg in peak V.

H.p.l.c. — H.p.l.c. was conducted at room temperature with a Waters 6000A pump system fitted with a differential refractometer (Knauer) and a Dynamax-60A NH_2 column (4.6 mm × 25 cm, Rainin). The solvent was acetonitrile–water (13:7, v/v) pumped at a flow rate of either 1.0 or 1.5 mL/min. Oligosaccharides that were eluted from the NH_2 column were concentrated, dissolved in water, and passed through a column (1 × 30 cm) of Sephadex G-15 to remove low-molecular-weight material(s). The yield of XG oligosaccharide was determined by the anthrone assay[20].

Reduction of XG oligosaccharides. — Each purified oligosaccharide (1 mg) was dissolved and reduced in 0.1 mL of M NH_4OH containing $NaBH_4$ (1 mg), as described[21]. After 1 h, the reaction mixture was chilled in an ice bath and mixed with 30 μL of 5M acetic acid (final pH ~4.9). The reduced XG oligosaccharides were then desalted on a column (1 × 30 cm) of Sephadex G-15, concentrated, and frozen.

Chemical analyses. — Glycosyl-composition and glycosyl-linkage analyses were determined as described[22,23]. A phthalate contaminant was found to co-chromatograph with arabinitol pentaacetate on the fused silica SP2330 capillary column used for glycosyl-composition analysis. Therefore, alditol acetate samples were passed through a Supelclean LC-Ph cartridge (Supelco) that was eluted with 70% aqueous methanol to remove this contaminant.

Fast-atom bombardment-mass spectrometry (f.a.b.-m.s.). — Positive-ion and negative-ion f.a.b.-m.s. spectra were obtained as described[10].

Nuclear magnetic resonance spectroscopy (n.m.r.). — Oligoglycosyl alditols were dissolved in deuterium oxide (99.6% ^2H, Aldrich Chemical Co.) in order to replace exchangeable protons with deuterons, and the solvent was evaporated. The sample was then dissolved in deuterium oxide (99.96% ^2H, Cambridge Isotope Laboratories, 0.6 mL). ^1H-n.m.r. spectra were recorded at 27° with a Bruker AM 500 n.m.r. spectrometer, as described[10].

RESULTS AND DISCUSSION

Isolation of undecasaccharide **5**. — XG oligosaccharides were obtained by digesting purified sycamore extracellular XG with *T. reseii* endo-β-D-(1→4)-glucanase to selectively hydrolyze unbranched 4-linked β-D-glucosyl residues[2,9]. The oligosaccharide products were separated into five major fractions by chromatography on Bio-Gel P-2 (Fig. 1). Peak III corresponded to a mixture of **3** and **4**, and peaks IV and V corresponded to **2** and **1**, respectively. Larger XG oligosaccharides[10,27] were eluted as peaks I and II. The use of refractive index to monitor oligosaccharide elution from the Bio-Gel P-2 column revealed peak III′, as a shoulder on the leading edge of peak III (Fig. 1), indicating the presence of another XG oligosaccharide larger than decasaccharide **4**. When peak III′ was analyzed by h.p.l.c. on the NH_2 column, a new oligosaccharide (later characterized as **5**) was detected in addition to nonasaccharide **3** and decasaccharide **4** (Fig. 2A). In order to obtain milligram amounts of the new oligosaccharide in a pure form, peak III′ was reapplied to the Bio-Gel P-2 column. The leading edge of the resulting peak was subjected to semi-preparative h.p.l.c. on the NH_2 column (Fig. 2B) to yield 2.8 mg of the novel undecasaccharide.

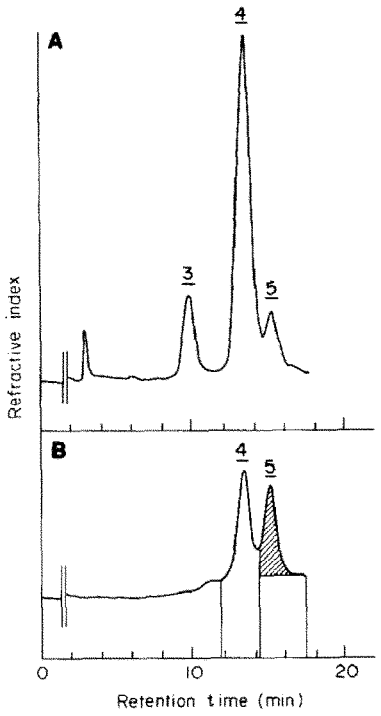
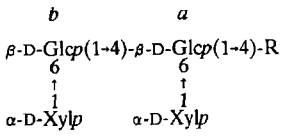


