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Structure of xylan from culms of bamboo grass (*Sasa senanensis* Rehd.)

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Abstract Xylan prepared from culms of kumaizasa (*Sasa senanensis* Rehd.), a representative species of bamboo grass, was hydrolyzed with β-xylanase of *Streptomyces olivaceoviridis* E-86. Four arabinoxylo-oligosaccharides and two glucuronoxylo-oligosaccharides were isolated from the enzymatic hydrolysate of the xylan by chromatography on a charcoal column, a Dowex 1-×8 column, a Toyopearl HW-40S column, and a LiChrospher 100 NH_2 column and on preparative paper chromatography. The results of the structural analyses of the saccharides showed that the isolated oligosaccharides had the structures of 3^2-α-L-arabinofuranosyl-xylobiose, 3^2-α-L-arabinofuranosyl-xylotriose, 3^2-α-[β-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl]-xylobiose, 3^3-α-[β-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl]-xylotriose, 2^3-α-4-*O*-methyl-D-glucuronosyl-xylotriose, and 2^3-α-D-glucuronosyl-xylotriose. From the structural analysis of the oligosaccharides derived from the xylan, kumaizasa xylan was concluded to be a kind of arabinoglucuronoxylan having not only stubs of single L-arabinose and single D-glucuronic acid but also stubs of disaccharide units such as β-D-xylopyranosyl-(1 → 2)-L-arabinofuranose.

Key words Bamboo grass · *Sasa senanensis* Rehd. · Xylan · Xylanase · Xylooligosaccharide

Introduction

Xylans, the most abundant component of hemicellulose, consist of a main chain of 1,4-β-linked xylopyranosyl residues. Acid or enzymatic hydrolysis of xylan has the capability of expanding the potential use of this polysaccharide for biomass.[1] The products of such hydrolysis of xylans may be converted to liquid fuels, solvents, and other chemicals by the fermentation of specific microorganisms.[1–3] However, xylans generally have different side chains depending on their origin. For example, the stubs of xylans from hardwood, softwood, and grasses are different.[4–6] Therefore, considering the industrial use of xylans, structural studies of xylans from various origins are essential. In previous papers we reported the structures of xylans from corncobs,[7,8] rice straw,[9] cotton-seed cake,[10] and hardwood[11] and the specificity of *Streptomyces olivaceoviridis* E-86 β-xylanase toward the xylans.[7–11]

Bamboo grasses (Bambusoideae) have received much attention for a potential biomass.[12] Bamboo grasses are widely distributed in Japn and have high xylan content. Though the bamboo grasses are considered abundant, renewable sources for the production of xylose and xylooligosaccharides, the structures of xylans from bamboo grasses have been poorly characterized.

In this study, xylan was prepared from culms of *Sasa senanensis* Rehd. (common name in Japan: kumaizasa), a representative species of bamboo grass. The xylan was hydrolyzed with β-xylanase from *Streptomyces olivaceoviridis* E-86. The heterooligosaccharides in the enzymatic hydrolysate were isolated and characterized to clarify the chemical structure of the xylan.

Materials and methods

Bamboo grass

Culms of kumaizasa (*Sasa senanensis* Rehd.) were harvested in the Kamikawa National Forest, Hokkaido, Japan, during September 1991. The air-dried culms were ground in a Willey mill. The air-dried meals (26 g, 42–60 mesh) were extracted with a mixture of ethanol and benzene (1:2, v/v).

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Preparation of xylan

The extractive-free meals (25 g) were delignified with sodium chlorite and acetic acid.[13] The holocellulose (20 g) was soaked in 200 ml of 6% potassium hydroxide at room temperature for 64 h, and the slurry was then filtered through a glass filter. After the solid material was washed with 200 ml of 6% potassium hydroxide and water, respectively, the filtrate and washings were combined. The extract was neutralized with 20 ml of acetic acid, filled up to 700 ml, and added to 3 l of ethanol. The precipitate was collected by centrifugation; washed with 80% ethanol (twice), ethanol (twice), and ether; and then dried. The yields of xylan were 22.3% and 28.1% based on the extractive-free meals and holocellulose, respectively.

