

NOTE

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## Semi-quantitative method to evaluate the $\alpha$ -carbonyl content in lignin

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**Abstract** A method to estimate the content of  $\alpha$ -carbonyl structures in lignin was developed. This method consists of two successive treatments: NaBD<sub>4</sub> treatment of pulp to reduce an  $\alpha$ -carbonyl structure in lignin, and nitrobenzene oxidation. NaBD<sub>4</sub> was used to convert an  $\alpha$ -carbonyl structure to a deuterium-labeled hydroxymethine structure. The ratio of D-vanillin [(HO)(H<sub>3</sub>CO)C<sub>6</sub>H<sub>3</sub>CDO] to H-vanillin [(HO)(H<sub>3</sub>CO)C<sub>6</sub>H<sub>3</sub>CHO] or that of their syringyl analogues obtained by nitrobenzene oxidation was used as the measure of the content of  $\alpha$ -carbonyl structure. Model experiments demonstrated that when sodium hydroxide was used as alkali for the nitrobenzene oxidation, the retention of deuterium at the side chain  $\alpha$ -position was very low due to the displacement of deuterium with hydrogen by an unknown reaction mechanism. In order to depress this unexpected displacement, the reaction conditions of the nitrobenzene oxidation were modified. The modified nitrobenzene oxidation employs 0.5 mol/l of lithium hydroxide as a reaction medium instead of 2.0 mol/l sodium hydroxide. By this modification, this method could successfully trace the formation and the degradation of the  $\alpha$ -carbonyl structure in milled wood lignins.

**Key words** Hydrogen peroxide · Bleaching · Lignin ·  $\alpha$ -Carbonyl structure · Nitrobenzene oxidation · Sodium borodeuteride

### Introduction

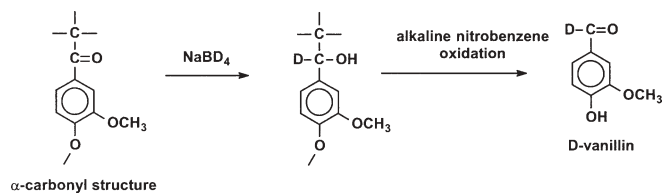
Because of the environmental concern over chlorinated organic compounds being discharged from pulp bleaching mills, the introduction of oxygen-based reagents for bleaching of kraft pulp has become essential for the industry to become environmentally compatible. Hydrogen peroxide is one of those reagents and is widely introduced in the nonchlorine bleaching sequences. In spite of its industrial importance, however, the roles of hydrogen peroxide in the degradation and the removal of lignin during bleaching of chemical pulp are still obscure. Heuts and Gellerstedt<sup>1</sup> evaluated its ability to degrade  $\beta$ -O-4 structures. However, this type reaction did not seem to play a decisive role in the degradation of residual lignin because of the quite low content of  $\beta$ -O-4 structures in kraft pulp residual lignin.<sup>2</sup>

It is widely assumed that the  $\alpha$ -carbonyl structure is one of the key structures of lignin that govern the reactivity of lignin toward nonchlorine bleaching chemicals such as oxygen, ozone, and hydrogen peroxide. The reactivity of lignin aromatics toward oxygen and ozone is very low when its side chain carries  $\alpha$ -carbonyl structure.<sup>3,4</sup> Therefore, the introduction of an  $\alpha$ -carbonyl structure during oxygen and ozone bleaching may depress the delignification reaction.<sup>5</sup> On the other hand, an  $\alpha$ -carbonyl structure provides a site for nucleophilic attack by hydroperoxy anion,<sup>6,7</sup> and thus, may play an important role when lignin is treated by alkaline hydrogen peroxide. This type reaction results in the fragmentation of lignin.<sup>6,8,9</sup>

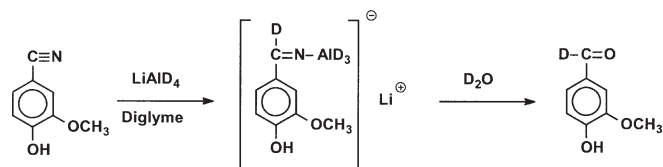
In spite of such an important contribution, however, there has been no precise information obtained about the content of  $\alpha$ -carbonyl structure in residual lignin in pulps. For this purpose, the authors examined a scheme consisting of two successive treatments (Fig. 1). The first step of our method is the reduction by sodium borodeuteride (NaBD<sub>4</sub>). By this treatment, an  $\alpha$ -carbonyl structure in lignin should be converted into an  $\alpha$ -hydroxymethine structure in which the C=O linkage is labeled as a C–D linkage. Then the sample containing lignin with the “ $\alpha$ -D labeled” side chain

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**Fig. 1.** Scheme for the estimation of  $\alpha$ -carbonyl structure in lignin



**Fig. 2.** Synthetic scheme for preparation of D-vanillin

is subjected to nitrobenzene oxidation. If the aldehyde group of a nitrobenzene oxidation product (vanillin or syringaldehyde) has a form of -CDO instead of -CHO, this product should be derived from an  $\alpha$ -carbonyl structure that had been converted into the  $\alpha$ -hydroxymethine structure by the preceding  $\text{NaBD}_4$  reduction.