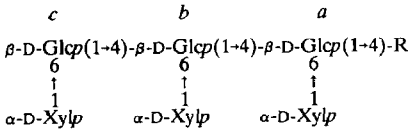
Fig. 2. H.p.l.c. of oligosaccharides **3**, **4**, and **5** on the Dynamax-60A NH_2 column. Panel A depicts h.p.l.c. of total peak III' oligosaccharides from the Bio-Gel P-2 column (Fig. 1). Panel B depicts h.p.l.c. of a fraction that was enriched in **5** by rechromatography of peak III' oligosaccharides on Bio-Gel P-2.

^{1}H-n.m.r. analysis of **10**. — Undecasaccharide **5** was converted into the corresponding decaglycosyl alditol **10** by reduction with $NaBH_4$ in order to obtain simpler ^{1}H-n.m.r. spectra[9]. The chemical shifts and scalar coupling constants of signals in specific subregions of the ^{1}H-n.m.r. spectra of **7–9**, which were prepared by $NaBH_4$ reduction of **2–4**, have been shown[9] to correlate with specific substructures in XG-derived oligoglycosyl alditols. Accordingly, the anomeric regions of the ^{1}H-n.m.r. spectra of the oligoglycosyl alditols **7–10** were divided into subregions[9] A–G (Fig. 3). This allowed the two doublets [δ 5.257 ($J_{1,2}$ 3.8 Hz) and δ 5.279 ($J_{1,2}$ 3.8 Hz)] in subregion A of the spectrum of **10** to be assigned as the anomeric protons of *two* terminal α-L-fucopyranosyl residues. The spectrum of **10** included a doublet at δ 5.125 ($J_{1,2}$ 3.5 Hz) in subregion C and a doublet at δ 5.145 ($J_{1,2}$ 3.7 Hz) that was between subregions B and C, as defined in the original study[9]. The observation of *two* α-L-fucopyranosyl residues in the molecule indicated that *both* of these doublets arise from H-1 of α-D-xylopyranosyl residues having a 2-*O*-(α-fucopyranosyl)-β-galactopyranosyl substituent at O-2, and therefore should be assigned to subregion C.

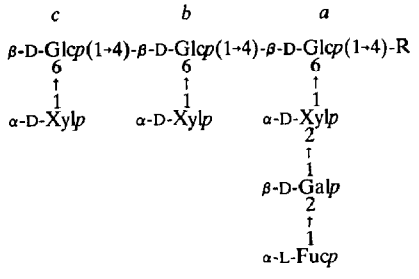
Doublets in subregion E correlate to the presence of terminal α-D-xylopyranosyl residues attached to O-6 of the β-D-glucopyranosyl residue (*i.e.*, Glc^c) that is not substituted at C-4. (See the Materials and Methods section for an explanation of the superscript a, b, and c nomenclature.) The chemical shift (δ 4.940) and vicinal coupling



