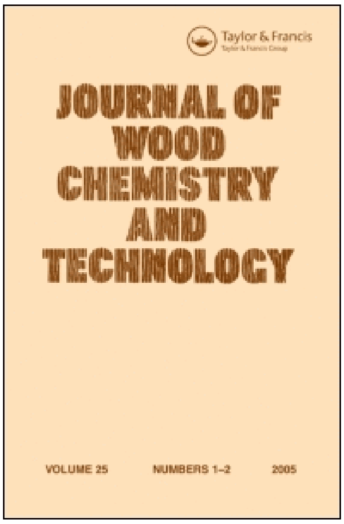

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# Interference of Carbohydrates in the Determination of the Methoxyl Content of Lignin in Woody Samples

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# Interference of Carbohydrates in the Determination of the Methoxyl Content of Lignin in Woody Samples

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**Abstract:** To evaluate the effect of the presence of carbohydrates on the determination of the methoxyl content of lignin, the mechanism of acid-catalyzed reaction of lignin methoxyl groups with iodide ion to form methyl iodide was evaluated using carbohydrate and lignin model compounds. Not only the iodide concentration but also the acid concentration was found to significantly affect the rate of formation of methyl iodide. This fact and Hammett plots of the relative reaction rates observed for several model compounds suggest an $S_N2cA$ reaction mechanism for methyl iodide formation. Carbohydrates interfered with the rate of formation of methyl iodide, probably by acting as Lewis bases. Interestingly, the study also revealed that a certain amount of methyl iodide could arise from carbohydrates even when the carbohydrates did not contain methoxyl groups as potential precursors to methyl iodide.

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These sources of error were significant when methoxyl content was determined for samples with low lignin content.

**Keywords:** Lignin, methoxyl content, carbohydrates, hydriodic acid, methyl iodide

## INTRODUCTION

The methoxyl content is one of the fundamental characteristics of lignin. The methoxyl content in lignin and its change could be used to characterize the amount, reaction, as well as the structure of lignin. For example, the methoxyl content of a lignin sample can provide much more reliable information on the syringyl/guaiacyl ratio[1,2] than nitrobenzene oxidation or thioacidolysis even though the possible presence of p-hydroxyl units always causes some uncertainty. The change in the methoxyl content in lignin during a chemical treatment is a good indication of the structural changes occurring in the lignin such as oxidation of its aromatic nuclei.[3–5] Recently, a finding of a low methoxyl content in the Klason lignin residue obtained from tree leaves led to the conclusion that "the true lignin content" of these leaves is much lower than indicated by the Klason lignin value.[6,7]

The methoxyl content determination often uses the reaction of the methyl ethers with hydriodic acid (HI) with the methyl iodide generated being determined in a variety of ways.[8–15] In spite of its usefulness, the determination of methoxyl content is always accompanied by some uncertainties. If the methyl iodide would be produced from some unknown substrates, or if the lignin methoxyl cleavage would be suppressed, errors in the determination could occur. When the methoxyl content is determined for a sample with low lignin content, the potential interfering effect of the carbohydrates cannot be ignored. Some hemicelluloses may contain methoxyl groups in the form of methyl ethers. Consequently, the methoxyl group in the carbohydrates could produce methyl iodide upon reaction with HI resulting in an overestimation of the lignin methoxyl content. In addition, preliminary experiments have indicated that carbohydrates can produce methyl iodide upon reaction with HI even when the carbohydrates do not contain any methoxyl groups.[16] To our knowledge, this phenomenon does not seem to have been addressed previously. In spite of these uncertainties, this method of methoxyl determination has been employed for pulp samples low lignin content.[3–5]

As described in this report, we have tried to evaluate the effect of carbohydrates on the quantitative determination of methoxyl groups in lignin. For this purpose, an improved method, similar to Baker's,[15] was developed that employs a closed system for the treatment of the sample with HI and the determination of the methyl iodide by gas chromatography (GC). The mechanism of methyl iodide formation during the determination was examined and possible sources of error were evaluated. Also, the formation of methyl iodide from carbohydrates, which do not contain methoxyl groups, and its effect on the methoxyl determination were quantitatively evaluated.