This article describes the establishment of the scheme shown in Fig. 1 by the use of model compounds and its application to variously treated milled wood lignins (MWLs). Although the method has been applied only to the soluble samples such as model compounds and MWLs in the present report, it seems to be applicable to the solid samples such as residual lignins remaining in pulps, which will be described in our next article.

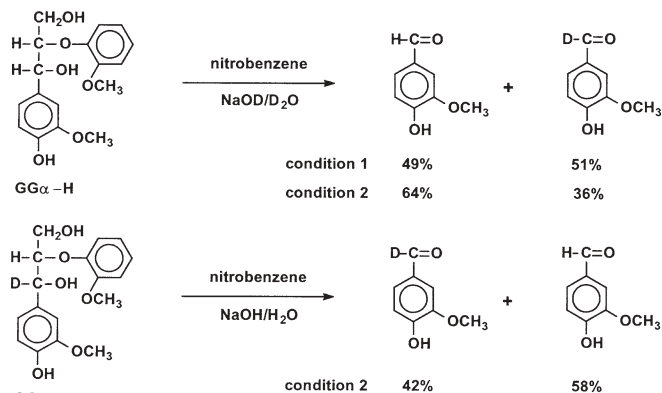
## Materials and methods

### Materials

$\alpha$ -(2-Methoxyphenoxy)- $\beta$ -hydroxy-propioquaiacone ( $\text{GG}\alpha=\text{O}$ ) was synthesized by Hosoya's method.<sup>10</sup> MWLs were prepared from Japanese cedar and birch. Vanillin-formyl-*d* (D-vanillin) was synthesized by  $\text{LiAlD}_4$  reduction of vanillonitrile according to a scheme used for the synthesis of vanillin from vanillonitrile (Fig. 2). DDQ-MWL ( $\alpha$ -carbonyl-enriched MWL) was prepared by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation of MWL.<sup>11</sup> Other reagents were commercially available.

### Nitrobenzene oxidation of vanillin and $\text{GG}\alpha\text{-H}$ with NaOD

Nitrobenzene oxidation of guaiacylglycerol- $\beta$ -guaiacyl ether ( $\text{GG}\alpha\text{-H}$ , see Fig. 3) in NaOD/ $\text{D}_2\text{O}$  was performed under several reaction conditions. Alkali concentra-



**Fig. 3.** Proportion of H-vanillin and D-vanillin in the products of alkaline nitrobenzene oxidation of two lignin model compounds. Condition 1: alkali concentration 2.0 mol/l, reaction time 2 h, temperature 170°C. Condition 2: alkali concentration 0.5 mol/l, reaction time 1 h, temperature 170°C. As alkali, NaOD in  $\text{D}_2\text{O}$  was used for  $\text{GG}\alpha\text{-H}$ , and NaOD in  $\text{D}_2\text{O}$  was used for  $\text{GG}\alpha\text{-D}$

tions were 2M, 1M, and 0.5M. After treatment, the reaction products were extracted and subjected to gas chromatography-mass spectrometry (GC-MS) as the acetone solution without derivatization. The retention of  $\alpha$ -hydrogen in vanillin was calculated based on the height of two peaks at  $m/z$  152 and 153.

### Nitrobenzene oxidation of $\text{GG}\alpha\text{-D}$ with different metal hydroxides

Deuterium-labeled GG ( $\text{GG}\alpha\text{-D}$ , see Fig. 3) was obtained by  $\text{NaBD}_4$  reduction of  $\text{GG}\alpha=\text{O}$ .  $\text{GG}\alpha\text{-D}$  was subjected to nitrobenzene oxidation with several metal hydroxides, such as lithium, sodium, potassium, cesium, and barium hydroxide. The alkali concentrations were 0.5M, 0.1M, and 0.05M, reaction temperature was 170°C, and the reaction time was 1 h. After treatment, the reaction products were extracted and subjected to GC-MS, and the retention of  $\alpha$ -deuterium was calculated by the same manner as above.

### Hydrogen peroxide treatment of $\alpha$ -carbonyl enriched MWL

$\alpha$ -Carbonyl enriched Japanese cedar MWL (DDQ-MWL, 5 mg) was treated with 5 mmol hydrogen peroxide in 30 ml of aqueous sodium hydroxide at pH 10 and pH 13 at 70°C for 60 min. After the hydrogen peroxide treatment, residual hydrogen peroxide was decomposed by the addition of sodium dithionite. The reaction mixture was neutralized and then concentrated to dryness. The dried matter was subjected to the scheme 1 to evaluate the amount of  $\alpha$ -carbonyl structure. As a control run, DDQ-MWL was treated without hydrogen peroxide at pH 13.