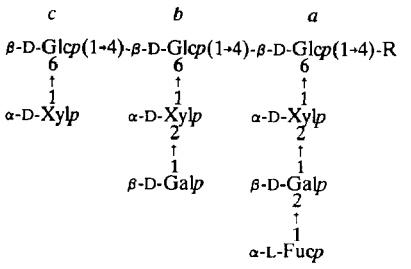
1 R = D-Glc
6 R = D-Glcol



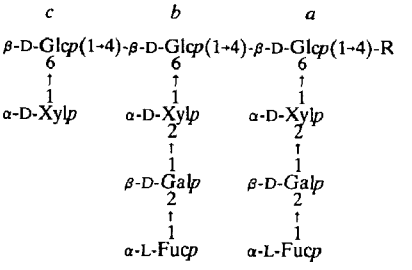
2 R = D-Glc
7 R = D-Glcol



3 R = D-Glc
8 R = D-Glcol



4 R = D-Glc
9 R = D-Glcol



5 R = D-Glc
10 R = D-Glcol

constant ($J_{1,2}$ 3.69 Hz) of the doublet in subregion E of the ^{1}H-n.m.r. spectrum of **10** indicated the presence of a single, nonreducing terminal α-D-xylopyranosyl residue. No anomeric signals were detected in subregions B and D of the spectrum of **10**, indicating that none of the α-D-xylopyranosyl residues of **10** had a terminal β-D-galactopyranosyl substituent at C-2 and that terminal α-D-xylopyranosyl residues were *not* present on either of the 4,6-linked β-D-glucopyranosyl residues (Glc^a and Glc^b) of **10** (ref. 9). The three doublets ($J_{1,2}$ 7.8–8.0 Hz) observed in subregion F could then be assigned as the anomeric protons of β-Glc^a, β-Gal^a, and β-Gal^b, and the two doublets ($J_{1,2}$ 7.8–8.0 Hz) in subregion G could be assigned as the anomeric protons of β-Glc^b and β-Glc^c. (Subregion G also contains signals assigned to H-5 of the two α-L-fucopyranosyl residues[9].) Thus the structure of **10** (and hence **5**) could be deduced from the assignment of these signals. The structure of **10** was confirmed by chemical analysis, f.a.b.-m.s., and selective glucosidase treatment as described below.

Confirmation of the structure of **10** *by f.a.b.-m.s. and chemical analyses.* — The glycosyl-residue and glycosyl-linkage compositions of **10** (Table I) were determined by standard chemical techniques[21–23]. Deviations from theoretical values for the relative