## EXPERIMENTAL

### Materials

All reagents used in this study were commercially available and reagent grade. Japanese cedar (*Cryptomeria japonica* D.Don) and Japanese birch (*Butula maximowiczii* Regel) wood meals, passing 80-Mesh, were extracted with ethanol-benzene (1:2, v/v) for 6 h. Parts of the wood meals were milled 120 h according to Björkman's method[17] to prepare milled wood lignins (MWL).

Softwood and hardwood kraft lignins (KL) were obtained from Tokai Pulp and Paper. The KLs were each dissolved in dioxane-water (9:1, v/v) and the solutions were drop-wise to ethyl ether. The precipitates were filtered, dried, and used as the kraft lignin samples.

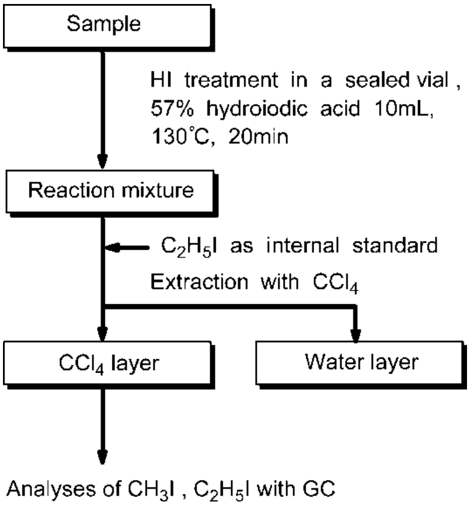
$\alpha$-Cellulose was prepared from fully bleached softwood kraft pulp (Kasugai Mill, Oji Paper). The pulp was extracted with acetone 1 h at 60°C to eliminate extractives, followed by sulfuric acid treatment (pH 1.3, 5% pulp consistency) for 3 h at 90°C to eliminate hexenuronic acids.[18] The resulting pulp was treated with 17.5% aqueous sodium hydroxide according to TAPPI standard T 203.[19] Acetic acid was not used to wash the $\alpha$-cellulose to exclude the possibility of producing methyl iodide from the residual acetic acid during the methoxyl determination.

### Methoxyl Determination

The procedure for determination of lignin methoxyl content was basically the same as Baker's method[15], as shown in Scheme 1. The sample (wood meals and lignin samples 10 mg, carbohydrates 100–1000 mg) was mixed with 57% HI (10 mL) in a vial with a pressure-resistant seal and held at 130°C for 20 min. Immediately after the treatment, the vial was cooled in ice-water and 1 mL of carbon tetrachloride containing ethyl iodide as an internal standard was added by syringe through the sealed cap. The reaction product (methyl iodide) and the internal standard were extracted with 10 mL of carbon tetrachloride, dried over anhydrous sodium sulfate, and analyzed by GC.

### HI Treatment of Samples with Various Concentrations of Acid and Iodide

Vanillin (10 mg, 0.066 mmol) was reacted with 10 mL of two different series of solutions by the procedure described earlier. The reaction solutions were as follows: (i) Solutions in which the HI concentration varied from 0.38 to



***Scheme 1.*** Method for determination of methoxyl content.

7.6 mol/L, but the iodide concentration was kept constant value at 7.6 mol/L by addition of sodium iodide. (ii) Mixed solutions of HI and hydrochloric acid, in which the iodide concentration was varied from 0.38 to 7.6 mol/L but the acid concentration was kept constant at 7.6 mol/L.

The methyl iodide that was generated was analyzed as described in the general scheme. and analyzed with GC.

### Treatments of Lignin Model Compounds with HI

Several lignin model compounds were treated with 5 mL of HI. The reaction time was 1–30 min, and the reaction temperatures were 85°C and 130°C. The methyl iodide generated was analyzed as described earlier. The lignin model compounds used in these experiments were as follows: Guaiacyl model compounds; vanillin, vanillyl alcohol, vanillic acid, creosol, and guiaicol. Syringyl model compounds; syringol, sinapic acid, and syringaldehyde. Other model compounds; methoxy *p*-quinone, methanol, methyl butyrate, and methyl *β*-D-glucopyranoside. Two wood meals, Japanese cedar (softwood) and Japanese birch (hardwood), and several monosaccharides as carbohydrate model compounds were also used.