Authentic oligosaccharides

Authentic 1,4-β-xylooligosaccharides, arabinoxylo-oligosaccharides, and glucuronoxylooligosaccharides were prepared by the method described in our previous papers.[7–11,14,15]

Streptomyces β-xylanase

A purified β-xylanase used in this study was prepared from the culture filtrate of *Streptomyces olivaceoviridis* E-86[14] according to the procedure described in our previous paper.[16] The enzyme assay and definition of the enzyme unit were also described in a previous paper.[17]

Measurement of sugar

Reducing sugar was determined by the method of Somogyi.[18] Total sugar was measured by the methods of orcinol-hydrochloric acid[19] and phenol-sulfuric acid.[20] Xylose was used as a standard.

Thin-layer chromatography

Thin-layer chromatography (TLC) was done by a double ascending method on a high-performance TLC (HPTLC) plate of cellulose (Merck, Darmstadt, Germany) with a solvent system of 1-butanol/pyridine/water (6:4:3, v/v/v). The sugars on the plate were detected with a 1% methanol solution of *p*-anisidine hydrochloride. TLC was performed on a TLC plate of silica gel 60 (Merck) with a solvent system of 1-butanol/acetic acid/water (2:1:1, v/v/v). The sugars were detected with concentrated sulfuric acid.

Enzymatic hydrolysis of bamboo grass xylan

Kumaizasa xylan (90 g containing 63.8 g of total sugar as xylose) was hydrolyzed with 1800 ml of *Streptomyces* β-xylanase (26300 units) at pH 5.7 and 55°C. After 24 h of the reaction, the hydrolysate was heated at 80°C for 30 min to inactivate the xylanase and then filtered through an Advantec No. 5C filter paper. Total sugar (TRS) and reducing sugar (DRE) levels of the filtrate (1860 ml) were 65.6 and 38.8 g, respectively.

Isolation of oligosaccharides in the enzymatic hydrolysate of kumaizasa xylan

Neutral oligosaccharides

Two-thirds of the filtrate (1240 ml containing 43.7 g TRS) was applied to a charcoal column (65 × 800 mm, 500 g active carbon for chromatography; Wako Pure Chemical, Osaka, Japan) at a flow rate of 250 ml/h. After washing with water to remove xylose and inorganic matters, the oligosaccharides were eluted with 15 l of 30% ethanol, followed by 15 l of 45% ethanol. The eluates were combined and concentrated to 985 ml. The concentrate (30.7 g TRS) was applied to a Dowex 1-×8 (CH_3COO^- form; Dow Chemical, Midland, Mi, USA) column (50 × 220 mm), equilibrated with water, at a flow rate of 214 ml/h. The column was then washed with water. The non-adsorbed fraction containing neutral sugars was collected and concentrated to 370 ml. The concentrate was applied to a charcoal column (65 × 800 mm) at a flow rate of 250 ml/h. After washing with water, the oligosaccharides were eluted from the column by a linear gradient from 0% to 45% of aqueous ethanol at a flow rate of 500 ml/h. The eluate was fractionated into 500-ml portions, and the sugar composition in the fraction was examined by TLC.

Fractions 39–47, which contained two major products designated AX-I and AX-II, in order of the R_f value on TLC, were combined and concentrated to 50 ml. They were separated by Toyopearl HW-40S gel filtration chromatography (GFC). Fractions 52–55 were combined and further purified by preparative paper chromatography. Then, one major product named AX-III with an R_f slightly lower than that of 1,4-β-xylobiose, was obtained. Fractions 61–66, which contained one major product, named AX-IV with an R_f slightly lower than that of 1,4-β-xylotriose, were combined and concentrated. The AX-IV in the concentrate was separated by preparative paper chromatography.