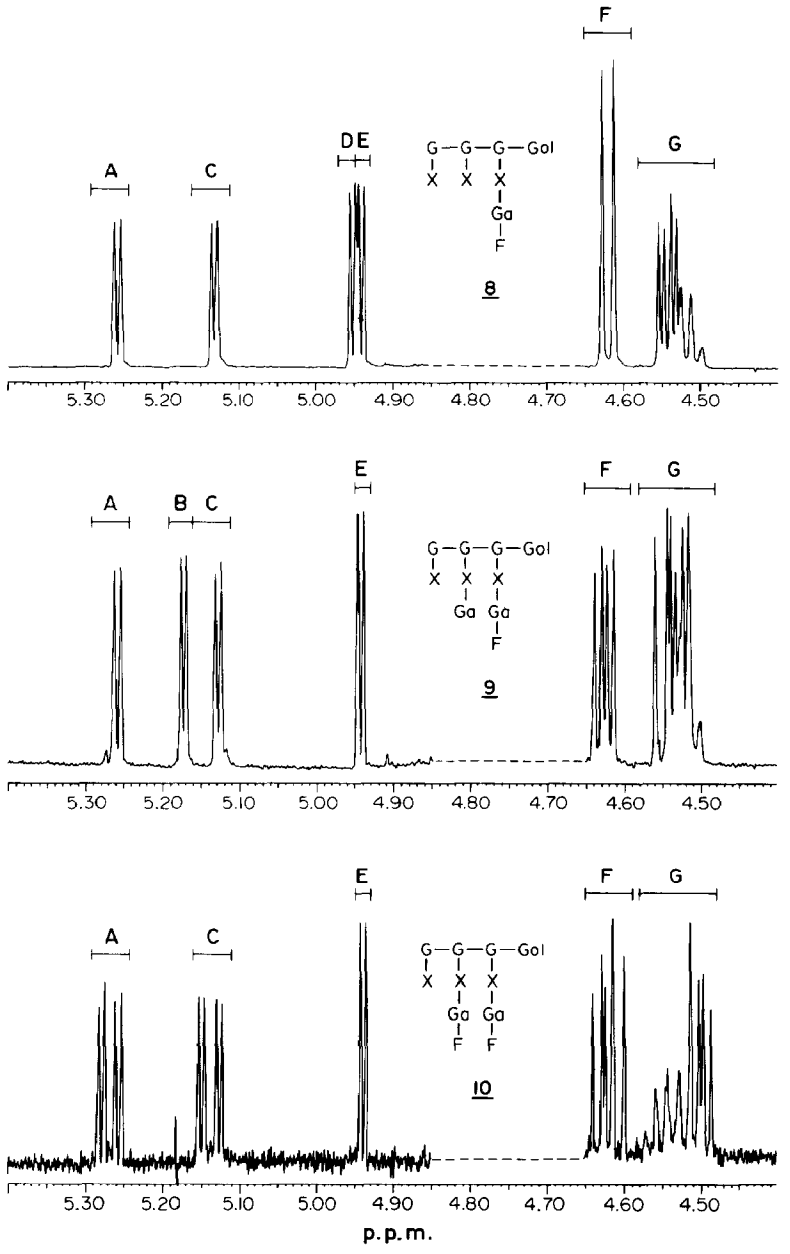


Fig. 3. Anomeric regions of the ^{1}H-n.m.r. spectra of oligoglycosyl alditols **8, 9,** and **10.** The HDO signal (δ 4.65–4.85) has been deleted for clarity. The anomeric signals could be assigned by arranging them in six subregions as defined by York *et al.*[9] The subregions encompass the following signals: A) H-1 of α-L-fucosyl residues; B) H-1 of α-D-xylosyl residues with a terminal β-Gal substituent at C-2; C) H-1 of α-D-xylosyl residues with an α-Fuc-(1→2)-β-Gal moiety at C-2; D) H-1 of non-reducing terminal α-Xyl^a or α-Xyl^b; E) H-1 of non-reducing terminal α-Xyl^c; F) H-1 of β-Glc^a and *2-linked* β-Gal^a and β-Gal^b; G) H-1 of β-Glc^b, β-Glc^c, non-reducing *terminal* β-Gal^a and β-Gal^b, and H-5 of α-Fuc. See the Materials and Methods section for a description of the superscript nomenclature indicating the positions of glycosyl residues in these oligoglycosyl alditols.

TABLE I

Glycosyl-residue and glycosyl-linkage compositions of **10**

A. Glycosyl-residue composition of **10**.

Glycosyl residue	*Molar ratio*
Fuc	1.7
Xyl	2.5
Gal	1.9
Glc	4.0[a]

B. Glycosyl-linkage composition of **10**.

Glycosyl residue	*Methylated position*	*Deduced linkage*	*Molar ratio*
Fuc	2,3,4	T-Fuc	1.5
Xyl	2,3,4	T-Xyl	0.8
Xyl	3,4	2-Xyl	1.7
Gal	3,4,6	2-Gal	1.8
Glc	1,2,3,5,6	4-Glcol	0.7
Glc	2,3,4	6-Glc	1.0
Glc	2,3	4,6-Glc	2.1

[a] Includes the Glcol residue.

amounts of the various derivatives reflect partial decomposition of glycosyl residues during chemical analysis[22,28]. The glycosyl-residue and glycosyl-linkage compositions obtained for **10** are nevertheless consistent with the structure of **10** as deduced by ^{1}H-n.m.r. spectroscopy.

Positive-ion f.a.b.-mass spectra of per-*O*-acetylated **10** were recorded using 1-thioglycerol as a matrix. The spectrum recorded after adding 1 μL of 1% NaOAc to the matrix[25] included a strong signal corresponding to the pseudomolecular ion $[M + Na]^+$ ($Fuc_2Gal_2Xyl_3Glc_3Glcol$, m/z 3005). The spectrum recorded without added NaOAc included signals corresponding to the pseudomolecular ion $[M + NH_4]^+$ ($Fuc_2Gal_2Xyl_3Glc_3Glcol$, m/z 3000) and "A" type[24,25] (non-reducing end) fragment ions

TABLE II

Positive-ion f.a.b.-m.s. per-*O*-acetylated **10**

Ion nominal mass	*Ion type*[a]	*Ion composition*
259	$[A]^+$	Xyl
273	$[A]^+$	Fuc
547	$[A]^+$	XylGlc
561	$[A]^+$	FucGal
777	$[A]^+$	FucGalXyl
1569	$[A]^+$	$FucGalXyl_2Glc_2$
3001	$[M + NH_4]^+$	$Fuc_2Gal_2Xyl_3Glc_3Glcol$
3005[b]	$[M + Na]^+$	$Fuc_2Gal_2Xyl_3Glc_3Glcol$

[a] Ions labeled $[A]^+$ are derived from the non-reducing end of the undecasaccharide[24]. [b] Observed upon addition of NaOAc to the ionization matrix[25].

