

# Lignin characteristics of bast fiber and core in kenaf, bark and wood of paper mulberry and mulberry

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**Abstract** The structural features of bast fiber and core lignins in kenaf (*Hibiscus cannabinus*), bark and wood lignin of paper mulberry (*Broussonetia papyrifera* (L.) Vent × *Broussonetia kazinoki* Sieb.) and mulberry (*Morus bombycis*) were characterized by alkaline nitrobenzene oxidation, ozonation and methoxyl group determination. Björkman lignins were isolated from bast fiber and core, and bark and wood fractions of the plant samples, and structural characteristics were investigated by <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopies. Kenaf bast fiber gave very high molar ratio of syringaldehyde to vanillin (S/V) of alkaline nitrobenzene oxidation products, while methoxyl content was about the same as that of the core fraction. Results of <sup>1</sup>H NMR and <sup>13</sup>C NMR of Björkman lignin suggested the presence of aliphatic fragments in lignins isolated from paper mulberry and mulberry bark, but not in kenaf bast fiber. The lower yield of alkaline nitrobenzene oxidation products from bast fiber and bark might be due to the higher content of condensed structure of lignin compared

to core fraction. Total yield of erythronic (*E*) and threonic (*T*) acids of ozonation products and the molar ratio of erythronic acid to threonic acid (*E/T*) of the bast fibers and bark were lower than the corresponding core and wood fractions, suggesting that the contents of arylglycerol-β-aryl ether intermonomer linkages in the bast fiber and bark lignin were lower than those of the core and wood fractions. Methoxyl content of bark lignin was lower than the corresponding wood lignin. The methoxyl content of the extract-free kenaf bast fiber was similar to that of the core fraction, while the values of paper mulberry and mulberry bark were about one-half of the corresponding wood fractions, respectively. In bark lignins, the methoxyl contents of Klason lignin and Björkman lignin from bark were lower than those of the extract-free barks. This result suggests that the purity of Klason lignin and Björkman lignins of bark may be rather low.

**Keywords** Kenaf bast fiber and core · Mulberry bark and wood · *E/T* ratio · *S/V* ratio · Methoxyl group

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## Introduction

Kenaf (*Hibiscus cannabinus* L.) and paper mulberry (*Broussonetia papyrifera* (L.) Vent × *Broussonetia kazinoki* Sieb.) are important fiber resources for paper making; mulberry (*Morus bombycis* Koidz.), which belongs to the same family, Moraceae, as paper mulberry, is also agriculturally and industrially important plant in agriculture and as feed of silkworms.

Fibers are divided into two large groups, xylary and extraxylary fibers. The latter exists in the various tissue systems outside the xylem [1]. The extraxylary fibers are sometimes combined into a group termed as bast fibers,

such as kenaf (*Hibiscus cannabinus*) bast fiber. Kenaf is an annual plant composed of bast and core [2]. Kenaf bast fiber has attracted a great deal of attention for its lignin characteristics: high syringyl to guaiacyl ratio in the aromatic composition of lignin [3–8]. Besides a high syringyl to guaiacyl ratio, kenaf bast fiber lignin is rich in arylglycerol- $\beta$ -ether linkage with predominantly *erythro* diastereomer [4].

Paper mulberry and mulberry are classified as Moraceae, which belongs to Angiospermae; these are composed of bark and woody xylem. In general, it has been documented that both the ratio of syringyl to guaiacyl nuclei of alkaline nitrobenzene oxidation products and methoxyl content of bark lignin are lower compared with those of woody xylem lignins [9–11]. However, no information on the characteristics of arylglycerol- $\beta$ -aryl ether intermonomer linkages in bark lignin, which is the essential structure to define lignin, is available yet.

In this study, the structural features of bast fiber and core lignins in kenaf, bark and wood lignin from paper mulberry and mulberry, the fibers of which are used as resources of high-quality paper and textile, were investigated. Aromatic structural feature of lignin was investigated by an alkaline nitrobenzene oxidation. The characteristic of *erythro* and *threo* forms of arylglycerol- $\beta$ -aryl ether intermonomer linkage was examined by an ozonation method. Methoxyl group content was also determined to discuss the difference in characteristics of lignin between bast fiber and core, and bark and wood. In addition, lignins prepared by the Björkman's procedure from bast fiber and core, and bark and wood fractions, and the structural characteristics were analyzed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopies.

