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Chemical structure of kenaf xylan

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Abstract

Methylation and partial acid hydrolysis of xylans from the bast and core of kenaf (*Hibiscus cannabinus*) showed that the main chain of these xylans consists of (1 → 4)-linked β-D-xylopyranosyl (Xyl*p*) residues, some of which carry a α-1,2-linked 4-*O*-methyl-glucopyranosyluronic acid (Me-GlcA*p*) and glucopyranosyluronic acid (GlcA*p*) residues as side chains. Partial hydrolysis of kenaf xylans afforded two series of aldouronic acids from aldobio- to aldotetraouronic acids. The acids of the first series composed of 4-*O*-Me-D-GlcA*p* and D-Xyl*p* residues: 4-*O*-Me-GlcA-Xyl_3, 4-*O*-Me-GlcA-Xyl_2 and 4-*O*-Me-GlcA-Xyl. The second series composed of D-GlcA*p* and D-Xyl*p*: GlcA-Xyl_3, GlcA-Xyl_2 and GlcA-Xyl.

In addition to these acids, another aldobiouronic acid, 4-*O*-(α-D-GalA*p*)-D-Xyl was found to be present in the partial hydrolysate.

The molar ratio of GalA, GlcA, 4-*O*-Me-GlcA, and Xyl residues was calculated to be 1.0:2.0:9.4:119 for the bast xylan and 1.0:1.3:7.9:99.4 for the core xylan.

Keywords: Kenaf; Xylan; Aldouronic acid; ^{13}C NMR spectroscopy; Ion exchange chromatography; Partial hydrolysis

1. Introduction

Kenaf (*Hibiscus cannabinus*) is an annual herbaceous plant which has attracted considerable attention as multipurpose plant having great potential for fiber, energy, feedstock, etc. Several workers have studied the structural features of components of kenaf, for example, lignin by Pascoal-Neto et al. (1996), and Lam, Hori, and Iiyama (2003), lignans by Seca et al. (2001) and xylan by Duckart, Byers, and Thompson (1988), Pascoal-Neto et al. (1996) and Nacos et al. (2006).

Kenaf contains high quantity of xylan (Cunningham, Carr, & Bagby, 1986). Based on the literatures cited above, kenaf xylan belongs to the category of hardwood xylan. It consists of a backbone of (1 → 4)-linked β-D-Xyl*p* residues. Every tenth D-Xyl*p* residue, on the average, is substituted at C-2 by a 4-*O*-methyl-D-glucopyranosyluronic acid residue (4-*O*-Me-D-GlcA*p*).

The structural varieties of xylan-type polysaccharides has been reported in detail by Ebringerova and Heinze (2000). Some acidic xylans with the GlcA side chain both in the 4-*O*-methylated and non-methylated forms are isolated from monocotyl plants (Lindberg et al., 1990).

Although hardwood xylans are only 4-*O*-methylglucuronoxylan, several structural details are, however, still open to discussion. One question is whether or not galacturonic acid (GalA), and rhamnose (Rha) are structural components of xylan. It has been proved that the reducing end of hardwood xylans from birch (*Betula platyphylla*) (Ericsson, Petersson, & Samuelson, 1977; Johansson & Samuelson, 1977; Shimizu, Ishihara, & Ishihara, 1976), and *Eucalyptus globulus* (Evtuguin, Tomas, Silva, & Pascoal-Neto, 2003), and of softwood xylan from spruce (Andersson, Samuelson, Ishihara, & Shimizu, 1983) has the structure -*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 3)-*O*-α-L-Rha*p*-(1 → 2)-*O*-α-D-GalA*p*-(1 → 4)-D-Xyl.

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In this report, the heteroxylans were isolated from the bast and core of kenaf (*H. cannabinus*) by direct extraction with 10% KOH. The structure of the xylans was studied by methylation analysis and partial acid hydrolysis. The acidic sugars formed on partial acid hydrolysis were separated by ion exchange chromatography and identified by ^{13}C NMR and ^{1}H NMR spectroscopy.

2. Experimental

2.1. General methods

Monosaccharides were analyzed by a Shimadzu LC-10AT high performance liquid chromatograph following the procedure of Nakamura, Hatanaka, and Nagamatsu (2000). A mixture of monosaccharides was chromatographed in a TSK-gel SUGAR AX1 column (TOSOH Co.) with 0.5 M borate −1.0% ethanolamine–HCl buffer at pH 7.9. Relative percentage amounts were calculated electronically. Gas liquid chromatography (G.l.c.) for the partially methylated, partially acetylated alditol was performed on a Shimadzu GC-9A chromatograph with glass column (200 × 0.26 cm) containing 2% ECNSS-M on Chromosorb WAW DMCS (100–120 mesh). Injector and detector temperatures were set at 220 °C. The oven temperature was held at 180 °C. Nitrogen was used as the carrier gas at a flow rate of 40 mL/min. Samples of 2.0 μl were injected manually. FT-IR spectra were recorded on JASCO Model FT/IR-410 spectrometer using a KBr disc containing 1% finely ground sample.

