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Sequencing of xyloglucan oligosaccharides by partial Driselase digestion: the preparation and quantitative and qualitative analysis of two new tetrasaccharides

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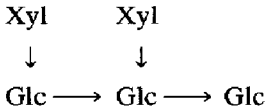
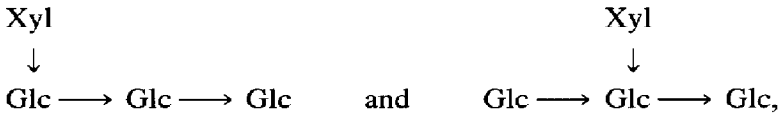
Abstract

The pentasaccharide (XXG),



obtained from *Rosa* xyloglucan, was converted to two isomeric tetrasaccharides, **a** and **b** ($Xyl_1 \cdot Glc_3$), by mild acid hydrolysis. During hydrolysis in 2 M trifluoroacetic acid at 90°C, optimal yields of **a** and **b** were obtained after 20–40 min. Each tetrasaccharide was purified by preparative paper chromatography and high-pressure liquid chromatography (HPLC). The two isomers were distinguished by the products of their partial digestion with Driselase, which hydrolyses the glucosidic bonds sequentially from the non-reducing terminus: **a** and **b** yielded cellobiose and Xyl → Glc → Glc, respectively, showing that they were



respectively. Tetrasaccharide **b** was chromatographically identical, upon HPLC on Dionex CarboPac PA1, with the tetrasaccharide produced from XXG by the action of *Tropaeolum*

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α-D-xylosidase, supporting the proposed structure. Xyloglucan oligosaccharides were assayed quantitatively by measurement of the yield of isoprimeverose (Xyl → Glc) after complete Driselase digestion.

Keywords: Cell wall (plant); Driselase; β-D-Glucosidase; Isoprimeverose; Xyloglucan

1. Introduction

Xyloglucan is a structural polysaccharide found in the primary cell walls of plants [1–3]. It has a backbone mainly composed of cellotetraose-based units, such as XXXG (for explanation of abbreviated names of oligosaccharides see the legend to Fig. 1). In addition to the β-D-Glc*p* and α-D-Xyl*p* residues, there are smaller numbers of β-D-Gal*p*, α-L-Fuc*p*, α-L-Ara*f*, β-D-Xyl*p*, and *O*-acetyl groups. In the plant cell wall, xyloglucan chains can hydrogen-bond to cellulosic microfibrils and may cross-link them, restraining cell expansion [2–4]. Therefore, enzymes such as cellulase [5] and xyloglucan endotransglycosylase [6,7] that cleave xyloglucan might loosen the cell wall and contribute to cell expansion and thus plant growth.

Some xyloglucan oligosaccharides (XGOs) that are acceptor substrates for xyloglucan endotransglycosylase (e.g., XXXG) [7–9] can, at concentrations of ca. 1 μM, promote the elongation of stem segments excised from etiolated pea seedlings [10–12]. A causal relationship has been proposed, in which the XGOs loosen the cell wall by increasing the rate at which load-bearing xyloglucan chains are cleaved by xyloglucan endotransglycosylase [13].

In preparation for testing the proposed relationship, it was of interest to define the structural requirements for XGOs to act as acceptor substrates for xyloglucan endotransglycosylase. XXG (Fig. 1) is the smallest XGO so far shown to possess appreciable activity as a xyloglucan endotransglycosylase acceptor substrate; both the Xyl residues were apparently required [13].

We therefore wished to prepare and test a range of smaller XGOs containing only xylose and glucose residues. Here we report some simple new methods for the preparation and analysis of the desired oligosaccharides. The activity of these XGOs as acceptor substrates for xyloglucan endotransglycosylase has recently been reported [13].

2. Experimental

Enzymes.—Driselase, a mixture of hydrolases from the fungus *Irpex lacteus*, was purchased from Sigma Chemical Co. and partially purified [14]. Driselase contains β-D-glucosidase activities including a xyloglucosidase that can hydrolyse a β-D-Glc*p* residue which is α-D-xylosylated at O-6. Driselase lacks α-D-xylosidase activity. *Trichoderma* cellulase was from Sigma Chemical Co., product No. C9422.