## Materials and methods

### Materials

Kenaf (*Hibiscus cannabinus* L.) and paper mulberry (*Broussonetia papyrifera* (L.) Vent  $\times$  *Broussonetia kazinoki* Sieb.) were harvested on 27 September 2005 (5 and 6 months old for kenaf and paper mulberry, respectively) at a farm (N33°32', W133°41') in the Kochi Prefecture, Japan. The height and diameter at the base of kenaf were 396 and 3.7 cm, respectively. Samples of kenaf were collected from a height of 190–210 cm (diameter 2.5 cm). Paper mulberry (plant height 250 cm, diameter at the base 2.2 cm), which was budded in the end of March 2005, was sampled from a height of 90–110 cm (diameter 1.5 cm). Mulberry (*Morus bombycis* Koidz.) was collected from the University Farm at Yayoi, Bunkyo-ku, Tokyo (N35°43', W139°45') of the University of Tokyo. The mulberry sample was harvested on 15 September 2005 from new

branches that had started growing during the middle of March 2005. The sample was collected from sections with diameter 1.0–2.0 cm.

Kenaf, paper mulberry and mulberry were separated into bast fiber and core, and bark and wood fractions and freeze-dried as soon as possible after harvest. The freeze-dried sample was ground in a Wiley mill to pass a 420- $\mu\text{m}$  sieve. The ground sample was extracted by boiling with 80% (v/v) ethanol for 1 h (three times), followed by water at 40°C overnight with shaking. The extract-free sample was washed with acetone (three times) and then dried in a vacuum oven at 40°C overnight. The extract-free bast fiber and bark was further extracted with 1% NaOH.

The extract-free sample was ground further using a mini-scale stainless steel vibratory ball mill (Retsch type MM200) for 15 min with a vibration rate of 30 s $^{-1}$  for alkaline nitrobenzene oxidation, ozonation and methoxyl group determination.

### Preparation of Björkman lignin

The bast fiber and core, and bark and wood residues free from 80% ethanol and water extract were ground finely with a stainless steel vibratory ball mill VS-2 (Irie Shokai Co. Ltd., Tokyo, Japan) for 72 h with cooling by tap water flow without using any solvents [12]. Lignin was extracted with dioxane-water (9:1, v/v). The Björkman lignin was prepared by purifying with 80% acetic acid, followed by a mixture of 1,2-dichloroethane and ethanol (2:1, v/v) [13].

### Chemical and spectroscopic analyses

#### Lignin content determination

Lignin content was determined according to the Klason procedure (Tappi Standard T 22 om-88, “acid insoluble lignin in wood and pulp”) with minor modifications. The sample was treated with 72% (w/w) H $_2$ SO $_4$  for 3 h at room temperature. The solution was diluted with water to 3% (w/w) concentration of H $_2$ SO $_4$ . The solution was boiled in an autoclave at 121°C for 30 min. After cooling, the insoluble fraction was collected by filtration using a glass filter. Klason residue was measured gravimetrically after drying at 105°C overnight. Acid-soluble lignin was determined by measuring UV absorption at 205 nm using an extinction coefficient of 110 l g $^{-1}$  cm $^{-1}$  [14]. Klason lignin content was expressed as the summation of Klason residue and acid-soluble lignin.

#### Alkaline nitrobenzene oxidation

Aromatic feature of lignin of the extract-free sample was examined by an alkaline nitrobenzene oxidation [15].

The reaction products were trimethylsilylated with N,O-bis(trimethylsilyl)acetamide (BSA). The products were analyzed by a Shimadzu GC-17A Gas chromatograph using NB1 capillary column (25 m × 0.25 mm id.) equipped with a flame ionization detector (FID). Both injector and detector temperatures were 280°C. The column temperature was kept at 150°C for 10 min and then programmed at 5°C min<sup>-1</sup> to 250°C. Ethyl vanillin was used as an internal standard.

#### Ozonation

Ozonation analysis was carried out according to the scheme of Akiyama et al. [16]. The ozonation products were trimethylsilylated with BSA and analyzed by a Shimadzu GC-18A Gas chromatograph using NB1 capillary column (25 m × 0.25 mm id.) equipped with FID. The both injector and detector temperatures were 280°C. The column temperature was kept at 120°C for 5 min, and then programmed at 4°C min<sup>-1</sup> to 170°C followed by 10°C min<sup>-1</sup> to 280°C. Erythritol was added as an internal standard.