### Treatment of α-Cellulose with Hydriodic Acid in the Presence or Absence of Vanillin

Vanillin and *α*-cellulose were treated with 5 mL of HI for 20 min at 130°C under the following two conditions. (i) Vanillin (10 mg, 0.066 mmol) and

$\alpha$-cellulose (0–2 g) were treated together. (ii) $\alpha$-cellulose (0.2–2 g) was treated alone.

### Deuteroiodic Acid Treatment

Because 57% of deuteroiodic acid was not available, the reaction medium was prepared on site by dissolving 0.5 g of sodium iodide in 1 mL of 7.6 mol/L deuterochloric acid (heavy water solution). The concentration of the iodide in the prepared reaction medium was still lower than 57% aqueous HI. Thus, the reaction time was increased to 1 h from the usual 20 min to compensate for the lower iodide concentration. The reaction products were extracted with carbon tetrachloride and subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

### Gas Chromatography

In GC analysis, a Shimadzu GC-14B chromatograph equipped with a flame ionization detector was used. The capillary column used was a Varian CP-SIL 13CB for halocarbons (25 m × 0.32 mm i.d., thickness: 1.2 μm). The flow rate was 1.1 mL helium/min. The column temperature was held at 40°C for 5 min, and then raised at 10°C/min to 180°C. The injection port and detector port temperatures were 200°C and 230°C, respectively.

In GC-MS analysis, a Shimadzu QP-5000 mass spectrometer equipped with a Shimadzu GC-17A chromatograph was used. Chemical ionization (CI) was used to detect parent ions. Other analytical conditions were the same as those of the GC analysis.

## RESULT AND DISCUSSION

### Mechanism of Methyl Iodide Formation

It is well known that the cleavage of ether linkages with concentrated HI is either an $S_N1$ or $S_N2$ reaction mechanism. Primary and secondary ether linkages usually react via an $S_N2$ mechanism and tertiary ethers typically react via an $S_N1$ mechanism. Thus it can be assumed that a methyl ether linkage of lignin reacts with HI via an $S_N2$ reaction. In this case, HI dissociates into ions in the aqueous media, so the reaction rate is thought to be dependent on the iodide concentration ($[I^-]$) and the substrate concentration. In order to observe the effect of $[I^-]$ on the formation of methyl iodide, vanillin was treated with HI solutions in which $[I^-]$ was varied but the acid concentration ($[H^+]$) was kept constant by the addition of hydrochloric acid (Figure 1). The effect of $[I^-]$ is clearly observed.

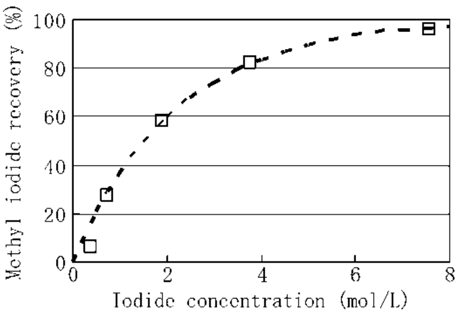


*Figure 1.* Effect of iodide concentration on methyl iodide recovery from vanillin (acid concentration 7.6 mol/L).

However, further experiments showed that the formation of methyl iodide was significantly affected not only by [I$^-$] but also by the [H$^+$]. Vanillin was treated for 20 min in solutions in which the HI concentration ([H$^+$]) were varied but [I$^-$] were kept at constant value of 7.6 mol/L by the addition of sodium iodide (Figure 2). These results demonstrate that in order to achieve effective conversion of methoxyl to methyl iodide a rather high concentration of acid is required.

