

## NOTE

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## Effect of alkali extraction on the lignin monomeric composition in *Eucalyptus*

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**Abstract** The effect of alkali extraction on the lignin monomeric composition was examined in *Eucalyptus camaldulensis* and *E. globulus* by thioacidolysis using extractive-free samples as a control. Results showed that the effect on *Eucalyptus* is different among species and among sample positions in the trunk, although a small amount of lignin is solubilized during the extraction in all samples. In addition, it was proved that lignin extracted by the alkali extraction is not always guaiacyl-rich, probably relating to the original lignin monomeric composition, which depends on the sample species or the sample position in the trunk.

**Key words** *Eucalyptus camaldulensis* · *E. globulus* · Alkali extraction · Lignin · Lignin monomeric composition

### Introduction

Polyphenols called “kino” interfere with the content determination of lignin using the Klason method for *Eucalyptus* species.<sup>1</sup> Therefore, the standard method recommends that the samples be preextracted with 0.1 N NaOH solution at 100°C for 1 h (Australian Pulp and Paper Industry and Technology Standard P2m-73) to remove kino.

The structural changes of lignin before and after the alkali extraction have been investigated by the lignin syringaldehyde/vanillin (S/V) ratio using nitrobenzene oxidation in *Eucalyptus* species.<sup>2,3</sup> Garland et al. compared the lignin S/V ratio between the original and samples treated with 0.1 N NaOH at 100°C for 1 h.<sup>2</sup> They found a 2%–8% in-

crease in the S/V ratio in both extracted heartwood and sapwood, and that the extract had a 70% lower S/V ratio than the extracted wood of *E. regnans* and *E. diversicolor*. They concluded that more guaiacyl units were solubilized during the alkali extraction. In contrast, Kawamura and Bland compared the S/V ratio between alcohol/benzene-extracted samples and those subsequently treated with 0.125 N NaOH at 100°C for 80 min.<sup>3</sup> They did not find any difference in *E. alba*, *E. tereticornis*, *E. camaldulensis*, *E. ovata*, or *E. regnans*. From these results, using original wood as a control, nonlignin components such as diarylpropane, which can be extracted by alcohol/benzene extraction, possibly affect the lignin S/V ratio.<sup>4</sup> Consequently, extractive-free samples as the control and an analytical method unaffected by nonlignin components are required to investigate the effect of alkali extraction on the lignin monomeric composition.

In this study we examined the effect of alkali extraction on the lignin monomeric composition in *E. camaldulensis* and *E. globulus* by thioacidolysis, which specifically cleaves  $\beta$ -O-4 bonds using extractive-free samples as the control.

### Materials and methods

#### Materials

About 2 × 2 × 6 cm blocks were sampled from three positions in the trunks of two trees. The samples were 0–2 cm from the pith and 1.3 m above the ground (“low inner”), 6–8 cm and 1.3 m (“low outer”), and 0–2 cm and 9.3 m (“high inner”) for *E. camaldulensis*. For *E. globulus*, the samples were 0–2 cm and 1.3 m (“low inner”), 8–10 cm and 1.3 m (“low outer”), and 0–2 cm and 13.3 m (“high inner”). Both trees were 14 years old, grown in western Australia. They were planted and grown at an annual average temperature of 15°–16°C and an annual average rainfall of 1000 mm. The estimated whole-tree heights and diameters at breast height after debarking for each tree were as follows: *E.*

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*camaldulensis* 15.2 m and 18.8 cm, respectively; and *E. globulus* 19.9 m and 24.4 cm, respectively.

### Alkali extraction

Twenty mesh pass wood meals were extracted by a Soxhlet apparatus with a sequence of toluene/ethanol, ethanol, and water to prepare extractive-free samples utilized as the control.<sup>5</sup> About 100 mg extractive-free samples were weighed and alkali-extracted in 5 ml of 0.1 N or 0.25 N NaOH solution at 40°, 70°, and 100°C for 1 h with occasional agitation. After the reaction, the samples were filtered with a pre-weighed 2-ml glass thimble (medium porosity) and washed with 10 ml of warm water at the extraction temperature. The extract was neutralized with 2 N HCl solution and freeze-dried to be used for subsequent lignin analysis. The extraction residues were washed further with 10 ml of warm water at the extraction temperature, 3.3 ml of 10% acetic acid solution, and 20 ml of warm water at the extraction temperature. Then they were high vacuum-dried to determine the alkali extractives and were used for the lignin analysis. Alkali extractive content was expressed on the

basis of the control value. The experiment was duplicated, and the data were averaged.