#### Methoxyl content determination

Methoxyl content was determined as described by Jin et al. [17]. The reaction products were analyzed using a CP-Sil 13 CB capillary column (25 m × 0.25 mm id.) chromatograph on a Shimadzu GC-17A system equipped with a flame-ionization detector. The injector and the detector temperatures were 200 and 230°C, respectively. The column temperature was kept at 40°C for 5 min, and then programmed at 10°C min<sup>-1</sup> to 180°C. Iodoethane (60 mg in 20 ml CCl<sub>4</sub>; 1 ml) was added as an internal standard.

#### <sup>1</sup>H NMR and <sup>13</sup>C NMR analyses

The Björkman lignin (around 100 mg) was acetylated with 15 ml of acetic anhydride with 0.1 ml pyridine as a catalyst at room temperature overnight with stirring. The acetylated sample was dissolved in CDCl<sub>3</sub> and <sup>1</sup>H NMR spectrum recorded using a JEOL JNM-A 500 Spectrometer (JEOL, Japan) with 128 scans. The original Björkman lignin (100 mg) was dissolved in (CD<sub>3</sub>)<sub>2</sub>SO (1 ml) and the <sup>13</sup>C-NMR spectrum was recorded using a JEOL JNM-A 500 Spectrometer (JEOL, Japan) with 20000 scan times.

## Results and discussion

### Contents of extracts and Klason lignin

The contents of extracts with 80% (v/v) ethanol and hot water of the kenaf bast fiber were similar to those of the

core fraction, while those of the bark of paper mulberry and mulberry were about twice of those of wood fractions (Table 1). The residues after the extraction of the bast fiber and bark samples were further extracted with 1% (w/v) NaOH aqueous solution. The contents of the alkaline extracts were 11.7, 15.5 and 25.8% of the extracted bast fibers and bark with ethanol and water for kenaf, paper mulberry and mulberry, respectively (Table 1). It was suggested that the extract with 1% NaOH would be composed of low molecular weight phenolic compounds together with oligosaccharides [18].

Klason lignin contents of bast fiber and bark fractions were lower than the corresponding core and wood fractions and increased after extraction with 1% NaOH (Table 2), probably because of removal of considerable amounts of sugar fragments by alkali extraction.

### Aromatic characteristics of bast fiber and bark lignin

Kenaf is an annual plant and fiber in the bast fiber was classified into groups called as a bast fiber. Alkaline nitrobenzene oxidation of coniferous wood lignin yields 24–28% vanillin, based on the Klason lignin, while non-coniferous wood lignin gives a total yield of 35–51% vanillin and syringaldehyde [19–21]. It is well known that the molar ratio of syringyl nuclei (syringaldehyde and syringic acid) to guaiacyl nuclei (vanillin and vanillic acid) (S/V) of non-coniferous wood lignin is 1–4, which is dependent on the wood species [19–21]. Kenaf core and bast fiber gave syringaldehyde and vanillin as major products on alkaline nitrobenzene oxidation, with total products yield of 438 and 369 mmol (200 g lignin)<sup>-1</sup>, respectively. Kenaf bast fiber showed significantly high S/V ratio compared to the core fraction (Table 2). The high S/V ratio of kenaf bast fiber is in agreement with the results in previous papers [2, 4, 6, 22]. Fibers are divided into two

**Table 1** Content of extracts of samples

	Boiling 80% (v/v) ethanol <sup>a</sup>	Water <sup>a</sup> at 40°C	1% (w/v) NaOH <sup>b</sup> at 25°C
Kenaf bast fiber	11.2	4.0	11.7
Paper mulberry bark	16.7	3.2	15.5
Mulberry bark	12.7	4.5	25.8
Kenaf core	11.2	3.2	–
Paper mulberry wood	9.5	1.3	–
Mulberry wood	6.5	1.5	–

Kenaf: *Hibiscus cannabinus* L., Paper mulberry: *Broussonetia papyrifera* (L.) Vent × *Broussonetia kazinoki* Sieb., Mulberry: *Morus bombycis* Koidz

<sup>a</sup> Ethanol and water extracts: % of oven-dried weight of original sample