Acidic oligosaccharides

The acidic oligosaccharides bound by the Dowex 1-×8 column were eluted with 2 M acetic acid. The eluate was collected and neutralized with 4 M sodium hydroxide. The sugar solution was desalted by passing it through a charcoal column according to the method described above. The salt-free solution was concentrated to 2 ml (containig 1.1 g of TRS). The acidic oligosaccharides were further separated using an autopreparative high-performance liquid chromatography (HPLC) system.

Paper partition chromatography

Paper partition chromatography (PPC) for preparative purposes was done by a descending method on No. 526 filter

paper (400 × 400 mm; Advantec, Tokyo, Japan) with a solvent system of 1-butanol/pyridine/water (6:4:3, v/v/v). After development, the saccharides on the paper were extracted with water.

Gel filtration chromatography

Gel filtration chromatography (GFC) was done on a Toyopearl HW-40S (Tosoh, Tokyo, Japan) column (40 × 800 mm), with water as eluent, at a flow rate of 18 ml/h. The eluate was fractionated into 9-ml portions.

HPLC

Preparative HPLC was done on an autopreparative HPLC system (Gilson, Villiers-le-Bel, France) equipped with a refractive index detector and a LiChrospher 100 NH_2 column (10 × 250 mm; Merck), and chromatographed with a solvent system of acetonitrile/water/acetic acid (25:75:1.5, v/v/v) at a flow rate of 3 ml/min. For analytical HPLC, a LiChrospher NH_2 column (4 × 250 mm, Merck) was used with the same solvent system at a flow rate of 1 ml/min.

Reduction of saccharides

The reduction of neutral mono- and oligosaccharides (1–5 mg) into the corresponding sugar alcohols was done with sodium borohydride (30 mg) at 30°C for 1.5 h. The resultant sugar solution was treated with Amberlite IR-200C (H^+ form; Organo, Tokyo, Japan) resin. The sugar solution after removal of the resin was evaporated with methanol to remove boric acid and then dried.

The conversion of acidic saccharide (oligosaccharide having uronic acid) into the corresponding neutral sugar was done by the method of Taylor and Conrad.[21] A 20-mg aliquot of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (WSC, Dojindo Laboratories, Kumamoto, Japan) was added to the sugar solution (2 ml including 1–5 mg of acidic saccharide), and the mixture was stirred at room temperature for 1 h. Throughout the reaction, the mixture was kept at pH 4.7 by titration with 0.005 N hydrochloric acid. Then 3 M sodium borohydride solution (2 ml) was added dropwise to the mixture over 30 min, keeping the pH at 7.0 with 1 N hydrochloric acid during this period. The resultant reaction mixture was treated with cation-exchange resin, evaporated, and dried as described above.

Analysis of component sugars of oligosaccharide

The oligosaccharide (1–5 mg) was hydrolyzed with 1 ml of 10% trifluoroacetic acid (TFA) at 100°C for 2 h. The hydrolysate was dried and then reduced with sodium borohydride. The sugar sample was acetylated with 1 ml of a mixture of an equal portion of pyridine and acetic anhydride. The alditol-acetate derivatives were analyzed by gas-liquid chromatography (GLC).

Methylation analysis

Oligosaccharides (1–5 mg) were methylated by the method of Ciucanu and Kerek.[22] The methylated sugar was hydrolyzed with 10% TFA at 100°C for 2 h and converted to the corresponding partially methylated alditol-acetate derivatives by the method described above. The derivatives were analyzed by GLC.

Gas chromatography

Gas chromatography (GC) was done on a Shimadzu GC-8A gas chromatograph (Shimadzu, Kyoto, Japan) with a stainless steel column of 3% ECNSS-M on Uniport HP (GL Sciences, Tokyo, Japan) at 180°C (for alditol-acetates) or 155°C (for partially methylated alditol-acetates) with nitrogen as the carrier gas at a flow rate of 50 ml/min.