2.2. Mass spectrometry

GLC–MS analysis of the methylated derivatives was performed on a DP-1 fused silica capillary column (30 m length, 0.25 mm ID) using helium as the carrier gas, at a flow rate of 50 mL/min. The GC–MS system consisted of a Shimadzu GC-17A-GCMS-QP5050 operating at an ionization potential 70 eV. Injector and MS interphase temperatures set at 260 °C. The column temperature was programmed at 5 °C/min from 150 → 250 °C. The components were identified based on the comparison of their relative retention times and mass spectra with those of standards.

2.3. NMR spectrometry

NMR spectra of oligosaccharides were recorded at 25 °C by taking samples in D_2O with a JEOL ECA 500FT-NMR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained at 500.16 and 125.77 MHz. The chemical shifts are reported relative to internal TSP. Standard pulse sequences were utilized to obtain COSY, NOESY, HMQC and HMBC spectra.

2.4. Materials

Kenaf (Tainung2, *H. cannabinus* Linn.) cultivated in the garden of Kochi University, Kochi Prefecture and harvested in February 2003 was kindly supplied by Prof. Kazuhiko Samejima. The dried sample was separated into bast fiber and stem core. They were milled with a cutting mill (Fritsch Japan Co., Ltd.) and the fraction 40–60 mesh was collected by sieving.

2.5. Chemical composition

The chemical compositions of the extractive-free bast fiber and stem core were determined by the standard method of Japan Wood Research Society (1985, chap. 4). Extractives were removed with the mixture of alcohol–benzene (1:2) by use of Soxhlet apparatus. Cold and hot water extraction were carried out at room temperature and 100 °C, respectively. Alkaline extracts were determined with 1% NaOH at 100 °C for 1 h. The lignin content was determined in extractives-free samples by Klason method. The holocellulose was determined by the acidic chlorite method. α-Cellulose was estimated from the residues by extraction of holocellulose with 17.5% NaOH. The neutral sugar composition was determined in the hydrolysate of Klason lignin by liquid chromatography as described above.

2.6. Alkaline extraction

Kenaf mills were exhaustively extracted with methanol in an extracting equipment. Extractives-free kenaf mill (20 g) was extracted with hot water at 100 °C for 3 h and after air-drying shaken with 200 mL of 3%, 5%, 8%, 10% and 12% KOH (w/v) at room temperature for 2 h under nitrogen. After filtration through a fine cloth, the residue was washed with the corresponding concentration of KOH (50 mL), and successively washed with water, 10% acetic acid, water and acetone. The air-dried residue was weighed and corrected for moisture content.

The filtrate and alkaline washings were acidified to pH 4.5 with acetic acid, and then 4 volumes of ethanol were added. After standing 24 h, the precipitates formed were collected by centrifugation, washed with successively with 80% aqueous ethanol, absolute ethanol and petroleum ether and dried in vacuum over phosphorous pentoxide.

2.7. Methylation analysis

Xylans (10 mg) were methylated with K+ methylsulphinyl-methanide anion and methyl iodide in dimethyl sulphoxide as described by Carpita and Shea (1989), a modification of the Hakomori (1964) procedure. The esterified uronic acid residues in the per-methylated polysaccharides were reduced with 1.0 M lithium triethylborodeuteride in tetrahydrofuran. The partially-*O*-methylated, partially-*O*-acetylated alditols were prepared by hydrolysis, reduction, and acetylation. The methylated and deuterated samples were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100 °C for 4 h, reduced with sodium borohydride

and *O*-acetylated with 1-methylimidazol and acetic anhydride (Blakeney, Harris, Henry, & Stone, 1983).

2.8. Analysis of sugar composition

In order to investigate the sugar composition of aldouronic acid, samples (1 mg) were hydrolyzed with 1 M TFA at 120 °C for 1 h. A part of the hydrolysate was injected to the chromatograph and eluted with borate buffer as described above. Another part, after saponification by keeping at pH 8 for 4 h with 0.1 M NaOH, was applied to the column of anion exchange resins Diaion (AcO^-, 5×650 mm) and elution with 0.08 M sodium acetate (pH 5.9). The ***Dv*** value of each acid was calculated in the usual way (Samuelson, 1963).