(Table II). The series of "A" type fragment ions at *m/z* 273 $[Fuc]^+$, *m/z* 561 $[FucGal]^+$, and *m/z* 777 $[FucGalXyl]^+$ confirmed that the fucosyl residue-containing sidechains of **10** have the same structure as those previously found[9] in XG oligosaccharides. The presence of a (FucGalXyl) sidechain on a per-*O*-acetylated XG oligoglycosyl alditol selectively inhibits cleavage of the glycosidic bond of the glucosyl residues bearing the (FucGalXyl) sidechain[27] during positive-mode f.a.b.-m.s. Accordingly, the abundance of the "A" type fragment ion $[FucGalXyl_2Glc_2]^+$ at *m/z* 1569 in the positive-ion f.a.b. mass spectrum of per-*O*-acetylated **10** was very low. Nevertheless, the "A" type fragment ion $[XylGlc]^+$ at *m/z* 547 indicated that neither of the two fucosyl residue-containing sidechains were present on Glc^c, and by default they must be present on both Glc^a and Glc^b. This was confirmed by negative-ion f.a.b.-m.s., as described next.

TABLE III

Negative-ion f.a.b.-m.s. of underivatized **10**

Nominal mass	*Ion type*[a]	*Ion composition*
783	$[B]^-$	$FucGalXylGlcGlcol$
1077	$[DB]^-$	$FucGalXyl_2Glc_2Glcol$
1239	$[B]^-$	$FucGalXyl_2Glc_3Glcol$
1371	$[B]^-$	$FucGalXyl_3Glc_3Glcol$
1385	$[B]^-$	$Fuc_2Gal_2Xyl_2Glc_2Glcol$
1533	$[B]^-$	$FucGal_2Xyl_3Glc_3Glcol$
1547	$[B]^-$	$Fuc_2Gal_2Xyl_2Glc_3Glcol$
1679	$[M - H]^-$	$Fuc_2Gal_2Xyl_3Glc_3Glcol$
1701	$[M - 2H + Na]^-$	$Fuc_2Gal_2Xyl_3Glc_3Glcol$
1814[b]	$[M - H + AG + CO_2]^-$	$Fuc_2Gal_2Xyl_3Glc_3Glcol$

[a] Ions labeled $[B]^-$ originate from the alditol end of the undecasaccharide by a single-cleavage event. Ions labeled $[DB]^-$ also originate from the alditol end, but by a double-cleavage ionization process[25]. These ions correspond in mass to the "Pathway B ions" described by Dell[25]. [b] The ion $[M + 134]^-$ is consistently seen when aminoglycerol is used as an ionization matrix for neutral oligoglycosyl alditols and has been tentatively assigned as an adduct consisting of the deprotonated oligoglycosyl alditol plus one aminoglycerol molecule plus one CO_2 molecule. Aminoglycerol appears to readily absorb atmospheric CO_2.

The negative-ion f.a.b.-m.s. spectrum of **10** was recorded using 1-amino-1-deoxyglycerol ("amino glycerol") as the matrix (Table III, Fig. 4). The intense $[M - H]^-$ ion at *m/z* 1679 in this spectrum confirmed that **10** is a decaglycosyl alditol ($Fuc_2Gal_2Xyl_3Glc_3Glcol$). Fragment ions were observed at *m/z* values corresponding to cleavage of glycosidic bonds between C-1 and O-1, and release of an anionic fragment derived from the alditol end of the molecule[9,25]. The fragment ions at *m/z* 1385 and 783 confirmed the positions of the two fucosyl residue-containing sidechains of **10**. Formation of the *m/z* 1385 ion $[Fuc_2Gal_2Xyl_2Glc_2Glcol]$ requires the loss of 294 a.m.u. (*i.e.*, one hexose and one pentose) from the $[M - H]^-$ ion. The structural features of the sidechains and backbone of **10**, as determined by positive-ion f.a.b.-m.s. and ^1H-n.m.r. spectroscopy (see above), preclude a loss of 294 a.m.u. from the $[M - H]^-$ ion except *via* a "single-cleavage" ionization process[9,25], and, therefore, the *m/z* 1385 ion was assigned