Results and discussion

Isolation of oligosaccharides from the enzymatic hydrolysate of kumaizasa xylan

Figure 1 shows the scheme for isolating oligosaccharides. Four neutral and two acidic oligosaccharides were isolated from the enzymatic hydrolysate of bamboo grass xylan. TLC of the isolated oligosaccharides is shown in Fig. 2. Each of the oligosaccharides produced a single spot on TLC, which was used for structural analysis.

Structural analysis of isolated oligosaccharides

Table 1 shows the results of analyses of component sugars and methylation. AX-I was composed of L-arabinose and D-xylose in a molar ratio of 1:2. The methylation analysis of this sugar gave 2,3,5-tri-*O*-methyl-arabinofuranose (2,3,5-Me-Ara*f*), 2,3-di-*O*-methyl-xylopyranose (2,3-Me-Xyl*p*), and 2,4-di-*O*-methyl-xylopyranose (2,4-Me-Xyl*p*) in a molar ratio of 1.0:0.9:0.7. Additionally, AX-I had the same R_f value as authentic 3^2-α-L-arabinofuranosylxylobiose on TLC (lane 4, Fig. 2A). Thus AX-I had the structure of 3^2-α-L-arabinofuranosyl-1, 4-β-D-xylobiose.

AX-II was composed of arabinose and xylose in a molar ratio of 1:3. The methylation analysis of this sugar gave 2,3,5-Me-Ara*f*, 2,3,4-Me-Xyl*p*, 2,3-Me-Xyl*p*, and 2-Me-Xyl*p* in a molar ratio of 1.0:1.0:1.2:1.1. Additionally, AX-II had the same R_f value as authentic 3^2-α-L-arabinofuranosylxylotriose on TLC (lane 5, Fig. 2A). Thus AX-II had the structure of 3^2-α-L-arabinofuranosyl-1, 4-β-D-xylotriose.

AX-III was composed of arabinose and xylose in a molar ratio of 1.0:2.8. The methylation analysis of this sugar gave 3,5-Me-Ara*f*, 2,3,4-Me-Xyl*p*, 2,3-Me-Xyl*p*, and 2,4-Me-Xyl*p*, in a molar ratio of 1.0:1.0:1.0:1.0. Additionally, AX-III had the same R_f value as authentic 3^2-α-[β-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl]-xylobiose

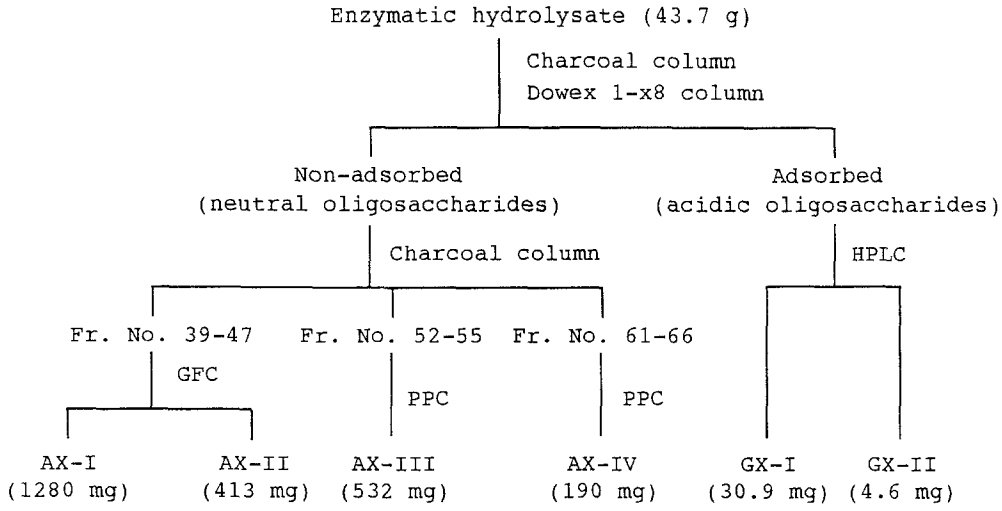


Fig. 1. Isolation of oligosaccharides from the enzymatic hydrolysate of bamboo grass xylan