<sup>b</sup> 1% NaOH extracts: % of ethanol and water extract-free residue

**Table 2** Lignin content, alkaline nitrobenzene oxidation and ozonation products

	Extracted with boiling 80% ethanol and water			Extracted with boiling 80% ethanol and water			Extracted with 1% NaOH		
	Kenaf core	Paper mulberry wood	Mulberry wood	Kenaf bast fiber	Paper mulberry bark	Mulberry bark	Kenaf bast fiber	Paper mulberry bark	Mulberry bark
Klason lignin (% of extract-free sample)									
KR	19.6	21.3	22.4	9.9	12.2	11.2	13.0	15.5	16.8
ASL	2.5	2.0	1.8	3.3	2.3	4.2	2.8	1.6	1.9
Total	22.1	23.3	24.1	13.2	14.5	15.4	15.8	17.1	18.7
Alkaline nitrobenzene oxidation products (mmol (200 g lignin) <sup>-1</sup> )									
H	7	7	7	ND	5	7	ND	5	6
HA	ND	ND	ND	ND	ND	ND	ND	ND	ND
V	151	154	119	68	13	35	76	16	68
VA	5	5	4	2	1	2	4	1	4
S	263	226	173	284	30	15	293	36	27
SA	12	11	6	14	2	1	26	3	2
Total	438	403	309	369	51	60	399	62	108
S/V	1.76	1.49	1.46	4.26	2.29	0.43	3.99	2.29	0.43
Ozonation products (mmol (200 g lignin) <sup>-1</sup> )									
E	212	194	157	125	15	23	110	22	41
T	92	89	77	50	13	20	46	12	32
Total	304	283	234	175	28	43	156	33	73
E/T	2.30	2.18	2.04	2.50	1.15	1.15	2.39	1.83	1.28

KR Klason residues, ASL acid-soluble lignin, H *p*-hydroxybenzaldehyde, HA *p*-hydroxybenzoic acid, V vanillin, VA vanillic acid, S syringaldehyde, SA syringic acid, S/V molar ratio of syringyl nuclei (S+SA) to guaiacyl nuclei (V+VA), E erythronic acid, T threonic acid, E/T molar ratio of erythronic acid to threonic acid

large groups, xylary and extraxylary fibers, which exist in various tissue systems outside the xylem [1]. It has been well documented that the secondary wall of xylary fiber is composed predominantly of syringyl units [23–29]. Kenaf bast fibers exhibit xylary fiber lignin characteristics.

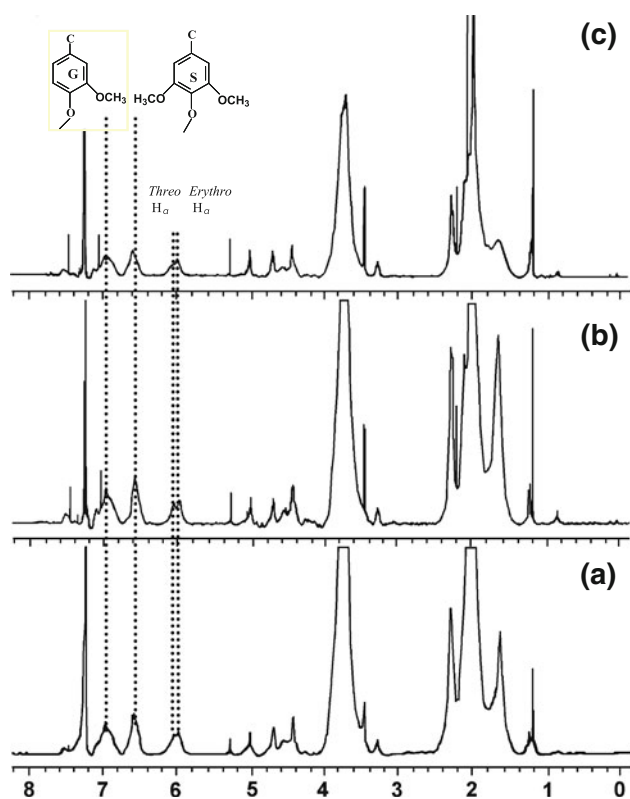
Total yields of alkaline nitrobenzene oxidation products from paper mulberry and mulberry barks were much lower than from corresponding wood fractions (Table 2). The low yields would be due to a high degree of condensation (Fig. 1, 2, 3), but not due to contaminants such as phenolic acids and suberin, because these contaminants also give aromatic aldehydes and aromatic acids by alkaline nitrobenzene oxidation. Alkaline nitrobenzene oxidation provides information on uncondensed guaiacyl and syringyl units present in lignin macromolecules, and the guaiacyl units are usually more condensed than syringyl units [20]. Bark lignins are known to be relatively rich in guaiacyl units, a significant portion of which is in the form of condensed structures resistant to alkaline nitrobenzene oxidation, resulting in low S/V ratio compared with wood lignin [10, 11, 18]. Although paper mulberry and mulberry belong to the same family, Moraceae, paper mulberry bark produced syringaldehyde as the major product (S/V ratio 2.29), while mulberry bark gave vanillin as the major

product (S/V ratio 0.43). The difference between paper mulberry and mulberry bark is caused by different proportion of lignified fiber in bark fractions.