2.9. Separation of acidic oligosaccharide

The xylans (1 g) from the core and bast were hydrolyzed with 2 M TFA at 120 °C for 30 min, respectively. The hydrolysates were kept at pH 8 for 4 h at room temperature to hydrolyze the lactone, and then applied to a column of Dowex 1×8 (AcO^-). The neutral sugars were eluted with water until the anthrone test was negative, and acidic sugars were eluted with 5 M acetic acid.

The acidic sugars were fractionated by chromatography on a preparative column of strongly acidic ion exchange resin Diaion (AcO^-, 15×930 mm) by elution with **A**, 0.08 M sodium acetate (pH 5.9) giving 9 fractions. Fractions 1–9 were rechromatographed on a preparative column of Aminex A-27 (10×830 mm) by elution with **B**, 0.25 M acetic acid, **C**, 0.5 M acetic acid and **D**, 1.0 M acetic acid. The both xylans gave same products. Each acid was identified by the volume distribution coefficients (***Dv***) calculated in the usual way (Samuelson, 1963), acid hydrolysis, subsequent identification of the hydrolysis products and ^{13}C NMR and ^{1}H NMR spectroscopy.

Fraction 1 representing aldotetraouronic acid gave three fractions, **Fr.1:S1, 1:S2 and 1:S3** on rechromatography in eluant **B** with ***Dv*** 4.63, 4.84 and 5.40, respectively. These acid gave same products, 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl*p*, 4-*O*-Me-GlcA and Xyl on acid hydrolysis with 2 M TFA at 120 °C for 2 h, suggesting that they were possible isomers. **Fr.1:S2** was a main peak and its ***Dv*** coincided with that of the authentic sample *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl. (Shimizu et al., 1976). Its ^{13}C NMR spectrum was identical with that reported by Nacos et al. (2006). **Fr1:S3** showed ***Dv*** identical with that of *O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl*p* (Shimizu, Hashi, & Sakurai, 1978). **Fr1:S1** may be other possible isomers, of which uronic acid attached to the central unit of xylotriose. But it was obtained in a small amount, so, further study was not done.

Fraction 2 gave also three fractions, **Fr.2:S1**, **2:S2** and **2:S3** in eluant **C**. These acids gave 2-*O*-(α-D-GlcA*p*)-D-Xyl*p*, GlcA and Xyl on hydrolysis with 2 M TFA at 120 °C for 2 h indicating that they were isomers of the aldotetraouronic acids consisting of GlcA and Xyl. **Fr.2:S1** and **S3** were, however, obtained in small amount and neglected in the present study. **Fr2:S2** having ***Dv*** 2.9 was identified as *O*-(α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl-(1 → 4)-D-Xyl on basis of ^{13}C and ^{1}H NMR spectra.

Fraction 3 representing aldotriouronic acid gave two fractions, **Fr.3:S1** and **Fr.3:S2** in eluant **C** with ***Dv*** 4.3 and 4.9, identical with those of the authentic samples *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p* and *O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl*p*, respectively (Shimizu et al., 1978). The ^{13}C NMR and ^{1}H NMR spectra of the former were identical with those reported by Cavagn and Deger (1984).

Fraction 4 gave one peak **Fr.4:S1** in eluant **D** with ***Dv*** 2.4 identical with that of the authentic sample *O*-(α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p* (Shimizu & Samuelson, 1973). This acid gave the same hydrolysis products as **Fr.2:S1**. Its ^{13}C NMR and ^{1}H NMR spectra were identical with those reported by Simas et al. (2004).

Fraction 5 gave two peaks **Fr.5:S1** and **Fr.5:S2** in eluant **D** with ***Dv*** 4.7 and 7.5, respectively. The latter was obtained in a trace amount and neglected in the present study. The former was identified as 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl based on ***Dv*** 4.7 and ^{13}C NMR and ^{1}H NMR spectra comparing with those of the authentic sample (Azuma & Koshijima, 1983; Shimizu & Samuelson, 1973).

Fraction 6 gave one peak **Fr.6:S1** in eluant **D** with ***Dv*** 7.6 identical with the authentic sample 4-*O*-(α-GalA*p*)-D-Xyl (Shimizu & Samuelson, 1973). This was confirmed by the fact that the acid gave GalA and Xyl on acid hydrolysis with 2 M TFA at 120 °C for 2 h.

Fraction 7 gave two peaks **Fr.7:S1** and **Fr.7:S2** in eluant **D** with ***Dv*** 6.0 and 12.5, respectively. The latter was obtained in a trace amount and neglected in the present study. The former acid **Fr.7:S1** had ***Dv*** identical with that of the authentic sample, 2-*O*-(α-D-GlcA*p*)-D-Xyl (Shimizu & Samuelson, 1973) and gave the starting material, GlcA and Xyl on acid hydrolysis with 2 M TFA at 120 °C for 2 h. The ^{13}C NMR and ^{1}H NMR spectra of this acid were identical with those reported by Simas et al. (2004).