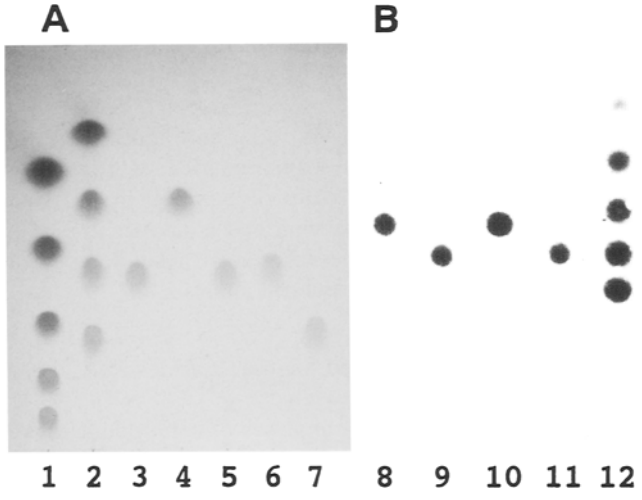


Fig. 2. Thin-layer chromatography (TLC) of the isolated oligosaccharides. **A** TLC on cellulose. **B** TLC on silica gel 60. Lanes 1 and 12: authentic xylose to xylopentaose from top to bottom. Lane 2: authentic *O*-α-L-arabinofuranosyl-(1 → 3)-D-xylose, 3^2-α-L-arabinofuranosyl-1,4-β-D-xylobiose, 3^2-α-[β-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl]-1,4-β-D-xylobiose, and 3^3-α-[β-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl]-1,4-β-D-xylotriose from top to bottom. Lane 3: authentic 3^2-α-L-arabinofuranosyl-1,4-β-D-xylotriose. Lane 4: AX-I. Lane 5: AX-II. Lane 6: AX-III. Lane 7: AX-IV. Lane 8: GX-I. Lane 9: GX-II. Lane 10: authentic 2^3-α-4-*O*-methyl-D-glucuronopyranosyl-1,4-β-D-xylotriose. Lane 11: authentic 2^3-α-D-glucuronopyranosyl-1,4-β-D-xylotriose

on TLC (lane 6, Fig. 2A). Thus AX-III had the same structure.

AX-IV was composed of arabinose and xylose in a molar ratio of 1.0:3.8. The methylation analysis of this sugar gave 3,5-Me-Ara*f*, 2,3,4-Me-Xyl*p*, 2,3-Me-Xyl*p*, and 2,4-Me-Xyl*p* in a molar ratio of 1.0:1.1:2.3:1.3. Additionally, AX-IV had the same R_f value as authentic 3^3-α-[β-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl]-xylotriose on TLC (lane 7, Fig. 2A). Thus AX-IV had the same structure.

GX-I was composed of 4-*O*-methyl-glucuronic acid (4-*O*-Me-GlcA) and xylose in a molar ratio of 1:3.1. 4-*O*-Me-GlcA was identified by comparing with authentic 4-mono-*O*-methyl-1,2,3,5,6-penta-*O*-acetyl-glucitol, which was derived from authentic 2-*O*-α-(4-*O*-methyl-glucopyranosyluronic acid)-xylotriose[10] after reduction with WSC and sodium borohydride, followed by hydrolysis and conversion to the alditol-acetate derivative. The methylation analysis of GX-I after reduction with WSC and sodium borohydride gave 2,3-Me-Xyl*p* (or 3,4-Me-Xyl*p*), 1,2,3,5-Me-xylitol, and 2,3,4,6-Me-Glc*p* in a ratio of 2.2:1.0:1.1. In addition, GX-I had the same R_f value as authentic 2^3-4-*O*-methyl-α-D-glucuronopyranosyl-xylotriose on TLC (lane 8, Fig. 2B). Moreover, GX-I (20.82 min) had the same retention time as authetic 2^3-4-*O*-methyl-α-D-glucuronopyranosyl-xylotriose (20.84 min) on HPLC analysis.[10] The above results show that GX-I had the structure of 2^3-4-*O*-methyl-α-D-glucuronopyranosyl-1,4-β-D-xylotriose.