In order to explain the effects of both [H$^+$] and [I$^-$], an $S_N2cA$ reaction mechanism was postulated (Scheme 2). As illustrated in Scheme 2, the oxygen atom in a methoxyl group is first protonated to produce the conjugate acid, and then the iodide ion attacks the methyl carbon from side opposite to the ether linkage. The most significant difference between a simple $S_N2$ reaction and an $S_N2cA$ reaction may be the effect of functional groups present on the aromatic ring. In case of an $S_N2$ reaction, electron withdrawing groups present on the aromatic ring will facilitate the attack of the iodide ion on the methyl carbon. Contrary to this, in the case of an $S_N2cA$ reaction, the initial protonation step will be suppressed by the presence of

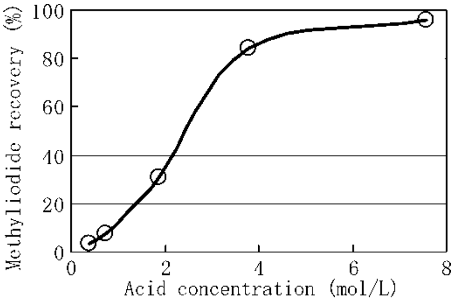
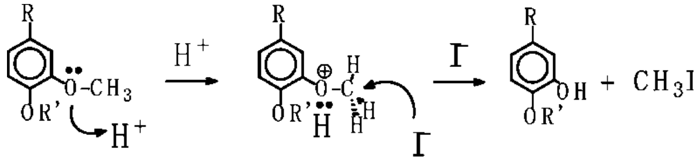


*Figure 2.* Effect of acid concentration on methyl iodide formation from vanillin (iodide concentration 7.6 mol/L).



*Scheme 2.* Mechanism of reaction between ligin methoxyl and hydriodic acid.

an electron withdrawing groups on the aromatic ring, and the attack of iodide on the methyl carbon will thus be retarded. From this point of view, the effect of ring substituents on the formation rate was examined by the use of several model compounds.

Table 1 shows the relative rate of methyl iodide formation from each model compound. In case of the guaiacyl models, as predicted from the $S_N2cA$ reaction mechanism, formation of methyl iodide from compounds with electron donating groups were faster than those possessing electron withdrawing groups. In these five guaiacyl models, a satisfactory Hammett's law relation was observed (Figure 3).

Compared with the guaiacyl models, the syringyl models reacted much more slowly. This was probably due to the low rate of the second methoxyl cleavage reaction in the syringyl compounds but further investigation was not made. Other compounds, such as methanol, methyl ester (methyl butyrate), and methylated saccharide (methyl $\beta$-glucopyranoside) reacted faster than the various lignin model compounds and quantitatively generated methyl iodide. Thus, if such types of compounds are present in a lignin sample, they will affect the methoxyl group determination, and should be removed completely before methoxyl analysis.

***Table 1.*** Relative formation rate of methyl iodide from lignin model compounds

| Model compounds | Relative reaction rate |
|---|---|
| Creosal | 1.36 |
| Vanillylalcohol | 1.04 |
| Guaiacol | 1.00 |
| Vanillic acid | 0.47 |
| Vanillin | 0.42 |
| Syringaldehyde | 0.12 |
| Syringol | 0.31 |
| Sinapic acid | 1.04 |
| Methanol | 7.25 |
| Methoxy *p*-quinone | 0.72 |
| Methyl butyrate | 3.62 |
| Methyl $\beta$-glucopyranoside | 5.80 |

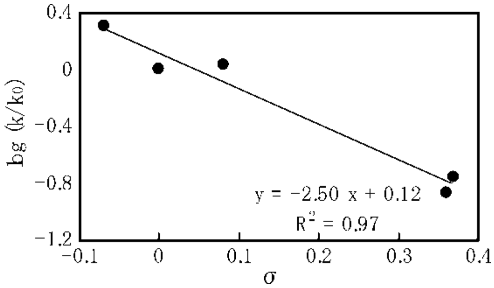


***Figure 3.*** Hammett plot for methoxyl cleavage in guaiacyl model compounds.

## Effect of Co-existing Carbohydrates on the Determination of Methoxyl Groups

As [H$^+$] significantly affects the formation of methyl iodide (Figure 2), any factors that possibly cause a change in the proton concentration may affect the methyl iodide formation. Therefore, it seemed necessary to evaluate the effect of carbohydrates because they could act as a Lewis base and lower the effective proton concentration. In order to examine the effect of carbohydrates, vanillin was treated with HI in the presence of different amounts of $\alpha$-cellulose. Figure 4 shows the formation of methyl iodide from the mixture of vanillin and $\alpha$-cellulose or from $\alpha$-cellulose alone during the HI treatment.