Fractions 8 and 9 gave one peak **Fr.8:S** with ***Dv*** 14.6 and **Fr.9:S1** with ***Dv*** 8.4 in eluant **D**, respectively. They were obtained in small amounts and identified as 4-*O*-Me-D-GlcA and GalA with their ***Dv*** values, respectively.

3. Result and discussion

3.1. General chemical composition

Table 1 shows the chemical composition of bast fiber and stem core. The bast fiber and stem core of kenaf contained alcohol–benzene extractives 3.1% and 5.5%, respectively. These extractives probably consist of sterols, triterpene, long chain fatty ester, lignans, etc. as reported by Seca et al. (2001). The contents of cold and hot water

Table 1
Chemical composition of the bast and core of kenaf (*Hibiscus cannabinus*) (% o.d. material)

	Alcohol–benzene[a]	Cold water[a]	Hot water[a]	Hot 1% NaOH[a]	Lignin	Holo-cellulose	α-Cellulose
Bast	3.1	13.9	14.9	27.8	10.8	82.5	57.3
Core	5.5	7.5	9.4	32.1	20.4	82.4	39.1

[a] Extractives.

extracts were very high compared with those of temperate woody species (Yonezawa et al., 1973). It has been reported that kenaf contains appreciable amounts of proteins (Jin, Nakagawa, Shimizu, & Ohi, 2006; Pascoal-Neto et al., 1996). The extracts with hot 1% NaOH reached about 30% in both samples indicating that appreciable amounts of hemicellulose were dissolved out. Although the holocellulose content is not different in bast and core, lignin content is lower and α-cellulose content is higher in bast than in core as observed by other researchers (Jin et al., 2006; Lam et al., 2003; Pascoal-Neto et al., 1996).

Table 2 shows the sugar compositions of the hot water extracts of bast fiber and stem core. The hot water extracts from the bast are a mixture of several hemicelluloses, whereas glucose is a predominant in the hydrolysate of core indicating the presence of starch. In fact, the hot water extracts of stem core reacted with iodine giving blue color.

Table 3 shows the neutral sugar composition of bast fiber and stem core. Glucose is the predominant sugar and xylose is second most abundant sugar indicating that xylan is the main hemicellulose in kenaf. Xylan content in core and bast can be calculated from their holocellulose contents to be 23.8% and 12.0%, respectively. Xylan is richer in core than in bast.

3.2. Isolation of xylan

In order to set the alkaline concentration giving a quantitative yield of xylan, the extractive-free bast fiber and stem core were extracted with 3–12% KOH. As shown in Fig. 1, the extracted amount increased with increasing concentration of KOH and reached plateau with 10% KOH. In the case of stem core, the extracted amounts with 3–12% KOH reached 27–32% and the yields of precipitate (mainly xylan) formed from the extracts by addition of ethanol were 19–25%, meaning that 70–80% of the extracts was precipitated by addition of ethanol. In the case of bast fiber, the extracted amount with the alkali was in the range of 16.2–24.3%, and the yield of precipitate was in the range of 2.3–12.8%. These facts were similar to those reported by Pascoal-Neto et al. (1996). These facts show that most of xylan is easily extracted with even weak alkali (5% NaOH) from core, but that at least 10% KOH is necessary to extract quantitatively xylan from the bast in spite of lower content of lignin than core. In general, the xylan extractable from cell wall with alkali is inversely proportional to lignin content in cell wall (Ishihara, Nojiri, Hayashi, & Shimizu, 1996).

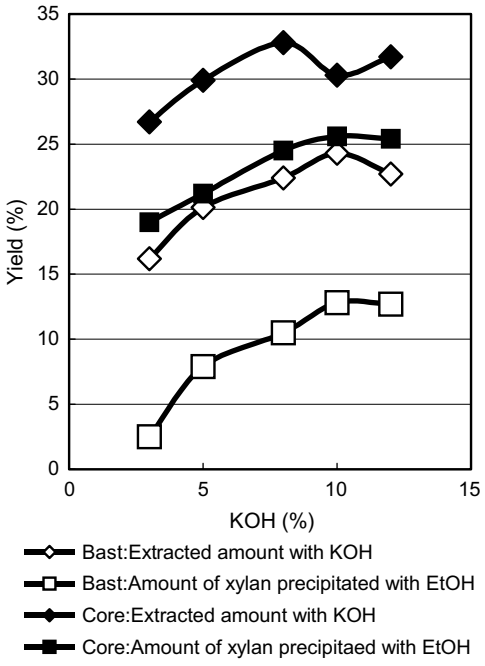

Fig. 1. Extraction of xylan from bast and core with 10% KOH.