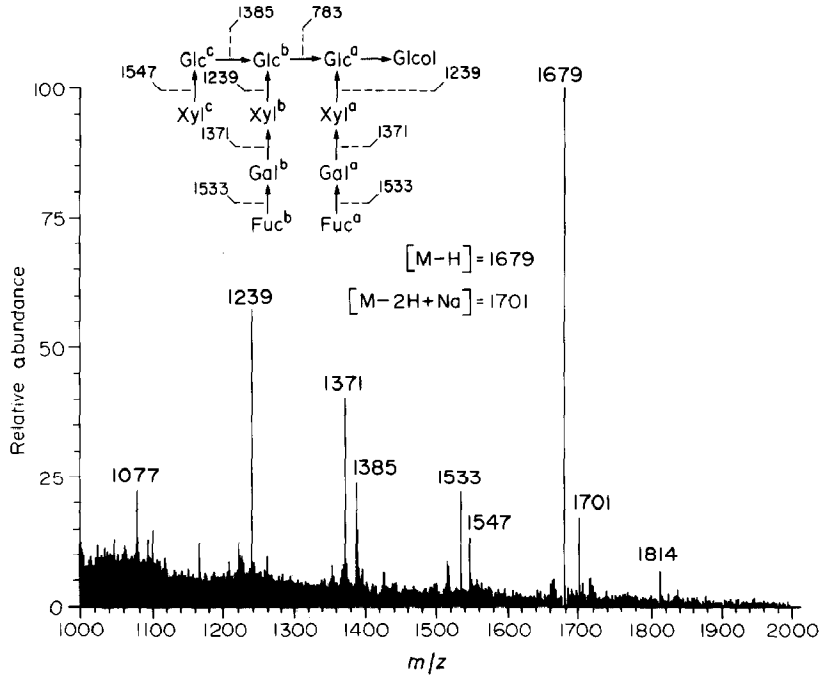


Fig. 4. Negative-ion f.a.b.-m.s. spectrum of **10**. Fragment ions were assigned as having formed by cleavage of the C-1/O-1 bond, releasing the alditol-containing fragment as an oxanion[9,25]. Ions are labelled with their nominal masses. The ion at *m/z* 1077 is assigned as a double-cleavage ion[9,25].

as "single-cleavage" ion. The *m/z* 783 ion was assigned as a "single-cleavage" ion by similar reasoning. Conversely, the ion at *m/z* 1077 was assigned as a "double-cleavage" ion, formed as previously described[9,25] by loss of either of the 2-*O*-(α-L-fucosyl)-β-D-galactosyl moieties from the *m/z* 1385 ion. This interpretation of the negative-ion f.a.b.-mass spectrum is consistent with results obtained by positive-ion f.a.b.-m.s. Thus, ^{1}H-n.m.r. analysis, chemical analyses, and f.a.b.-m.s. analyses firmly established the structure of the **10**.

Confirmation of the structure of **10** *by selective glycosidase treatment.* — Kato *et al.* have described a fungal enzyme that is very useful in structural studies of XG oligosaccharides[7,8]. This oligoxyloglucan hydrolase cleaves α-D-Xyl*p*-(1→6)-D-Glc from the non-reducing end of XG oligosaccharides when the α-D-Xyl*p*-(1→6)-D-Glc moiety is attached *via* a β-D-glucopyranosyl linkage to O-4 of another β-D-glucopyranosyl residue of the oligosaccharide, but only when the α-D-xylosyl residue of the α-D-Xyl*p*-(1→6)-D-Glc moiety itself is terminal[7]. Therefore, the α-D-Xyl*p*-(1→6)-D-Glc moiety at the non-reducing end of **10** should be susceptible to cleavage by this hydrolase. An immobilized enzyme preparation containing this hydrolase, along with α-L-fucosidase and β-D-galactosidase contaminants, was prepared (see Materials and Methods section). The activity of α-L-fucosidase was inhibited[19] by addition of 1,5-dideoxy-1,5-imino-L-fucitol. The β-D-galactosyl residues of **8** and **10** have an α-L-fucosyl substituent