When the mixture of vanillin and $\alpha$-cellulose was treated (solid line of Figure 4), the effect of $\alpha$-cellulose was bi-functional. In the presence of relatively low amounts of $\alpha$-cellulose, methyl iodide formation was suppressed as the $\alpha$-cellulose increased, but when the $\alpha$-cellulose increased beyond a certain

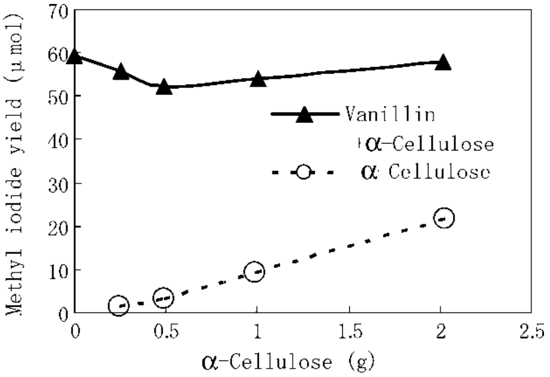


***Figure 4.*** Effect of $\alpha$-cellulose on methyl iodide formation from vanillin (vanillin content 0.066 mmol).

point (0.5 g), methyl iodide formation from the mixture increased with the increase of $\alpha$-cellulose. The initial decrease in methyl iodide formation was probably due to a decrease in effective proton concentration due to the presence of the $\alpha$-cellulose. On the other hand, the increase in methyl iodide formation from a certain point seemed to indicate the formation of methyl iodide from the $\alpha$-cellulose itself. In order to confirm this, $\alpha$-cellulose was treated with HI. As is shown by a dotted line in Figure 4, $\alpha$-cellulose produced methyl iodide in proportion to its amount. The relation between the amount of $\alpha$-cellulose and methyl iodide yield is indicated as:

$$\text{methyl iodide } (\mu\text{mol}) = 11.5 \times \alpha - \text{cellulose(g)} - 2.0 \quad (R^2 = 0.997)$$

It is well known that certain carbohydrates, in particular hemicelluloses, contain methoxyl groups that can interfere with the determination of methoxyl groups in lignins. However, the origin of the methyl iodide observed in Figure 4 does not seem to be associated with methoxyl groups present in the carbohydrate because the rate of formation of methyl iodide from this $\alpha$-cellulose is quite low. Gran suggested that glucose and other carbohydrates give rise to "apparent" methoxyl contents on acid catalyzed reactions but formation of methyl iodide in a methoxyl analysis was not confirmed.[20,21] In this study, methyl iodide was detected and "apparent" methoxyl content might be truly generated from carbohydrates.

Table 2 shows the time-dependency of methyl iodide formation from a variety of samples. In the samples containing methoxyl groups the formation of methyl iodide was very rapid and did not increase in a detectable scale when the treatment was conducted longer than 5 min. Conversely, when the $\alpha$-cellulose was treated, the formation of methyl iodide was very slow but