GX-II was composed of glucuronic acid and xylose in a molar ratio of 1:3.2. The methylation analysis of GX-II gave 2,3-Me-Xyl*p* (or 3,4-Me-Xyl*p*), 1,2,3,5-Me-xylitol, and 2,3,4,6-Me-Glc*p* in a ratio of 2.1:1.0:1.2. Additionally, GX-II had the same R_f value as authentic 2^3-α-D-glucuronopyranosyl-xylotriose on TLC (lane 9, Fig. 2B). Moreover, GX-II (29.81 min) had the same retention time as authetic 2^3-α-D-glucuronopyranosyl-xylotriose (29.78 min) on HPLC analysis.[10] The above results show that GX-II had the structure of 2^3-α-D-glucuronopyranosyl-1,4-β-D-xylotriose.

The possible structures of the isolated oligosaccharides are shown in Fig. 3.

Structure of bamboo grass xylan

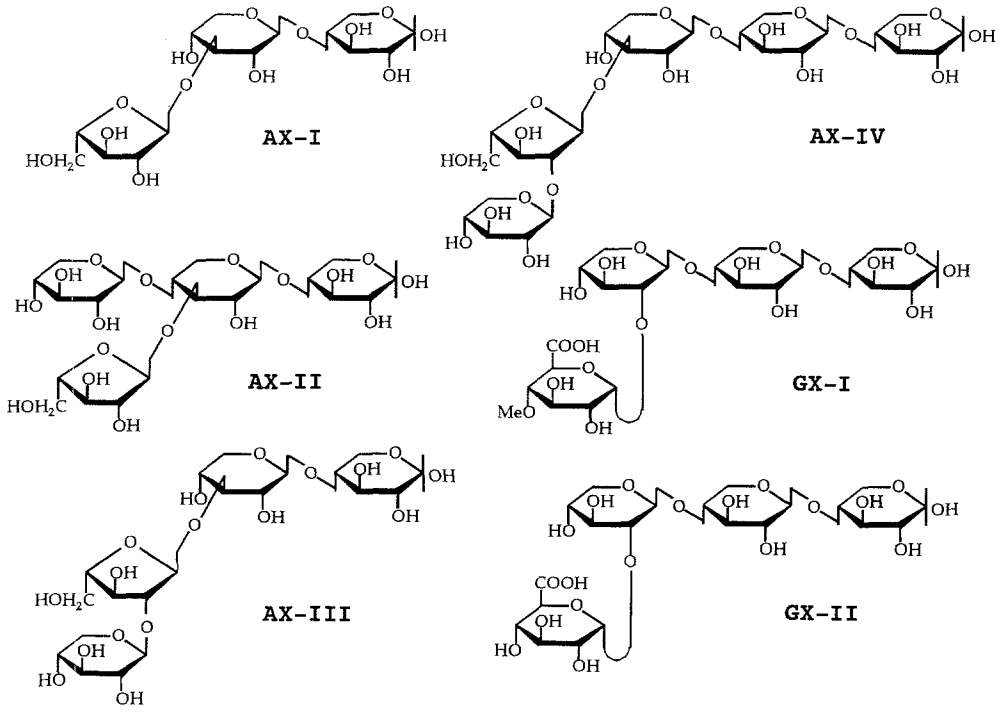
Figure 4 shows the proposed structure of bamboo grass xylan based on the structural analysis of the oligosaccharides released by xylanase digest. The bamboo grass