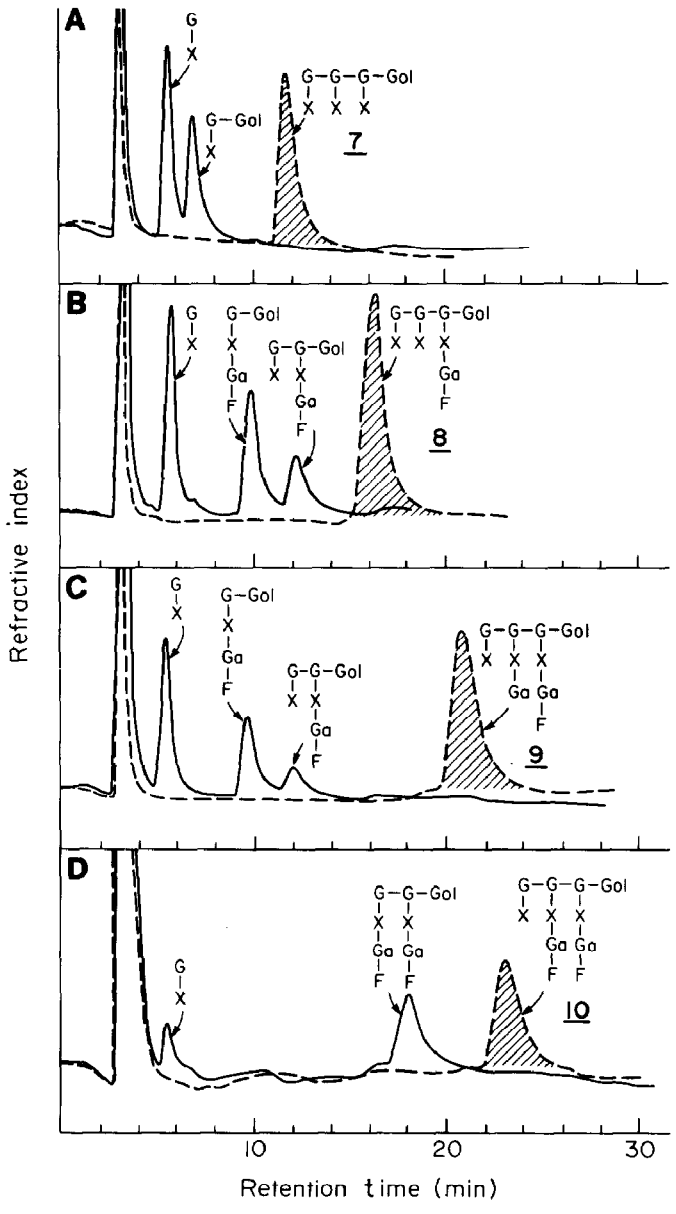


Fig. 5. Comparison of h.p.l.c. elution profiles of oligoglycosyl alditols **7–10**, before and after treatment with immobilized oligoxyloglucan hydrolase in the presence of an α-L-fucosidase inhibitor. The elution profiles of the untreated oligoglycosyl alditols is shown by dashed lines and shaded peaks, and the elution profiles of the products of enzyme treatment are shown by solid lines.

at C-2 and are not susceptible to cleavage by the contaminating β-D-galactosidase unless the α-L-fucosyl residues are first removed. Therefore, inhibition of the α-L-fucosidase also prevented cleavage of the β-D-galactosyl residues of **8** and **10**.

The h.p.l.c. elution patterns of the products formed by digestion of **7–9** with the immobilized hydrolase in the presence of the fucosidase inhibitor corresponded to release of either one or two α-D-Xyl*p*-(1→6)-D-Glc moieties from the non-reducing end, depending on the distribution of side-chains in the oligoglycosyl alditol (Fig. 5). Two equivalents of α-D-Xyl*p*-(1→6)-D-Glc were released from **7** and **8** as expected. The terminal β-D-galactosyl residue of **9** was cleaved by contaminating β-D-galactosidase when incubated with the immobilized enzyme preparation, allowing two α-D-Xyl*p*-(1→6)-D-Glc moieties to be released from **9** by the hydrolase. Treatment of **10** with the hydrolase produced two products, identified by their h.p.l.c. retention times and glycosyl-composition analyses as α-D-Xyl*p*-(1→6)-D-Glc and an octaglycosyl alditol ($Fuc_2Gal_2Xyl_2Glc_2Glcol$). In the absence of fucosidase inhibitor, the contaminating α-L-fucosidase in the hydrolase preparation removed fucosyl residues from **10**, exposing the β-D-galactosyl residues, thus making them susceptible to cleavage by β-D-galactosidase. Accordingly, when **10** was digested with the hydrolase but without the fucosidase inhibitor, three peaks were detected, with retention times corresponding to a mixture of monosaccharides (L-fucose and D-galactose), α-D-Xyl*p*-(1→6)-D-Glc, and a diglycosyl alditol (XylGlcGlcol). These results indicated that the backbone of **10** consists of cellotetraitol substituted with α-D-xylosyl residues at O-6 of each of the three glucopyranosyl residues and that the Xyl residue attached to O-6 of Glc^c was terminal. Thus, the structure of **10** was confirmed by h.p.l.c. analysis of the products obtained after treatment of **10** with the oligoxyloglucan hydrolase.

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