Tropaeolum seed α-D-xylosidase, which attacks XGOs to remove only the single α-D-Xyl*p* residue furthest from the reducing terminus [15,16a], was a generous gift of Dr. C. Fanutti, Universty of Stirling, UK.

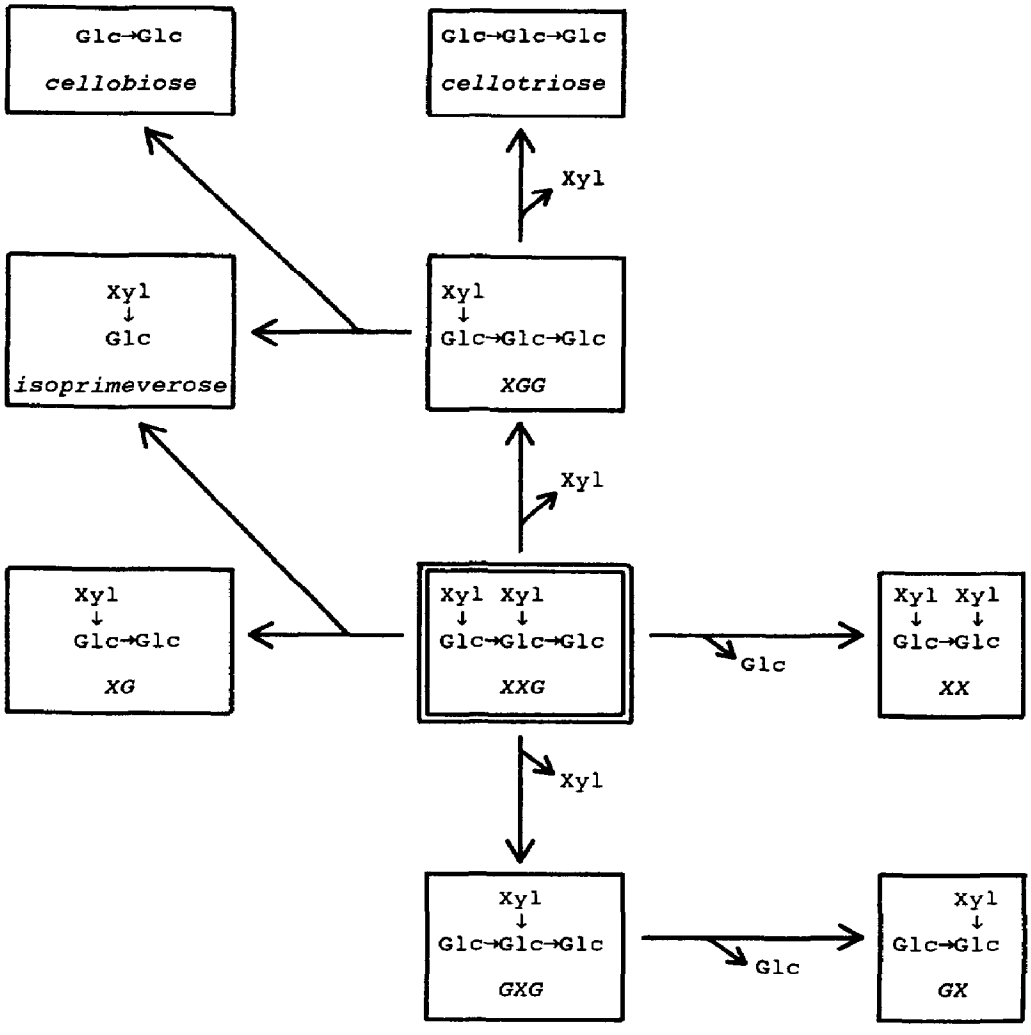


Fig. 1. The predicted effects of partial acid hydrolysis on XXG. Each of the ten plausible products can be generated by a one- or two-step degradation of the initial pentasaccharide; only the simplest route to each product is shown. In the abbreviated nomenclature [20] of xyloglucan oligosaccharides, X represents an isoprimeverose residue [α-D-Xyl*p*-(1→4)-β-D-Glc*p*-...], and G represents a non-xylosylated β-D-Glc*p*-... residue. The symbols X and G are listed sequentially from the non-reducing terminus of the oligo-β-glucan backbone of the oligosaccharide. Thus, for example, XGG represents the tetrasaccharide α-D-Xyl*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-D-Glc.