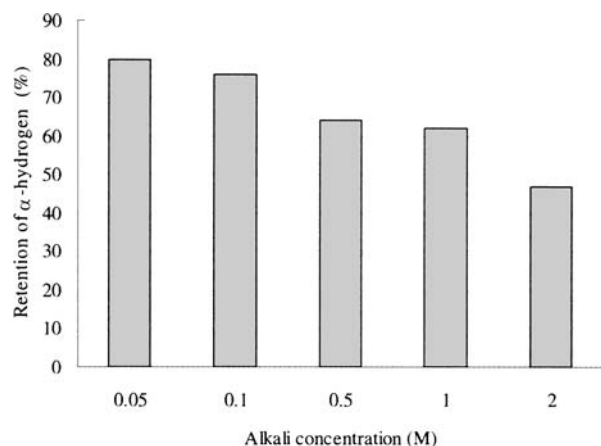
The same treatment was applied to birch DDQ-MWL, but 7.5 mg of sample was used for one experiment.

## Results and discussion

Exchange of hydrogen in methine group at side chain  $\alpha$ -position during nitrobenzene oxidation

In order to estimate the content of  $\alpha$ -carbonyl structure according to the scheme shown in Fig. 1, the deuterium atom introduced to the  $\alpha$ -position of side chain has to be retained in vanillin after nitrobenzene oxidation. Therefore, it was necessary to examine whether the hydrogen atom in the methine group at the side chain  $\alpha$ -position or in the formyl group of vanillin is retained. For this purpose, a model compound, guaiacylglycerol- $\beta$ -guaiacyl ether (GG $\alpha$ -H, Fig. 3), was subjected to nitrobenzene oxidation in NaOD/D<sub>2</sub>O system under the usual conditions (reaction time: 2 h, temperature: 170°C, alkali concentration: 2.0 mol/l). Contrary to our expectation, the hydrogen atom at the  $\alpha$ -position was not stable. A considerable amount of D-vanillin was produced from GG $\alpha$ -H by nitrobenzene oxidation, indicating that the hydrogen atom at the  $\alpha$ -position exchanged with deuterium. Although the extent of H $\rightarrow$ D exchange measured here could be different from that of D $\rightarrow$ H exchange due to the isotope effect, it was obvious that the deuterium atom introduced to the  $\alpha$ -position of the side chain would not be well retained in the final product, vanillin, when the method in Fig. 1 is applied.

The effect of reaction conditions on the H $\rightarrow$ D exchange was analyzed in more detail by subjecting GG $\alpha$ -H to nitrobenzene oxidation in the NaOD/D<sub>2</sub>O system under different conditions. Because the yield of vanillin (data not shown) reached its maximum after 1 h, a reaction time of 1 h seemed to be necessary. The alkali concentration significantly affected the H $\rightarrow$ D exchange (Fig. 4). The H $\rightarrow$ D exchange was effectively suppressed by lowering the concentration of sodium hydroxide, but the concentration of 0.5 mol/l was necessary in order not to sacrifice the vanillin yield. If alkali concentration was under 0.5 mol/l, the yield of vanillin decreased to about one tenth of that of normal conditions. When the reaction temperature was



**Fig. 4.** Retention of hydrogen in formyl group of vanillin obtained by nitrobenzene oxidation of GG $\alpha$ -H under different alkali (NaOD) concentration (170°C, 1 h)

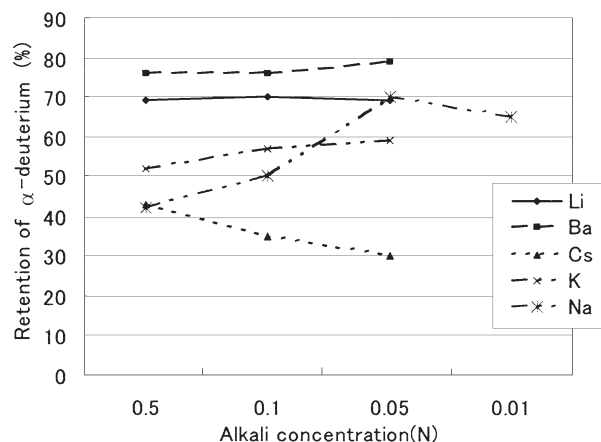
changed in the range between 150° to 190°C, both the H $\rightarrow$ D exchange ratio and vanillin yield were not affected (data not shown).