### Lignin determination

The lignin content and lignin syringyl/guaiacyl ratio as the lignin monomeric composition were determined by the modified Klason method called the "small-scale method" and the modified thioacidolysis method described elsewhere, respectively.<sup>5,6</sup> The lignin content in the extract was based on the amount of thioacidolysis products. Lignin content was expressed on the basis of the control value. The experiment was duplicated, and the data were averaged.

## Results and discussion

The results for *E. camaldulensis* and *E. globulus* are summarized in Tables 1 and 2, respectively. In *E. camaldulensis* the lignin content in the residues after alkali extraction from all samples decreased with the increase in treatment sever-

**Table 1.** Effect of alkali extraction on the lignin monomeric composition in *Eucalyptus camaldulensis*

Sample and trait	Control	Treated					
		0.1 N			0.25 N		
		40°C	70°C	100°C	40°C	70°C	100°C
<b>Low inner</b>							
Alkali extractives (%)	–	4.1	12.3	18.8	6.1	15.8	22.9
Lignin (%)							
Residues	33.2	29.3	27.6	25.1	28.8	27.0	23.8
Extract	–	0	0.2	0.5	0.1	0.2	0.2
S/G ratio							
Residues	1.95	2.18	2.44	2.58	2.36	2.44	2.82
Extract	–	0.90	1.88	2.11	0.37	1.91	1.85
<b>Low outer</b>							
Alkali extractives (%)	–	2.5	17.8	11.8	3.1	9.8	12.5
Lignin (%)							
Residues	30.1	29.0	25.2	26.3	28.7	27.6	27.1
Extract	–	0.1	0.1	0.5	0.1	0.2	0.2
S/G ratio							
Residues	1.82	2.25	2.19	2.27	1.99	2.11	2.31
Extract	–	1.44	1.48	1.36	1.39	1.32	1.26
<b>High inner</b>							
Alkali extractives (%)	–	2.5	10.7	16.5	3.4	12.5	19.0
Lignin (%)							
Residues	29.0	27.8	26.7	24.9	27.8	26.4	23.5
Extract	–	0.2	0.3	0.4	0.1	0.3	0.3
S/G ratio							
Residues	2.03	2.39	2.19	2.68	2.33	2.46	2.45
Extract	–	1.69	1.81	1.54	1.69	1.54	1.56

Control comprises extractive-free samples. The 0.1 N and 0.25 N are the mean concentrations of the NaOH solution

Low inner, Low outer, and High inner represent samples from a position 0–2 cm from the pith at 1.3 m above ground, 6–8 cm at 1.3 m, and 0–2 cm at 9.3 m, respectively

S/G ratio is the lignin syringyl/guaiacyl molar ratio

Residues and extracts are after alkali extraction

All data are expressed on the basis of the control values

**Table 2.** Effect of alkali extraction on the lignin monomeric composition in *Eucalyptus globulus*

Sample and trait	Control	Treated					
		0.1 N			0.25 N		
		40°C	70°C	100°C	40°C	70°C	100°C
<b>Low inner</b>							
Alkali extractives (%)	–	5.9	8.1	12.4	7.0	9.2	15.5
Lignin (%)							
Residues	24.6	22.2	22.0	22.2	21.7	21.9	19.2
Extract	–	0.3	0.4	0.7	0.2	0.6	0.5
S/G ratio							
Residues	3.57	3.61	3.65	3.55	3.62	3.47	3.62
Extract	–	3.12	3.20	3.17	3.20	3.55	3.23
<b>Low outer</b>							
Alkali extractives (%)	–	4.1	6.0	10.3	5.1	7.2	12.7
Lignin (%)							
Residues	21.4	21.6	20.9	20.9	20.2	20.1	18.4
Extract	–	0.2	0.3	0.4	0.1	0.4	0.6
S/G ratio							
Residues	3.02	3.08	3.19	3.28	3.36	3.29	3.64
Extract	–	2.08	2.18	2.24	2.30	2.28	2.24
<b>High inner</b>							
Alkali extractives (%)	–	3.9	7.1	11.3	6.3	8.1	13.8
Lignin (%)							
Residues	25.6	23.4	24.6	22.5	22.3	21.8	20.6
Extract	–	0.2	0.5	0.7	0.3	0.4	0.7
S/G ratio							
Residues	3.02	3.11	3.13	3.37	3.34	3.35	3.52
Extract	–	2.59	2.43	2.62	2.44	2.49	2.63