Xyloglucan-derived hepta-, penta-, and tri-saccharides.—The heptasaccharide XXXG was obtained by digestion of *Rosa* xyloglucan with *Trichoderma* cellulase, and converted to the pentasaccharide XXG by partial digestion with Driselase [13]. The trisaccharide XG [α-D-Xyl*p*-(1→6)-β-D-Glc*p*-(1→4)-D-Glc] was a generous gift of Dr. Y. Kato (Hirosaki University, Japan). The disaccharide isoprimeverose [α-D-Xyl*p*-(1→6)-D-Glc] was obtained by complete digestion of *Tropaeolum* xyloglucan with Driselase and purified by preparative PC [16b].

Preparative paper chromatography (PC).—The sample solution (containing ~3.5 mg of carbohydrate) was loaded as a 15-cm streak on Whatman 3MM paper. The chromatogram

was developed by the descending method in 10:4:3 EtOAc–pyridine–water for 41 h, during which time cellobiose migrated ~29 cm. External markers were stained with aniline hydrogen-phthalate [17]. Compounds of interest were eluted in distilled water [18].

High-pressure liquid chromatography (HPLC).—Semi-preparative HPLC was performed on a 220×7 mm column of amino-substituted silica (Brownlee Amino-Spheri-5) with isocratic elution at 1.5 mL/min in 70% MeCN [19]. Oligosaccharides were detected by refractive index, and 1-min fractions were collected.

Analytical HPLC was performed on a 250×4 mm column of Dionex CarboPac PA1. Oligosaccharides were eluted with a linear gradient (1 mL/min) of 25 → 50 mM NaOAc in 100 mM NaOH over 60 min. Oligosaccharides were detected with a pulsed amperometric detector (PAD) fitted with a gold electrode. Peak areas were estimated by multiplication of height by width at half height.

Sequencing of XGOs by partial Driselase digestion.—An aqueous solution (90 μL; ~100 μM) of the oligosaccharide was mixed with 10 μL of 0.2% (partially purified) Driselase in buffer (1:1:98 pyridine–AcOH–water) and incubated at 25°C. At intervals (10 min, 1 h, and 16 h), 33-μL portions of the solution were boiled for 6 min, stored frozen, and later analysed by Dionex HPLC.

Quantitative analysis of XGOs by complete Driselase digestion.—The 16-h digestion products were mainly glucose and isoprimeverose. The yield of the latter was assayed after HPLC (on CarboPac PA1) by peak area using an empirical response factor determined for pure isoprimeverose.

Preparation of GXG by digestion of XXG with α-D-xylosidase.—To a sample of XXG (140 μg) in 100 μL of 50 mM acetate (NH_4^+) buffer, pH 5.0, was added 4.5 μg of α-D-xylosidase. After incubation for 3.5 h at 30°C, the solution was boiled for 10 min and the products were examined by HPLC on CarboPac PA1.

3. Results

Kinetics of acid hydrolysis of XXG.—HPLC of the pentasaccharide preparation revealed a major peak (XXG; t_R ~24–25 min) with minor contaminants (Fig. 2a). To optimise the conversion of XXG to the tetrasaccharides, XGG and GXG, we monitored the production of fragments by 2 M CF_3CO_2H at 90°C. The products included peaks that could be provisionally ascribed to all ten plausible structures (Fig. 1). This is illustrated for the sample taken after 20-min hydrolysis (Fig. 2b). The identity of the monosaccharides generated (glucose and xylose, not resolved under the HPLC conditions used) was verified by PC. Both the disaccharides (isoprimeverose and cellobiose) and two of the trisaccharides (XG and cellotriose) were available as authentic standards; the proposed identity of these di- and tri-saccharide peaks in partial acid hydrolysates was confirmed by co-chromatography in samples spiked with internal markers (e.g., Figs. 2c and d). Two of the three tetrasaccharides, XGG and GXG, were characterised as will be described below. Only small amounts of the third putative tetrasaccharide, XX, and the third putative trisaccharide, GX, were generated, and the identity of these peaks remains tentative.