After the preparatory experiment described above, xylan was extracted from the extractive-free and hot water-extracted bast fiber and stem core with 10% KOH, respectively. Their yields and neutral sugar compositions are shown in Table 4. Almost all of the xylan was extracted from core and also from bast with 10% KOH. Neutral sugar composition show that the xylan fraction is contaminated with some glucan such as cellulose and starch. Mannose, arabinose and galactose was detected in relatively small amounts. It should be noticed that Rha is also present in small but not negligible amounts.

Table 2
Yield and neutral sugar composition of hot water extracts from the bast and core of kenaf (*Hibiscus cannabinus*) (%)

	Rha	Man	Ara	Gal	Xyl	Glc
Bast	4.8	10.6	31.8	21.1	7.5	24.1
Core	1.1	6.5	5.8	8.0	4.8	73.8

Table 3
Neutral sugar composition (%) of the bast and core of kenaf (*Hibiscus cannabinus*)

	Rha	Man	Ara	Gal	Xyl	Glc
Bast fiber	0.8	2.3	2.4	2.4	14.5	77.6
Stem core	0.7	2.2	–	1.0	28.9	67.1

Table 4
Yield and neutral sugar composition of xylan extracted from the bast and core of kenaf (*Hibiscus cannabinus*)

	Yield	Rha	Man	Ara	Gal	Xyl	Glc
Bast	12.0	1.2	2.3	2.8	3.9	82.1	7.6
Core	21.8	1.7	0.5	0.7	1.4	89.2	6.5

3.3. FT-IR spectra

A portion of xylans from core and bast was treated for a few minutes at 0 °C with 50% aqueous methanol containing 5% HCl, giving the free acid form of the polysaccharide (Bryant, Timell, Zimbo, Goring, & Yean, 1968). Their FT-IR spectrum (Fig. 2) shows the typical spectrum for 4-*O*-Me-D-xylans (Marchessault & Liang, 1962; Sun & Tomkinson, 2002). The signals in the region 3600–2800 cm^{-1} are due to OH and CH stretchings. A sharp band at 897 cm^{-1} is attributed to C1 group frequency or ring frequency indicating that β-glycosidic linkage is dominant in the xylans. Bands between 1125 and 1000 cm^{-1} are typical of xylans due to C—O stretching. The absorption at 1735 cm^{-1} is due to C=O stretching (acid) indicating the acid form of uronic acid. The absorption at 1646 cm^{-1} is associated with absorbed water.

3.4. Methylation analysis

The xylans from bast and core were subjected to methylation analysis to determine the glycosyl linkage giving the same g.l.c. chromatogram. Fig. 3 shows the chromatogram of partially-*O*-methylated, partially-*O*-acetylated alditols obtained from the core xylan. The four alditols were identified by GC–MS using their retention times and mass spectra (Carpita & Shea, 1989; Shimizu, Teratani, & Miyazaki, 1971), as (1) 1,5-di-*O*-acetyl-2,3,4-*O*-methyl-xylitol, (2) 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-xylitol, (3) 1,5,6-tri-*O*-acetyl-(6,6-dideuterio)-2,3,4-tri-*O*-methyl-glucitol and (4) 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-xylitol. These facts indicate that the xylans have a backbone of (1 → 4)-linked β-Xyl*p* residues some of which are substituted at C-2 by GlcA*p* residue.

3.5. Separation of acidic oligosaccharides

The xylans from bast and core were hydrolyzed with 2 M TFA at 120 °C for 30 min. The neutral and acidic sugars were separated in the usual way (Shimizu & Samuelson, 1973), and the acidic sugars were fractionated by ion exchange chromatography using 0.08 M NaOAc as eluant. The chromatogram (Fig. 4) shows the fractionation of acidic sugars formed on hydrolysis of the core xylan. The bast xylan gave nearly same chromatogram. Nine **Fractions** were obtained, together with small fractions which were neglected in the present study. **Fraction 1** representing aldotetraouronic acids was rechromatographed in eluant **B**, giving three fractions, **Fr.1:S1**, **1:S2 and 1:S3**. **Fraction 2** was rechromatographed in eluant **C**, giving also three fractions, **Fr.2:S1**, **2:S2 and 2:S3**. **Fraction 3** representing aldotriouronic acids was rechromatographed in eluant **C** giving two fractions, **Fr.3:S1** and **Fr.3:S2**. **Fractions 4** was rechromatographed in eluant **D** giving one peak **Fr. 4:S1**. **Fraction 5–8** were purified in eluant **D**, giving fractions **5:S1**, **6:S1**, **7:S1**, **8:S1** and **9:S1**. The yields of main fractions are given in Table 5.