The characteristics of arylglycerol- $\beta$ -aryl ether intermonomer linkage of bast fiber and bark lignin

The presence of arylglycerol- $\beta$ -aryl ether intermonomer linkage in bast fiber lignins was confirmed by acidolysis [30]. The arylglycerol- $\beta$ -aryl ether intermonomer linkage can be either *erythro* or *threo* forms, and the molar ratio of *erythro* to *threo* forms (E/T ratio) is an important structural characteristic of lignin. The aromatic nuclei of lignin are degraded completely by ozone and side-chain parts are released as simple organic acids. Erythronic and threonic acids are obtained from *erythro* and *threo* forms of arylglycerol- $\beta$ -aryl ether structure, respectively. The E/T ratio can be measured on the basis of the yields of these two acids produced by ozonation [16, 31].

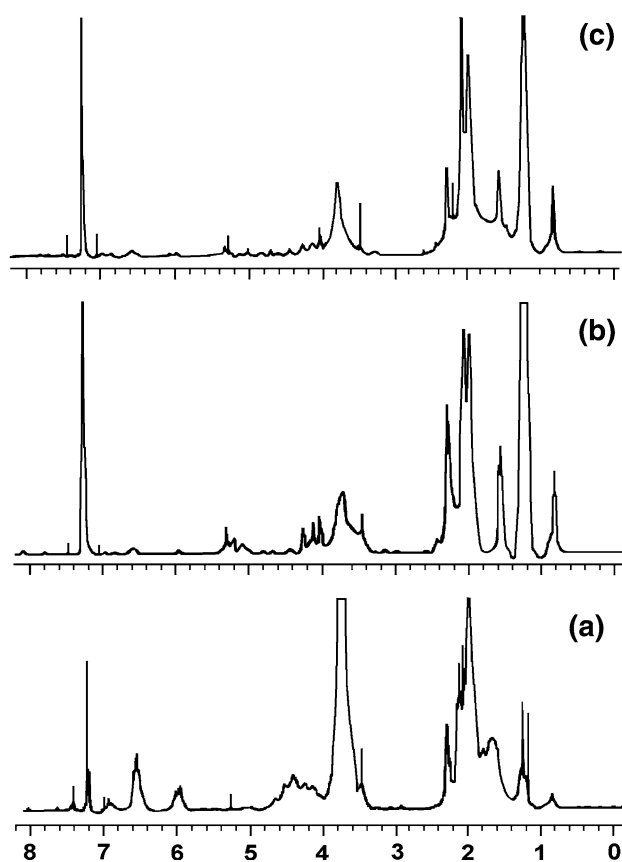
The *erythro* form is the predominant form of arylglycerol- $\beta$ -aryl ether intermonomer linkages in non-coniferous wood lignins, while coniferous wood lignins contain approximately equal amounts of *erythro* and *threo* forms of arylglycerol- $\beta$ -aryl ether intermonomer linkages [16, 31, 32].



**Fig. 1**  $^1\text{H}$  NMR spectra of acetylated lignins prepared by the Björkman's procedure from kenaf core (a), paper mulberry wood (b) and mulberry wood (c) fraction

The *E/T* ratio of the extract-free kenaf core and wood fractions of paper mulberry and mulberry ranged in non-coniferous wood lignin characteristics (Table 2). The *E/T* ratio of kenaf bast fiber was 2.5, suggesting that *erythro* form was the predominant form of arylglycerol- $\beta$ -aryl ether intermonomer linkages in kenaf bast fiber. Ralph also reported that kenaf bast fibers had very high *S/V* ratio, and high in  $\beta$ -ether units with predominantly *erythro* type [4]. In wood lignin, the *E/T* ratio seems to be strictly controlled by the aromatic ring type of phenylpropane units in wood lignin, which reflects stereo regulation during biosynthesis. Namely, lignin with high *S/V* ratio has also high *E/T* ratio [32]. Kenaf bast fibers exhibited high correlation between *S/V* ratio and *E/T* ratio, while the correlation between *S/V* and *E/T* ratio could not be found in paper mulberry and mulberry bark (Table 2).