Low inner, Low outer, and High inner represent samples from a position 0–2 cm from the pith at 1.3 m above ground, 8–10 cm at 1.3 m, and 0–2 cm at 13.3 m, respectively. See note in Table 1 also

ity, which caused an increase in the amount of alkali extractives. This increase is possibly caused by the progressive removal of polyphenols, which disturbs lignin quantification.<sup>1</sup> On the other hand, the alkali extract certainly contained thioacidolysis products. In other words, lignin derived from  $\beta$ -O-4 bonds was solubilized during the alkali extraction, although the amount does not represent all of the solubilized lignin. A small amount of lignin expressed on the basis of the control values may be solubilized, as reported by Garland et al.<sup>2</sup> Furthermore, the amount of the solubilized fraction increased with a rise in treatment temperature.

Lignin S/G ratios in the residues after alkali extraction in all samples generally increased with a rise in treatment temperature, although the magnitude of the S/G ratio change depended on the sample. Furthermore, lignin S/G ratios in treated samples were higher than those in the control. Here, thioacidolysis is not affected by the residual amount of polyphenols after alkali extraction because the polyphenols do not contain  $\beta$ -O-4 bonded cinnamyl alcohol.<sup>7</sup> Consequently, it is suggested that more guaiacyl units are solubilized than syringyl units during the alkali extraction. This is in accordance with the report in which the effect of alkali extraction on the S/V ratio was examined by extractive-free wood as a control in tropical species (i.e., *Duabanga grandiflora* and *Callicarpa poilanei*).<sup>8</sup> On the other hand, lignin S/G ratios in the alkali extract increased with a rise in treatment temperature in the case of 0.1 N

NaOH treatment, although they were rather stable in the case of 0.25 N NaOH treatment, except the “low inner” samples at 40°C. Lignin content in the extract increased with a rise in treatment temperature in the case of 0.1 N NaOH treatment. Compared to this, lignin content did not vary from 70°C to 100°C in the case of 0.25 N NaOH treatment and was often lower than that with 0.1 N NaOH, although 0.25 N NaOH treatment is more severe than that with 0.1 N NaOH. Considering that the lignin S/G ratio in residues under the 0.25 N NaOH condition is generally higher than those under 0.1 N NaOH, it is concluded that guaiacyl units would be solubilized more than the syringyl units by the alkali extraction; however, solubilized guaiacyl units would be decomposed further and were not detected by thioacidolysis.

In *E. globulus*, the difference of lignin content among the residues of alkali extraction and the control was small regardless of the treatment condition. This may be due to the smaller amount of polyphenols in *E. globulus* than in *E. camaldulensis*, as no difference in colored heartwood formation was observed between *E. globulus* and *E. camaldulensis*.<sup>9</sup> However, a small amount of lignin was certainly solubilized, as lignin assessed by thioacidolysis products was detected in the alkali extract.

Lignin S/G ratios in both the residue and the extract from the “low inner” samples did not change with the treatment condition. In contrast, in other samples lignin S/G ratios in the residue increased with the increase in treat-

ment severity, although those in the extract were rather stable. Furthermore, lignin content in the extract increased with the increase in treatment severity. Moreover, the extract's lignin S/G ratios were always lower than those in the residue. Based on these results, it is concluded that guaiacyl and syringyl units were solubilized almost homogeneously, and that guaiacyl units were decomposed more readily than syringyl units under various conditions for the "low inner" samples. In contrast, more guaiacyl units were solubilized than syringyl units with the increase in treatment severity. However, solubilized guaiacyl units are believed to decompose more readily than syringyl units with the increase in treatment severity, producing similar lignin S/G ratios in the extract regardless of the treatment conditions for other samples. Consequently, within-tree variation was observed in the behavior of lignin monomers during the alkali extraction for *E. globulus*. This may be caused by the structural difference of lignin, as "low inner" samples had a higher lignin S/G ratio than others. Further study is required.

It is concluded that the effect of alkali extraction on the lignin monomeric composition in *Eucalyptus* is different among species and among sample positions in the trunk, although a small amount of lignin is solubilized during the extraction in all samples. In addition, it has been proven that lignin extracted by alkali extraction is not always guaiacyl-rich, probably relating to the original lignin monomeric composition, which depends on the sample species or the sample position in the trunk.

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