Each fraction was identified by ***Dv*** values (Shimizu et al., 1978; Shimizu & Samuelson, 1973), hydrolysis products and ^{13}C NMR and ^{1}H NMR spectroscopy. The anomeric configurations of the glycosyl residues were determined by means of ^{1}H NMR and ^{13}C NMR spectrom-

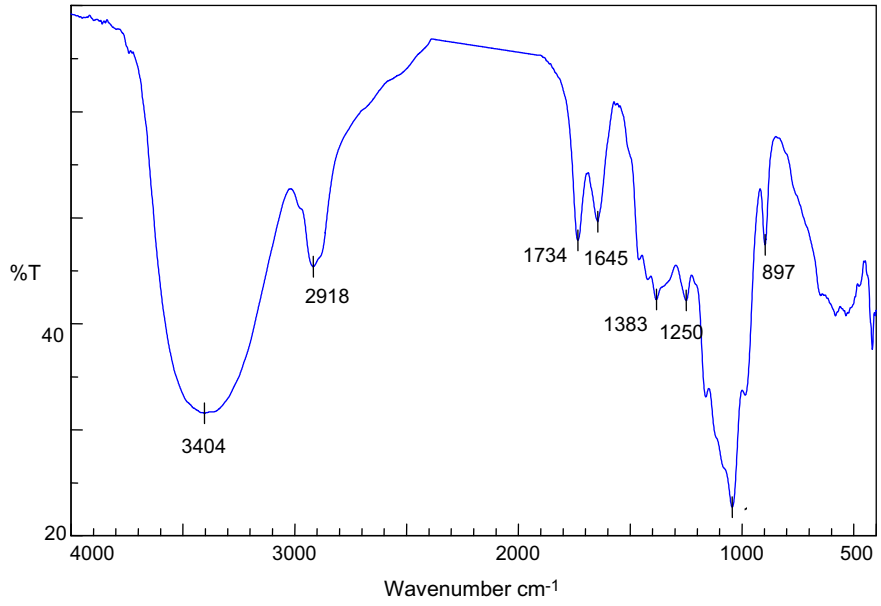

Fig. 2. FT-IR spectrum of xylan (free acid form) extracted from kenaf core.

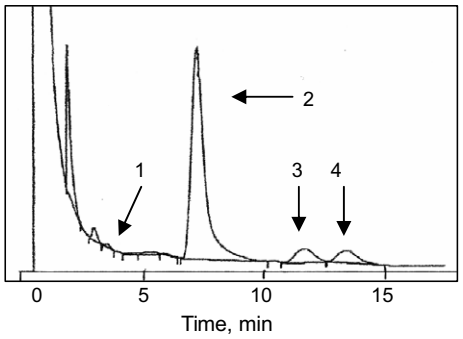

Fig. 3. Gas chromatogram of partially-*O*-methylated, partially-*O*-acetylated alditols derived from xylan of bast. 1, 2,3,4-tri-*O*-Methyl-D-xylitol acetate; 2, 2,3-di-*O*-methyl-D-xylitol acetate; 3, 2,3,4-tri-*O*-methyl-D-glucitol acetate; 4, 3-*O*-methyl-D-xylitol acetate.

etry. Assignments of signals were carried out using 2D NMR spectrometry and based on published data for some related compounds (Azuma & Koshijima, 1983; Cavagn & Deger, 1984; Gast, Attala, & McKelvey, 1980; Nacos et al., 2006; Simas et al., 2004). The carbon shifts of the main aldouronic acids purified are summarized in Table 6.

Fraction 5:S1 and **8:S1** were 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl and 4-*O*-Me-D-GlcA, respectively. Their ***Dv*** values were identical with those of the authentic samples from the acid hydrolysate of birch xylan (Shimizu & Samuelson, 1973). The ^{13}C NMR spectrum of **Fr.5:S1** (Table 6) was identical with that reported by Azuma and Koshijima (1983). The aldouronic acids in **Fractions 1** and **3** afforded, on hydrolysis, D-Xyl and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, and 4-*O*-Me-D-GlcA. They were a homologous series of acidic oligosaccharides and mixtures of possible isomers. **Fractions 1:S2** and **3:S1** identified as *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl and *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p*, respectively. Their ^{13}C NMR spectra (Table 6) coincided with those reported in the literatures (Cavagn & Deger, 1984; Nacos et al., 2006). The aldobtriouronic acid **3:S1** was a main product in the hydrolysis condition (2 M TFA, 120 °C for 30 min) used in the present study (Table 5).