Akiyama et al. [32] reported that the total yield of erythronic and threonic acids of wood lignin ranged from 220 to 370 mmol (200 g lignin) $^{-1}$ . Total yields of ozonation products from the extract-free kenaf core and paper mulberry and mulberry wood samples were in the range of wood lignins, while those of corresponding bast fibers and bark were significantly low (Table 2). The low yields of ozonation products from the bast fiber and bark lignins suggest lower arylglycerol- $\beta$ -aryl ether intermonomer



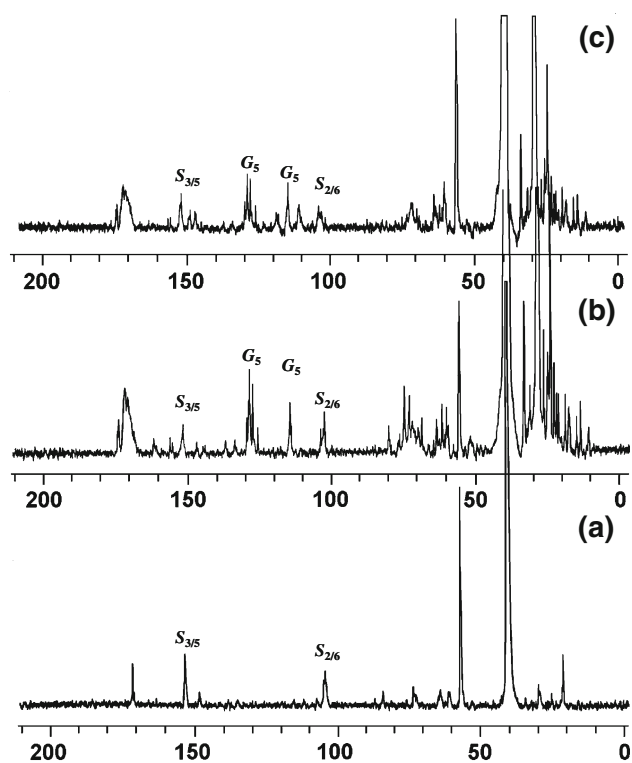
**Fig. 2**  $^1\text{H}$  NMR spectra of acetylated lignins prepared by the Björkman's procedure from kenaf bast fiber (a), paper mulberry bark (b) and mulberry bark (c)

linkages compared with lignin of the core and wood fraction. These results agreed with the results of alkaline nitrobenzene oxidation as being rich in condensed structure in bark lignins.

#### Spectrometric characteristics of bast fiber and bark lignin

Lignins were prepared according to Björkman's procedure from extract-free kenaf core and bast fiber, paper mulberry and mulberry wood and bark fractions to study structural feature of lignin in detail. In  $^1\text{H}$  NMR spectra of acetylated lignins in chloroform- $d_1$ , signals assigned to aromatic protons appear at about  $\delta$  6.90 for gymnosperm lignin (guaiacyl protons), and at  $\delta$  6.90 and 6.65 (syringyl protons) for angiosperm lignin [33]. The acetylated Björkman lignins of the core and wood fractions exhibited two strong signals at the aromatic region,  $\delta$  6.65 and 6.90, in the  $^1\text{H}$  NMR spectra (Fig. 1). In the  $^1\text{H}$  NMR spectra of kenaf bast fiber Björkman lignin, the signal at  $\delta$  6.65 was stronger than that at  $\delta$  6.90 (Fig. 2a), suggesting that the lignin was composed of a relatively high proportion of syringyl than guaiacyl nuclei. For the Björkman lignins from paper mulberry and





**Fig. 3**  $^{13}\text{C}$  NMR spectra of lignins prepared by the Björkman's procedure from kenaf bast fiber (a), paper mulberry bark (b) and mulberry bark (c). Lauric acid originated from fatty acid fragments of suberin

mulberry bark, signals at the aromatic region were very weak, indicating a high proportion of condensed aromatic structure in bark lignin. Results from  $^1\text{H}$  NMR spectroscopy were also in agreement with low total yields of alkaline nitrobenzene oxidation and ozonation products.