***Table 2.*** Time dependency of methyl iodide formation from various samples upon HI treatment

| Samples reaction time (min) | Methyl iodide yield (mmol/g) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 30 | 60 | 120 |
| Japanese cedar | | | | | | |
| Wood meal | 1.3 | 1.3 | 1.4 | 1.4 | 1.4 | 1.3 |
| MWL | 3.5 | 3.5 | 3.6 | 3.5 | 3.4 | 3.3 |
| Japanese birch | | | | | | |
| Wood meal | 1.6 | 1.6 | 1.8 | 1.8 | 1.8 | 1.7 |
| MWL | 5.0 | 5.0 | 4.9 | 4.8 | 4.7 | 4.3 |
| Softwood kraft lignin | 3.1 | 3.2 | 3.1 | 3.1 | 3.1 | 3.0 |
| Hardwood kraft lignin | 4.7 | 4.7 | 4.6 | 4.5 | 4.7 | 4.4 |
| $\alpha$-Cellulose ($\times 10^{-3}$) | 2.4 | 3.1 | 5.5 | 4.6 | 7.2 | 10.8 |

MWL, milled wood lignin.

the yield continuously increased with reaction time (Table 2). If the origin of methyl iodide produced from $\alpha$-cellulose were methoxyl groups, such a phenomenon would likely not have been observed. Also this phenomenon suggested that the $\alpha$-cellulose may have reacted in a complicated way to form methyl iodide.

To confirm the origin of the unexpectedly produced methyl iodide, deuteroiodic acid treatment was conducted. If methyl iodide is produced from methoxyl-group-free compounds, it may contain a deuterium as C-D linkage, because all the carbon atoms except methyl carbons carry at the most only two hydrogens. If this is the case, the methyl iodide originating from methoxyl groups could be distinguished from that produced from methoxyl-group-free compounds, when the reaction is carried out by the use of deuteroiodic acid in heavy water. Namely, methyl iodide produced from methoxyl-group-free compounds will contain deuterium and, thus, the $m/z$ of its parent ion peak may be bigger than that of ordinary methyl iodide, 142. First, it was confirmed that $m/z$ of the parent ion of methyl iodide did not change from $m/z$ 142 by 1 h of deuteroiodic acid treatment of methyl iodide itself (Figure 5). This result indicated that deuterium-hydrogen exchange in methyl iodide had not occurred during the treatments. Compounds carrying methoxyl groups such as lignin samples and lignin model compounds also produced methyl iodide with $m/z$ 142 as a predominant peak (Figure 6). Thus, it was confirmed that those originating from methoxyl groups are supposed to show their parent ion peak at $m/z$ 142. On the contrary to these, when carbohydrates were subjected to the reaction, the produced methyl iodide showed quite different $m/z$ distribution (Figure 5). The $m/z$ values of these parent ion peaks were higher than 142.