The aldouronic acids in **Fractions 2**, **4** and **7** gave 2-*O*-(α-D-GlcA*p*)-D-Xyl, GlcA and D-Xyl on acid hysrolysis indicating that these acids are another homologous series consisting of GlcA*p* and D-Xyl. **Fractions 2** and **4** were also

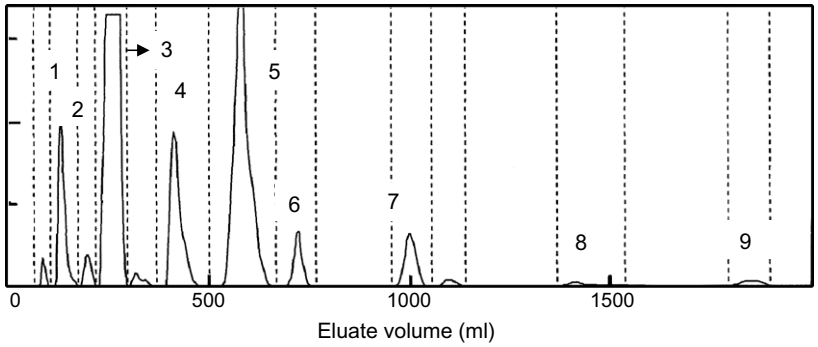

Fig. 4. Fractionation of uronic acids formed on hydrolysis of xylans from kenaf bast and core; column (15 × 900 mm) of diaion (AcO^-) eluted with 0.08 M NaOAc at 1.5 mL/min.

Table 5
Yield of sugars obtained from partial hydorolysates of xylans of kenaf bast and core

Fr. No.	Sugars	Bast		Core	
		Yield (mg)	Molar ratio[a]	Yield (mg)	Molar ratio[a]
1:S1	Isomer				
1:S2	*O*-(4-*O*-Me-α-D-GlcAp)-(1 → 2)-*O*-β-D-Xylp- (1 → 4)-*O*-β-D-Xylp-(1 → 4)-D-Xyl	12.7	9.4	11.1	7.9
1:S3	*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl*p*				
3:S1	*O*-(α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl-(1 → 4)-D-Xyl				
3:S2	*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl*p*				
5:S1	2-*O*-(4-*O*-Me-α-D-GlcAp)-D-Xyl	44.2		63.8	
8:S1	4-*O*-Me-D-GlcA	3.8		2.3	
2:S2	*O*-(α-D-GlcAp)-(1 → 2)-*O*-β-D-Xylp- (1 → 4)-*O*-β-D-Xyl-(1 → 4)-D-Xyl	6	2.0	2.4	1.3
4:S1	*O*-(α-D-GlcAp)-(1 → 2)-*O*-β-D-Xylp- (1 → 4)-D-Xylp	13.4		15.4	
7:S1	2-*O*-(α-D-GlcAp)-D-Xyl	14.5		8.0	
6:S1	4-*O*-(α-GalAp)-D-Xyl	4.0	1.0	7.1	1.0
9:S1	D-GalA	5.3		6.1	
	D-Xyl	615.0	119.0	639.0	99.4

[a] Molar ratio of anhydro Xyl:GaIA:GIcA:4-*O*-Me-GIcA.

Table 6
Assignments of signals in the ^{13}C NMR spectra of aldouronic acids obtained from partial hydrolysate of kenaf xylan (ppm)

		G(OMe)-X		G-X		G(OMe)-X′-X		G-X′-X		G(OMe)-X″-X′-X		G-X″-X′-X	
		β	α	β	α	β	α	β	α	β	α	β	α
X	1	97.53	90.39	97.61	90.49	97.28	92.75	97.42	92.81	97.33	92.85	97.18	92.70
	2	79.38	77.24	79.54	77.39	74.75	72.10	74.85	72.22	74.82	72.20	74.66	71.63
	3	74.98	71.80	75.09	71.93	74.76	71.75	74.85	71.89	74.74	72.20	74.61	71.63
	4	70.18		70.25		76.96	77.07	77.02	77.13	77.24	77.39	77.00	77.17
	5	65.68	61.44	65.75	61.49	63.70	59.48	63.79	59.59	63.81	59.65	63.57	59.69
X′	1					102.38	102.28	102.59	102.55	102.50		102.35	
	2					77.71		77.36		73.50		73.34	
	3					75.11		75.34		74.57		74.40	
	4					70.25		70.31		76.94		76.75	
	5					65.74		65.83		63.73		63.75	
X″	1									102.40		102.28	
	2									77.83		77.58	
	3									75.15		75.05	
	4									70.30		70.13	
	5									65.80		65.64	
G	1	98.49	97.47	98.69	97.68	98.54		98.47		98.63		98.51	
	2	71.69		71.78		71.73		72.00		71.78		72.25	
	3	72.84	72.80	73.20		73.04		73.53		73.09		74.58	
	4	82.30	82.26	72.18		82.49		72.92		82.46		73.28	
	5	70.23	70.34	71.75		70.69		72.92		70.54		72.04	
	6	174.37	174.29	174.30	174.18	174.89		177.93		174.67		175.23	
	OMe	60.76				60.81				60.87			