Of the starting material, XXG, 50% had disappeared within ca. 17.5 min. It is uncertain whether the pulsed amperometric detector gives equal responses for all the oligosaccharides

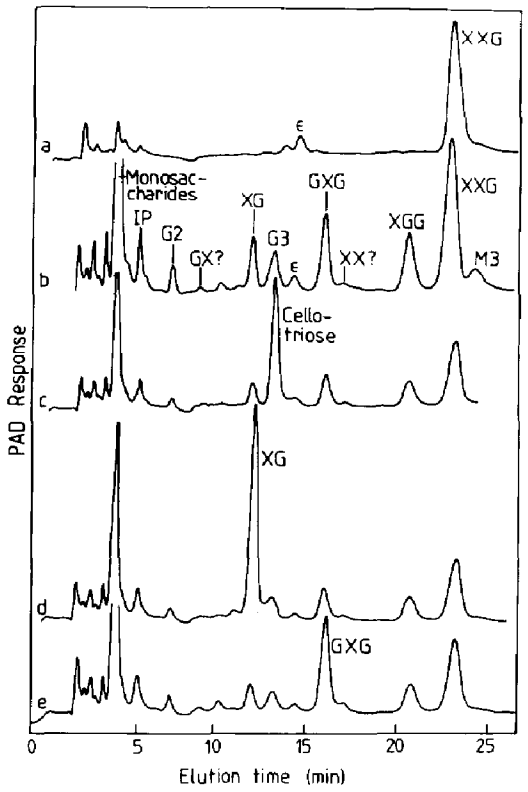


Fig. 2. HPLC analysis (on CarboPac PA1) of the pentasaccharide XXG and products of its partial acid hydrolysis. (a) Untreated XXG; (b) 20-min hydrolysis products spiked with maltotriose (M3); (c) 20-min hydrolysis products spiked with cellotriose; (d) 20-min hydrolysis products spiked with the trisaccharide XG; (e) 20-min hydrolysis products spiked with the tetrasaccharide obtained by α-D-xylosidase digestion of XXG. The peak marked ε is an unidentified contaminant present in the XXG preparation.

resolved; therefore, it is not possible to state with certainty which were major hydrolysis products and which were minor. However, assuming that peak area does give an indication of relative molar concentration of different oligosaccharides, we note that for the first 5–20 min the main products (Fig. 3) were the fragments generated by hydrolysis of one xylosyl linkage (GXG and XGG) rather than one glucosyl linkage (isoprimeverose, XG, and XX), indicating that the Xyl*p* bonds were substantially more labile than the Glc*p*. Indeed, at 10 min, the next most abundant product after XGG and GXG was cellotriose, the product of hydrolysis of both Xyl*p* bonds of XXG. The kinetics of formation of the two desired tetrasaccharides, XGG and GXG, were very similar; the yield of both peaked at 20–40 min (Fig. 3).

Prepapartion of tetrasaccharides XGG and GXG.—A larger sample (3.45 mg) of XXG was heated in 600 μL of 2 M CF_3CO_2H at 90°C for 30 min to provide an optimal yield of the two main tetrasaccharides and the hydrolysate was fractionated by preparative PC. Strips (1 cm) were eluted with water and each eluate was analysed for oligosaccharides by HPLC on CarboPac (Fig. 4). Selected eluates were pooled as shown (*A* and *B*) in Fig. 4 and dried in vacuo.

XGG, the main component of PC pool *A*, was found to be eluted from the amino column in the 26–30-min fractions (data not shown), which were therefore combined for use as