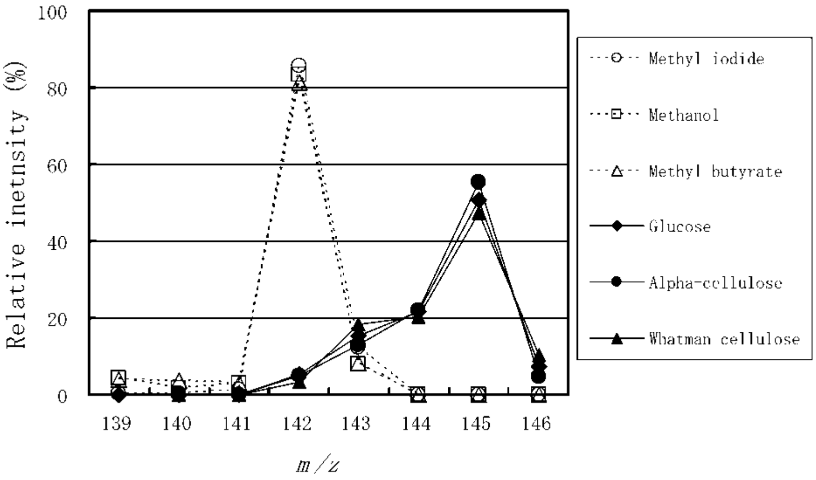


***Figure 5.*** $m/z$ distributions of parent ion peaks of methyl iodide with various origins.

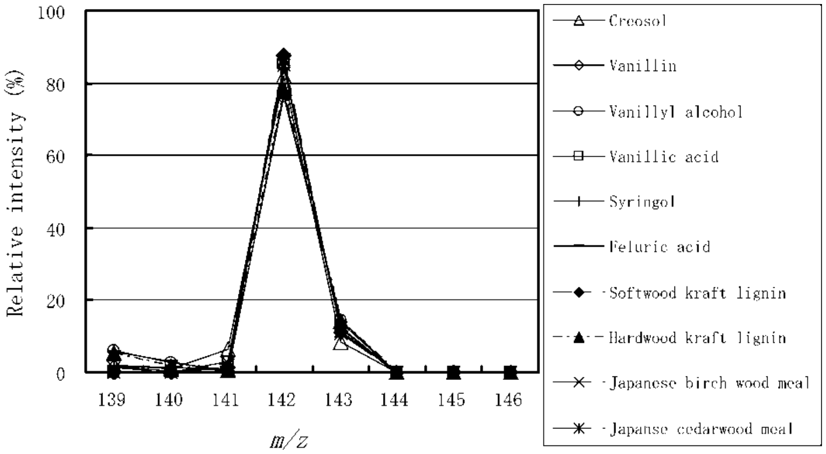


***Figure 6.*** $m/z$ distribution of parent ion peaks of methyl iodide produced-from lignin samples.

This result proved that methyl iodide could be produced from non-methyl carbons. From this fact, the methyl iodide formation from carbohydrates did not seem to be derived from methoxyl group-containing contaminants but from methoxyl group-free compounds, namely, carbohydrates themselves. The $m/z$ value of the most intensive peak observed was $m/z$ 145, which indicated that methyl iodide was produced in the form of $CD_3I$. Although the formation mechanism of $CD_3I$ is unknown, it is obvious that the $CD_3I$ formation requires not only the incorporation of deuterium from the solvent but also some deuterium-hydrogen exchange steps. Table 2 shows that the yield of methyl iodide from $\alpha$-cellulose was not high. In Table 3, yields of methyl iodide from monosaccharides as carbohydrate model compounds are shown. From a monosaccharide unit, $1.1-1.5 \times 10^{-3}$ (molecules) of methyl iodide were liberated in 20 min of treatment under standard condition and a

***Table 3.*** Methyl iodide yields in the hydriodic acid treatment of monosaccharides and $\alpha$-cellulose

| | Methyl iodide ($\mu$mol/g) | Methyl iodide molecules from 1 molecules ($\times 10^{-3}$) |
|---|---|---|
| Glucose | 7.8 | 1.4 |
| Galactose | 8.6 | 1.5 |
| Arabinose | 8.6 | 1.3 |
| Xylose | 9.5 | 1.4 |
| Mannose | 6.0 | 1.1 |
| $\alpha$-cellulose | 5.5 | — |

Reaction time: 20 min, reaction temperaature: 130°C.

significant difference among different monosaccharides was not found. About 6–11 (μmol/g) of methyl iodide was produced from 1.0 g of carbohydrates during the methoxyl group determination. Therefore, methyl iodide produced from carbohydrates may not interfere with the determination of methoxyl group content in lignin when the lignin content of the sample was high. However, when a sample with low lignin content such as a chemical pulp was analyzed, the formation of methyl iodide from carbohydrates that do not contain methoxyl groups cannot be ignored. As indicated earlier, if about 6–11 (μmol/g) of methyl iodide is produced from 1 g of carbohydrates during the methoxyl group determination in a chemical pulp, a softwood chemical pulp with 2% lignin content (kappa number about 15) may produce about 100 μmol of methyl iodide liberated from lignin, while 6-11 μmol are produced from carbohydrates.

## CONCLUSION

Even when carbohydrates do not contain any methoxyl groups, their presence could lead to error in the determination of methoxyl group content in lignin by two different ways; first, carbohydrates decrease the effective proton concentration by acting as a Lewis base, suppressing the formation of methyl iodide from the methoxyl groups according to the $S_N2cA$ mechanism. This effect will result in an underestimation of methoxyl group content. Second, carbohydrates themselves can produce methyl iodide of non-methoxyl group origin, which will result in an overestimation of methoxyl group in the lignin. In samples with low lignin content such as low kappa number pulps, those effects cannot be ignored.

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