Table 1. Component sugars and methylation analysis of isolated oligosaccharides

Parameter	Oligosaccharides					
	AX-I	AX-II	AX-III	AX-IV	GX-I	GX-II
Component sugars						
Araf[a]	1.0	1.0	1.0	1.0		
4-*O*-Me-GlcA					1.0	
GlcA						1.0
Xyl*p*	2.0	3.0	2.8	3.8	3.1	3.2
Methylation analysis						
2,3,5-Me-Ara*f*[b]	1.0	1.0				
3,5-Me-Ara*f*			1.0	1.0		
2,3,4,6-Me-Glc*p*					1.2	1.2
1,2,3,5-Me-xylitol					1.0	1.0
2,3,4-Me-Xyl*p*		1.0	1.0	1.1		
2,3-(or 3,4-)Me-Xyl*p*	0.9	1.2	1.0	2.3	2.2	2.1
2,4-Me-Xyl*p*	0.7		1.0	1.3		
2-Me-Xyl*p*		1.1				

Each number in this table indicates the molar ratio obtained from the results of gas chromatography analysis of each oligosaccharide.
[a] Ara*f*, L-arabinofuranose; GlcA, D-glucopyranosyluronic acid; Xyl*p*, D-xylopyranose; Me, methyl.
[b] 2,3,5-Me-Ara*f* denotes 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinose

Fig. 3. Possible structures of isolated oligosaccharides



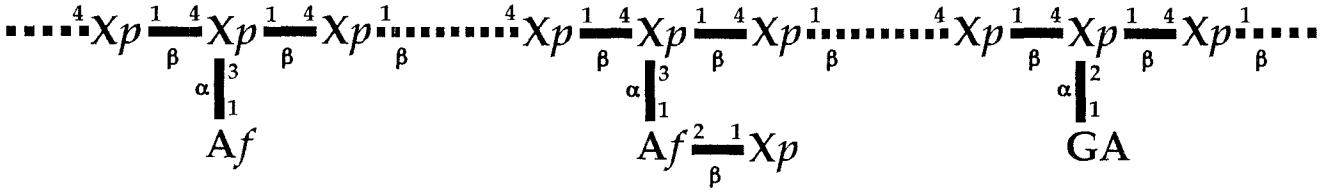


Fig. 4. Proposed structure of bamboo grass xylan. *Af*, L-arabinofuranose; *GA*, 4-*O*-methyl-D-glucopyranosyluronic acid or D-glucopyranosyluronic acid; *Xp*, D-xylopyranose

xylan is a kind of arabinoglucuronoxylan composed of D-xylopyranose, L-arabinofuranose, 4-*O*-methyl-D-glucopyranosyl uronic acid, and D-glucopyranosyl uronic acid. The arabinoglucuronoxylan is composed of the main chain of (1 → 4)-linked β-D-xylopyranosyl residues to which are directly attached α-L-arabinofuranosyl or α-(2-*O*-β-D-xylopyranosyl-L-arabinofuranosyl) stubs at the *O*-3 and 4-*O*-methyl-D-glucuronopyranosyl or D-glucuronopyranosyl stubs at the *O*-2 of the xylosyl residues of the main chain. The corncob xylan also has the α-(2-*O*-β-D-xylopyranosyl-L-arabinofuranosyl) stubs at the *O*-3 position of the xylosyl main chain. Thus the structure of bamboo grass xylan is similar to that of corncob xylan.[8] On the other hand, the bamboo grass xylan is somewhat different from those of rice straw[9] and bamboo[23] xylans because the latter do not have the α-(2-*O*-β-D-xylopyranosyl-L-arabinofuranosyl) stubs.

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