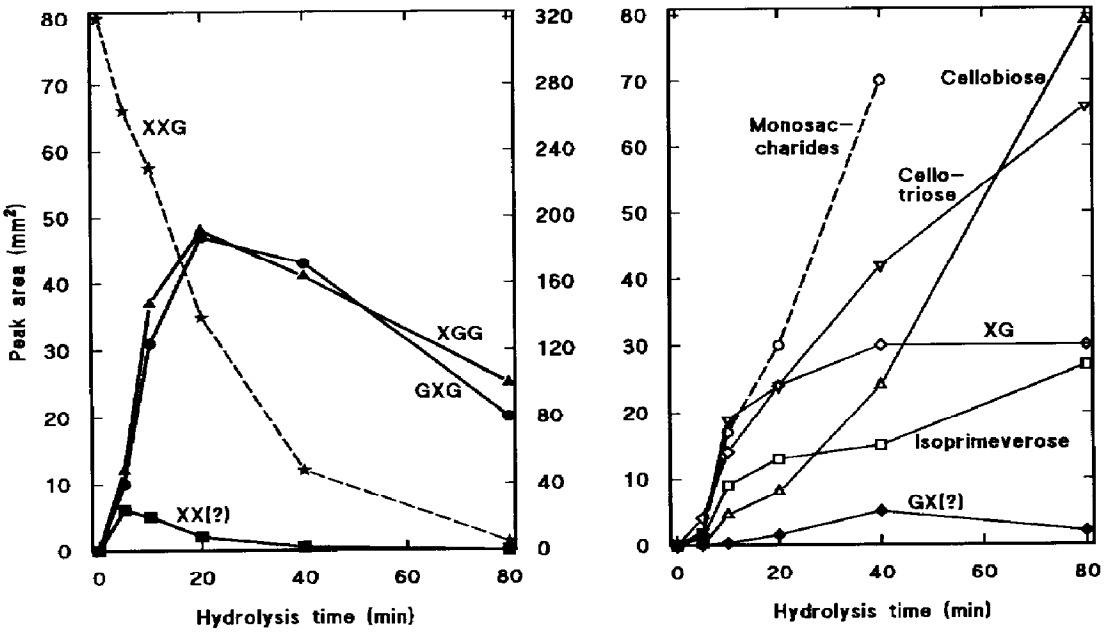


Fig. 3. Kinetics of hydrolysis of XXG in 2 M CF_3CO_2H at 90°C. Samples were removed at intervals and analysed by HPLC as in Fig. 2. Each peak area (mm^2 on the PAD output) was estimated by multiplication of height by width at half height: *, XXG; ▲, XGG; ●, GXG; ■, XX; ◇, XG; ◆, GX; ▽, cellotriose; △, cellobiose; □, isoprimeverose; ○, monosaccharides (Glc + Xyl, not resolved). The small peaks of isoprimeverose and monosaccharide apparently present at time 0 (see Fig. 2a) have been subtracted. The left-hand ordinate (0–80 mm^2) is used for all curves except XXG. Question marks indicate tentative identifications.

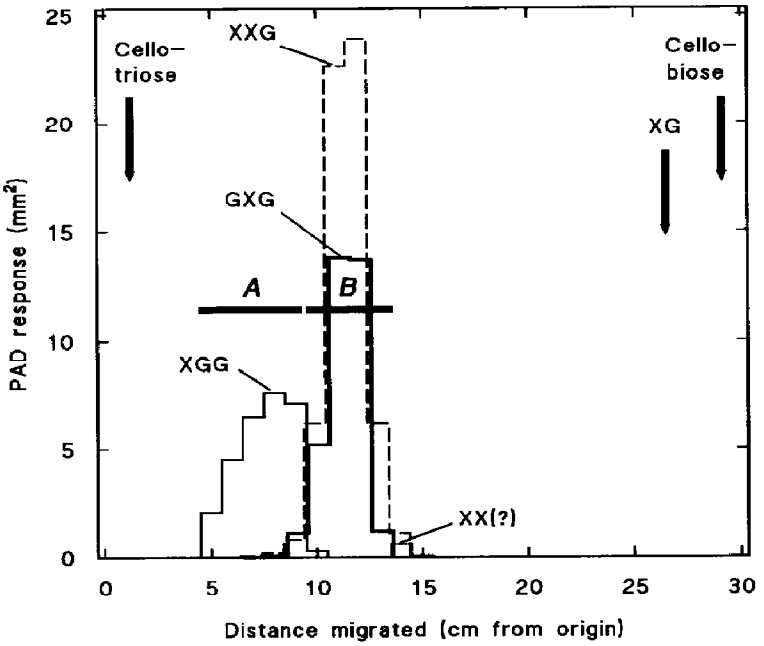


Fig. 4. Preparative paper chromatography of the products of XXG after 30 min hydrolysis in 2 M CF_3CO_2H at 90°C. Each strip of the paper was eluted with water and a small proportion of the eluate analysed by HPLC on CarboPac PA1. The histograms show the positions of (a) XGG, (b) GXG, (c) XX, and (d) XXG. Arrows show the positions of external markers run on the same paper chromatogram.