The signals at  $\delta$  6.01 and 6.06 of  $^1\text{H}$  NMR spectrum are assigned to the  $\text{H}_\alpha$  in *erythro* form and *threo* form of arylglycerol- $\beta$ -aryl ether intermonomer linkages, respectively [34]. Spectra of acetylated Björkman lignins of three species of core and wood fractions and kenaf bast fiber exhibited stronger signals at  $\delta$  6.01 than those at  $\delta$  6.06, suggesting that the *erythro* form was the predominant form of arylglycerol- $\beta$ -aryl ether intermonomer linkages. The results of  $^1\text{H}$  NMR coincide with those of ozonation. Björkman lignin from bark fractions of paper mulberry and mulberry also gave signals at  $\delta$  6.01 and 6.06, but the intensities were significantly low (Fig. 2b, c). The most prominent signals in the spectra of paper mulberry and mulberry bark lignin appeared around  $\delta$  1.2, which were assigned to methylene protons in saturated aliphatic compounds. The high intensity of signals around  $\delta$  1.2 indicated the presence of a greater number of aliphatic compounds. The existence of saturated aliphatic compounds was also confirmed in  $^{13}\text{C}$  NMR spectra of paper mulberry and mulberry bark lignins (Fig. 3b, c).

Paper mulberry and mulberry bark lignins gave strong signals at  $\delta$  20–35 in the  $^{13}\text{C}$  NMR spectra (Fig. 3b, c), which were assigned to alkyl carbon atoms of saturated aliphatic compounds. The saturated aliphatic compounds originated from suberin. Björkman lignin isolated from bark contains suberin [35]. Suberin consists of aliphatic polyester domains covalently attached to  $\gamma$ -positions of phenylpropane units through ester linkage [36]. The saturated aliphatic carbon and aliphatic ester carbonyl carbon in suberin contributed to signals at  $\delta$  20–35 and  $\delta$  168–175, respectively. The broad signals of  $\delta$  168–175 in  $^{13}\text{C}$  NMR spectra of paper mulberry and mulberry bark lignins suggest the presence of aliphatic ester carbonyl carbons. Kenaf bast fiber lignin gave signals similar to beech wood lignin [37].

The aromatic carbon atoms in lignin give signals in the range between  $\delta$  104 and 160 [38, 39]. The  $^{13}\text{C}$  NMR spectrum of kenaf bast fiber lignin was relatively simple in aromatic region showing strong signals at  $\delta$  105 and 154, which can be assigned to the  $\text{C}_{2/6}$  and  $\text{C}_{3/5}$  in syringyl units (Fig. 3a), respectively [38, 39]. Compared with kenaf bast fiber lignin,  $^{13}\text{C}$  NMR spectra of paper mulberry and mulberry bark lignins were more complicated. In addition to signals at  $\delta$  105 and 154, paper mulberry and mulberry bark lignins gave intensive signals at  $\delta$  116 and  $\delta$  125–131 (Fig. 3b, c). Guaiacyl units of coniferous wood lignin give signals at  $\delta$  116 for the  $\text{C}_5$  [37, 39, 40]. Based on studies of lignin model compounds, the signal of the  $\text{C}_5$  in substituted (condensed) guaiacyl units shifts to downfield and the  $\delta$ -values of the carbon atoms in 5-5 and 5-O-4 structures vary to a great extent depending on their substituents at the  $\text{C}_1$  being etherified at their phenolic groups [41, 42]. The strong signals at  $\delta$  116 and  $\delta$  125–131 suggested that guaiacyl nuclei predominated in paper mulberry and mulberry bark lignins, and the majority of guaiacyl nuclei were condensed. These signals were absent from the kenaf bast fiber lignin, suggesting that major aromatic units of lignin in kenaf bast fibers are the syringyl nuclei.

In addition to the methoxyl signal at  $\delta$  56.1, three main signals around  $\delta$  73.1–73.3,  $\delta$  85.0–86.2 and  $\delta$  60.6–62.0 in the  $\delta$  50–90 region of  $^{13}\text{C}$  NMR spectra can be assigned to carbon atoms at the  $\text{C}_\alpha$ ,  $\text{C}_\beta$  and  $\text{C}_\gamma$  in arylglycerol- $\beta$ -aryl ether intermonomer linkages, respectively [38, 43]. Three clear signals around  $\delta$  84–85,  $\delta$  71–74 and  $\delta$  60–61 in the spectrum of kenaf bast fiber lignin suggest that arylglycerol- $\beta$ -aryl ether intermonomer linkages are predominant. In addition to these signals, the Björkman lignin of kenaf bast fiber was characterized by the signal at  $\delta$  62–64, which would be due to acetylation at the  $\text{C}_\gamma$ . It was reported that signals of the  $\text{C}_\gamma$  in arylglycerol- $\beta$ -aryl ether intermonomer linkages shift downfield  $\delta$  2–4 by acetylation [42, 44]. Ralph reported that the lignin of kenaf bast fiber was characterized by arylglycerol- $\beta$ -aryl ether intermonomer