mixtures of possible isomers. Main **Fr.2:S2** was identified as *O*-(α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl. The ^{13}C NMR spectrum of **Fr. 2:S2** is shown in Fig. 5. Signal for the carbon of methoxyl around 60.8 ppm is absent confirming the absence of 4-*O*-Me-D-GlcA and the signal for C4 of GlcA shifted to higher field compared with that of C4 substituted with methoxyl group (Table 6). **Fr.4:S1** and **7:S1** were identified as *O*-(α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p* and 2-*O*-(α-D-GlcA*p*)-D-Xyl, respectively, by comparison of their ***Dv*** with those of authentic samples (Shimizu & Samuelson, 1973). Their ^{13}C NMR and ^{1}H NMR spectra were identical with those reported by Simas et al. (2004).

Fractions 6:S1 and 9:S1 were obtained in small amounts and identified as 4-*O*-(α-GalAp)-D-Xyl and D-GalA by comparing with ***Dv*** values of authentic samples in eluant **A** and **D** (Shimizu & Samuelson, 1973).

The molar ratio of D-GalA, D-GlcA, 4-*O*-Me-D-GlcA and D-Xyl residues in the xylans was calculated to be 1.0:2.0:9.4:119 for the bast xylan and 1.0:1.3:7.9:99.4 for

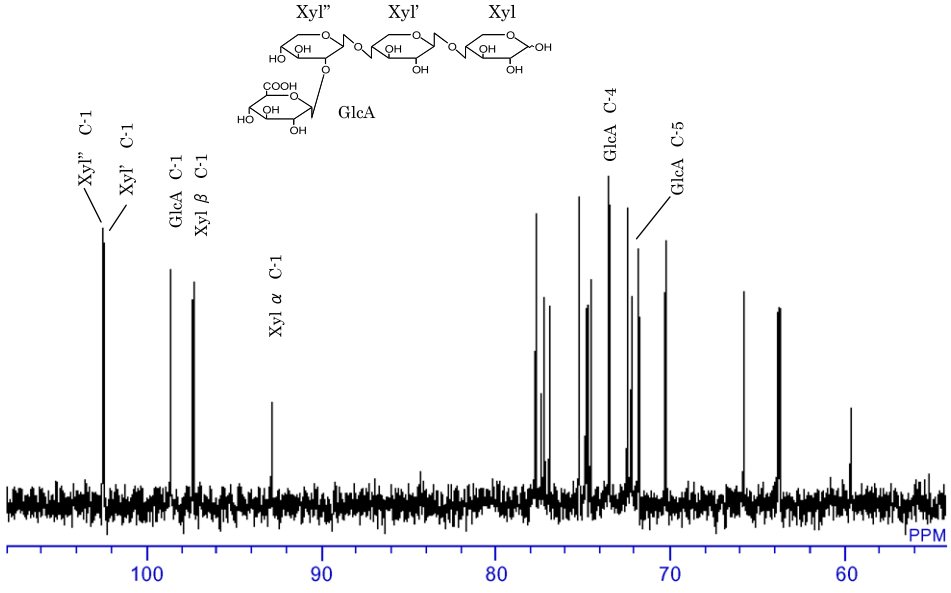

Fig. 5. ^{13}C NMR spectrum of *O*-(α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl (**Fr.2:S2**).

the core xylan based on the yield of each acid from 1 g of xylans (Table 5). In addition to 4-*O*-Me-D-GlcA residues, an appreciable amount of D-GlcA residue was found to be present. The ratio of D-GlcA (including 4-*O*-Me-D-GlcA) to D-Xyl residue is 1:10.4 for the bast xylan and 1:10.7 for the core xylan, respectively, indicating that the content of uronic acids is same level as that of hardwood xylan. Furthermore, the presence of D-GalA residue was noticed. The molar ratio of D-GalA to D-Xyl is 1:119 for the bast xylan and 1:99 for the core xylan. It has been reported that wood xylan has the terminal structural fragment [-*O*-β-D-Xylp-(1 → 3)-*O*-α-L-Rhap- (1 → 2)-*O*-α-D-GalAp-(1 → 4)-D-Xyl]. It can be considered that kenaf xylans have same structure in the backbone. In fact, the acidic oligosaccharide *O*-β-D-Xylp-(1 → 4)-*O*-β-D-Xylp-(1 → 3)-*O*-α-L-Rhap-(1 → 2)-*O*-α-D-GalAp-(1 → 4)-D-Xyl was isolated from the enzymatic hydrolysates of the bast and core xylans. We would like to report this fact in the next paper.

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