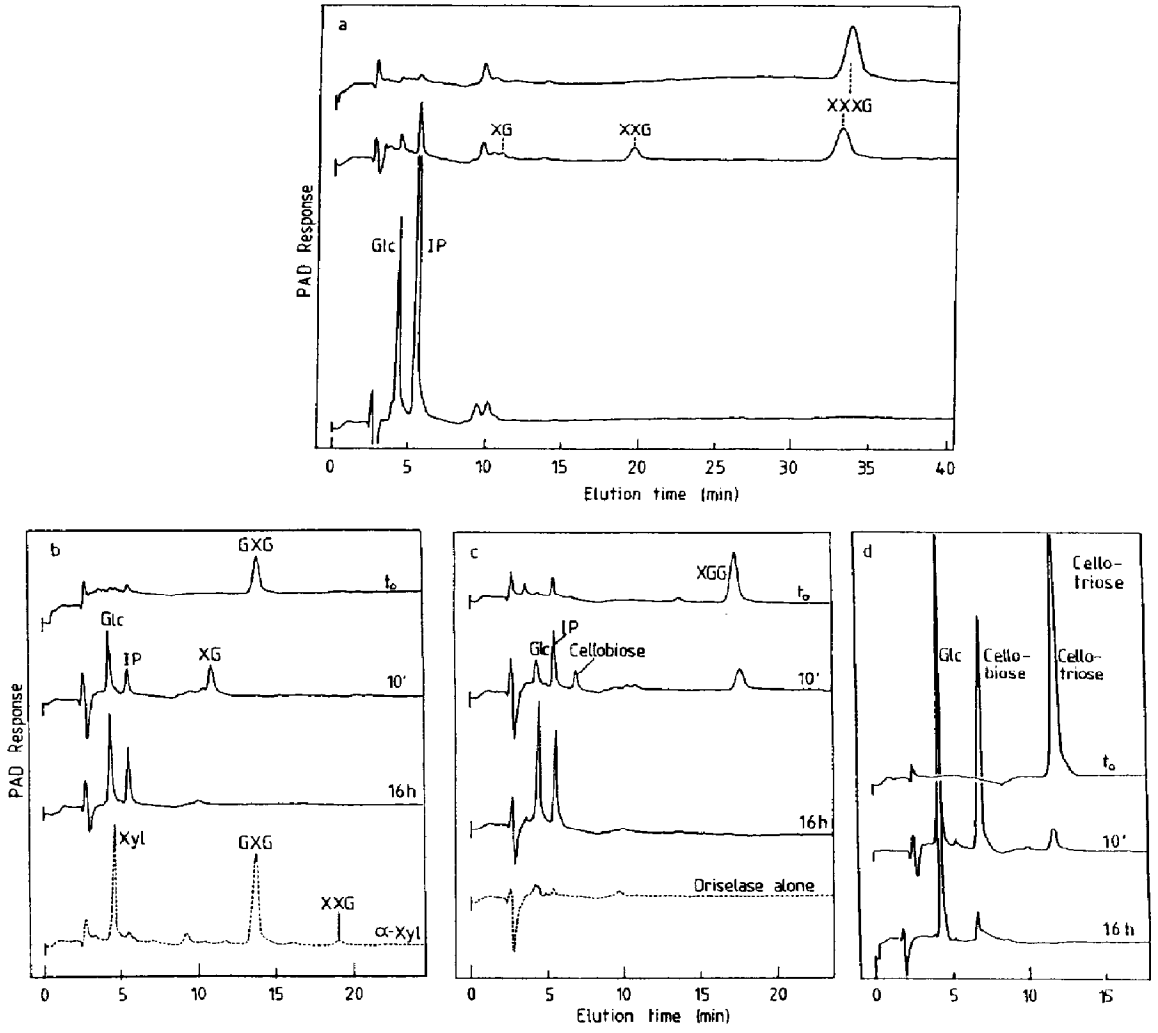
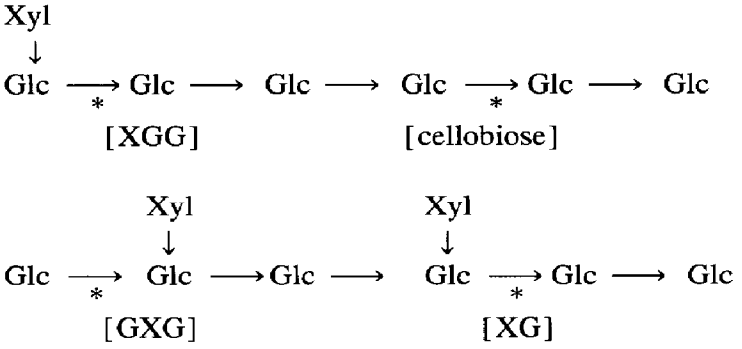