Based on these observations, we selected the following reaction conditions to apply for GG $\alpha$ -D: alkali concentration; 0.5 M, reaction time; 1 h, reaction temperature; 170°C (condition 2 in Fig. 3). When GG $\alpha$ -H was subjected to nitrobenzene oxidation in a NaOD/D<sub>2</sub>O system under these conditions, the retention of  $\alpha$ -H was about 65% (Fig. 4). However, when GG $\alpha$ -D was subjected to the reaction in NaOH/H<sub>2</sub>O under the same conditions, the retention of  $\alpha$ -D was only about 40%. These data demonstrated that deuterium introduced to label  $\alpha$ -carbonyl structure was not stably retained during nitrobenzene oxidation without sacrificing the vanillin yield when sodium hydroxide was used as alkali.

Suppression of D $\rightarrow$ H exchange by the use of different metal hydroxides

In a preliminary experiment, it was found that the C-H exchange could be suppressed when sodium hydroxide was displaced with other alkalis. Based on these findings, several kinds of alkali were applied for the nitrobenzene oxidation of GG $\alpha$ -D and the degree of D $\rightarrow$ H exchange was examined (Fig. 5). Alkalis examined were lithium hydroxide, potassium hydroxide, cesium hydroxide, and barium hydroxide. When barium hydroxide was used as alkali, D $\rightarrow$ H exchange was most effectively suppressed. The second best was lithium hydroxide. From a practical point of view, barium hydroxide was not favorable because of the formation of insoluble barium carbonate, which may disturb the quantitative extraction of oxidation products. Therefore, lithium hydroxide was selected as the alkali for nitrobenzene oxidation.

The retention of  $\alpha$ -deuterium in vanillin by the use of lithium hydroxide was about 70% (Fig. 5). The established conditions for nitrobenzene oxidation were as follows: reaction time 1 h, temperature 170°C, and 0.5 mol/l lithium



**Fig. 5.** Retention of deuterium in formyl group of vanillin obtained by nitrobenzene oxidation of GG $\alpha$ -D by the use of several metal hydroxides as alkali (170°C, 1 h)

**Table 1.** Proportion of D-vanillin among total vanillin obtained by nitrobenzene oxidation of variously treated milled wood lignins (MWLs)

	Original MWL	DDQ-MWL	DDQ-MWL treated by		
			H <sub>2</sub> O <sub>2</sub> at pH 13	H <sub>2</sub> O <sub>2</sub> at pH 10	Alkali at pH 13
Japanese cedar	5.7%	28%	6.8%	20%	22%
Birch	0.31%	33%	7.4%	25%	32%

Proportion of D-vanillin (%) = (D-vanillin)/(D-vanillin + H-vanillin) × 100

hydroxide. When a lignin model compound (Guaiacylglycerol  $\beta$ -guaiacyl ether: GG) and MWL were subjected to nitrobenzene oxidation under these conditions, the yields of expected products were more than 90% of those under the regular condition of 2.0 mol/l sodium hydroxide.

#### Application to variously treated MWLs

MWLs of Japanese cedar and birch were subjected to the oxidation by DDQ in order to increase the  $\alpha$ -carbonyl content (DDQ-MWL). DDQ-MWLs were then treated with hydrogen peroxide at pH 10 or 13. As a blank run, DDQ-MWL was simply treated with alkali at pH 13 in the absence of hydrogen peroxide. Those MWLs were then subjected to the scheme shown in Fig. 1 under the conditions established in the previous section.

Because the deuterium was introduced to the  $\alpha$ -position of the side chain as C–D bonding by the NaBD<sub>4</sub> reduction of the  $\alpha$ -carbonyl structure, the proportion of D-vanillin among total vanillin (D-vanillin and H-vanillin) in nitrobenzene oxidation products could be used as the indication of the  $\alpha$ -carbonyl content if D–H exchange is effectively suppressed. As summarized in Table 1, the proportion of D-vanillin obtained for DDQ-MWLs was much higher than those obtained for the corresponding original MWLs. The yield of vanillin was decreased by about 25% during treatment. The higher proportion must be explained by the enrichment of  $\alpha$ -carbonyl structure in MWLs by DDQ-oxidation.

Reactivity of hydrogen peroxide toward the carbonyl structure was expected to depend on alkalinity during the treatment, because of the enhanced reactivity of ionized hydrogen peroxide.<sup>6,7</sup> The results of decreased  $\alpha$ -carbonyl structure by hydrogen peroxide (Table 1) coincide well with this general theory of organic chemistry. Hydrogen peroxide seemed to be effective to remove  $\alpha$ -carbonyl structure from lignin only when it was applied under sufficiently alka-

line conditions, pH 13. The data for syringaldehyde is not shown in this article because of the lack of a calibration curve for D-syringaldehyde.

These results not only confirmed the usefulness of the new method proposed in the present report, but also indicate the effectiveness of hydrogen peroxide to degrade carbonyl structures when present in ionized form.

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