linkages with predominantly *erythro* isomer and extensive acetylation at their  $C_\gamma$  position [4]. It was indicated by the absence of corresponding  $C_\beta$  signals and significantly complicated signals at  $\delta$  69–75 and  $\delta$  59–65 that arylglycerol- $\beta$ -aryl ether intermonomer linkage are not the main intermonomer linkages in lignin of the paper mulberry and mulberry bark (Fig. 3b, c).

#### Methoxyl group content of lignin

The methoxyl group is a major functional group of lignin, which could serve as a useful means to provide an approximate measure of the lignin content [17]. Methoxyl content of the extract-free kenaf bast fiber was similar to that of the core fraction, while the value of paper mulberry and mulberry bark was about one-half of the corresponding wood fraction (Table 3). The methoxyl content of bark lignin was lower than the corresponding wood lignin [9–11, 30, 45].

Methoxyl contents of the extract-free core and wood fractions were similar to the value of Björkman lignins and those of the corresponding Klason residues (Table 3). This result was in agreement with previous studies. Obst reported that methoxyl contents of Björkman lignin and Klason lignin coincided with the methoxyl content of the whole fiber and whole ray cells [46]. Methoxyl contents of 21 coniferous wood and non-coniferous wood meals were similar with corresponding Klason lignins in the range of 0.88–1.66 equiv.·(200 g lignin)<sup>-1</sup> for wood meal and 0.97–1.49 for Klason lignin, respectively [32]. However, the methoxyl contents of both Klason and Björkman lignins of the bark samples were lower than those of corresponding extract-free bast fiber samples (Table 3). These results may suggest that the purity of Klason lignin and Björkman lignins from bark as lignin is rather low. The bark contains substantial quantities of low methoxyl content phenolic acids such as *p*-hydroxybenzoic and *p*-coumaric acids, and suberin, which is composed of guaiacyl propane units with long aliphatic chain [47, 48], but these nonlignin components would contribute to low methoxyl content in bark Klason lignin and Björkman lignin.

**Table 3** Methoxyl group content (mol/200 g lignin) of samples

	Original sample	Klason residue	Björkman lignin
Kenaf core	1.42	1.18	1.13
Paper mulberry wood	1.26	1.15	1.13
Mulberry wood	1.03	1.05	1.06
Kenaf bast fiber	1.28	0.96	1.02
Paper mulberry bark	0.46	0.14	0.19
Mulberry bark	0.53	0.26	0.40

#### Conclusion

1. Kenaf bast fiber gave very high molar ratio of syringaldehyde to vanillin (*S/V*) of alkaline nitrobenzene oxidation products, while methoxyl content was about the same as that of the core fraction. The results of <sup>1</sup>H NMR and <sup>13</sup>C NMR of Björkman lignin suggested the presence of aliphatic fragments, probably of suberin, in lignins isolated from paper mulberry and mulberry bark, but not in kenaf bast fiber.
2. The lower yield of alkaline nitrobenzene oxidation products from bast fiber and bark might be due to the higher content of condensed structure of lignin compared to core and wood fractions. The total yield of erythronic (*E*) and threonic (*T*) acids of ozonation products and the molar ratio of erythronic acid to threonic acid (*E/T*) of the bast fibers and bark were lower than corresponding core and wood fractions, suggesting that the contents of arylglycerol- $\beta$ -aryl ether intermonomer linkages in the bast fiber and bark lignin were lower than those of the core and wood fractions.
3. Methoxyl content of bark lignin was lower than the corresponding wood lignin. Methoxyl content of the extract-free kenaf bast fiber was similar to that of the core fraction, while the values of paper mulberry and mulberry bark were about one-half of the corresponding core fractions, respectively. In bark lignins, the methoxyl contents of Klason lignin and Björkman lignin were lower than those of the extract-free bark. This result suggests that the purity of Klason lignin and Björkman lignins of bark may be rather low.
4. Thus, the structural characteristic of lignin was significantly different between bast fibers of kenaf and bark of paper mulberry, although bast fiber of kenaf and bark of paper mulberry are used effectively as resources for paper making. In addition, those of mulberry were also different from paper mulberry, though both plants belong to the same family, Moraceae.

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