Fig. 5. Sequencing of xyloglucan-related oligosaccharides by partial Driselase digestion, and their quantitation by complete digestion. The oligosaccharides were digested for 0 min (top curve), 10 min (second curve), or 16 h (third curve), and then analysed by HPLC on CarboPac PA1. The oligosaccharides tested were (a) XXXG, (b) putative GXG, (c) putative XGG, and (d) cellotriose. The dashed profile in (b) shows the products obtained by treating XXG with α-D-xylosidase. The dashed profile in (c) shows the autolysis products obtained by incubation of Driselase for 16 h in the absence of added oligosaccharides. Abbreviation used: IP, isoprimeverose.

purified XGG. PC pool *B* contained GXG and XXG, which eluted from the amino column in the 28–33-min and ≥ 32-min fractions, respectively (data not shown); therefore the 28–31-min fractions were combined for use as purified XGG.

Sequencing and quantification of XGOs by Driselase digestion–HPLC.—The kinetics of oligosaccharide hydrolysis by Driselase were studied (Fig. 5). In all the XGOs tested, the predominant products at 16 h were isoprimeverose and glucose. Analysis of intermediate (e.g., 10-min; Fig. 5) products of digestion of XXXG, XXG, and cellotriose, indicated a progressive cleavage of glucosyl linkages from the non-reducing terminus, regardless of the presence of xylose residues, as expected from the known enzymic complement of Driselase. Thus, the digestions proceeded as follows: XXXG → XXG → XG → Glc and

cellotriose → cellobiose → Glc. On the same basis, the predicted routes for digestion of XGG and GXG are shown in the following scheme (in which the glucosyl bond susceptible to hydrolysisis marked thus *):



Cellobiose is resolved from the trisaccharide, XG, so these two different routes of digestion can be easily distinguished. The data (Fig. 5) strongly support the proposed structures of the two isomeric tetrasaccharides.

In addition, since each XGO has a predictable molar yield of isoprimeverose after Driselase digestion, the peak area of this disaccharide can serve to quantify any newly characterised XGO even though the PAD response factor of the intact novel XGO is unknown.

Confirmation of identity of GXG by α-xylosidase digestion of XXG.—The tetrasaccharide with the lower retention time (Dionex HPLC) was found to be chromatographically identical to the product obtained by digestion of XXG with *Tropaeolum* α-D-xylosidase (Fig. 2e). This enzyme is known to act on XGOs removing only the single xylose residue furthest from the reducing terminus [15,16] and would therefore be expected to convert XXG to GXG rather than to XGG.

4. Discussion

This paper reports three useful, simple methods: (*a*) The preparation of partially dexylosylated XGOs by optimised partial acid hydrolysis. (*b*) The use of partial Driselase digestion to sequence XGOs from the non-reducing terminus. Driselase contains α-L-fucosidase and β-D-galactosidase activities, so XGOs possessing Fuc and Gal residues would yield more complex mixtures of products; however, this problem could be overcome if inhibitors of α-L-fucosidase and β-D-galactosidase (e.g., the monosaccharides themselves, aldonolactones, or specific nojirimycin derivatives) were included in the digest. (*c*) The use of complete Driselase digestion to quantify traces of novel XGOs by converting them to a predictable yield of isoprimeverose.

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