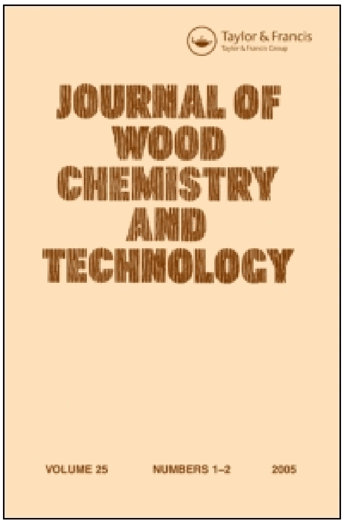

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Matsumoto, Yuji , Ishizu, Atsushi , Nakano, Junzo and Terasawa, Kazuo(1984) 'Residual Sugars in Klason Lignin', Journal of Wood Chemistry and Technology, 4: 3, 321 — 330
To link to this Article: DOI: 10.1080/02773818408070652
URL: http://dx.doi.org/10.1080/02773818408070652

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 4(3), 321-330 (1984)

RESIDUAL SUGARS IN KLASON LIGNIN

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Dedicated to Prof. J. L. McCarthy
on the occasion of his 70th birthday

ABSTRACT

The Klason method--dissolution of carbohydrates in sulfuric acid--has been widely used for the determination of lignin content in woody materials. It was an unsolved problem, however, whether Klason lignin still contains carbohydrates. We observed previously that Klason lignin becomes almost completely soluble in water after ozone treatment. In this work, the residual sugar content of Klason lignin was determined on the basis of the above observation.

Water-soluble lignin was prepared by ozone treatment (25 and 45 min) of finely-crushed Klason lignin obtained from spruce and birch wood meals. After hydrolysis with 2N-trifluoroacetic acid followed by sodium borohydride reduction, residual sugars were determined by GLC as their alditol acetates.

Klason lignin ozonated for 25 min contained 0.75% and 0.95% neutral sugars for spruce and birch, respectively. Most of the residual sugars were derived from hemicelluloses. Because ozone degrades carbohydrates, the prolonged ozonation was found to decrease the content of residual sugars. Therefore, the values obtained here are the minimum ones for the residual sugar content in Klason lignin.

INTRODUCTION

The Klason method, and its modifications, are widely used for the determination of lignin content in woody materials[1].

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These methods commonly consist of two successive acid treatments: pretreatment with concentrated sulfuric acid, and refluxing in dilute sulfuric acid. Treating conditions were designed to obtain the minimum yield and the maximum methoxyl content of resulting residue.

The inability to dissolve Klason lignin in any solvent makes it difficult to analyse its structural characteristics. On the basis of recent model experiments, Yasuda et al[2] revealed the occurrence of condensation reactions of lignin during the sulfuric acid treatment. Acid-catalyzed condensation reactions of lignin were discussed in detail by Lai and Sarkanen[3]. On the other hand, the authors[4] found that ozonation and then saponification of Klason lignin, as well as of milled wood lignin and other isolated lignins, gave erythronic and threonic acids as predominant products, indicating that some of the arylglycerol-β-aryl ether structures remain intact in Klason lignin. As for the Klason lignin itself, it is an interesting problem whether or not it contains carbohydrates and their denaturated products. Our previous work revealed that ozone preferentially decomposes lignin into aliphatic carboxylic acids[4], thus making it possible to study this subject. This report deals with the determination of residual sugars in Klason lignin according to the scheme shown in Fig. 1.

EXPERIMENTAL

Preparation of Klason Lignin

Spruce (*Picea jezoensis Carr.)* and birch(*Fagus crenata Blume*) wood meals (40-60 mesh, 2.5g) were treated with 50 mL of 72% sulfuric acid at 20°C for 4 h and then refluxed in 1960 mL of 3% sulfuric acid for 2 h. Residues (Klason lignin) were recovered by filtration on 1G-3 (spruce) and 1G-4 (birch) glass filters and washed enough with water.

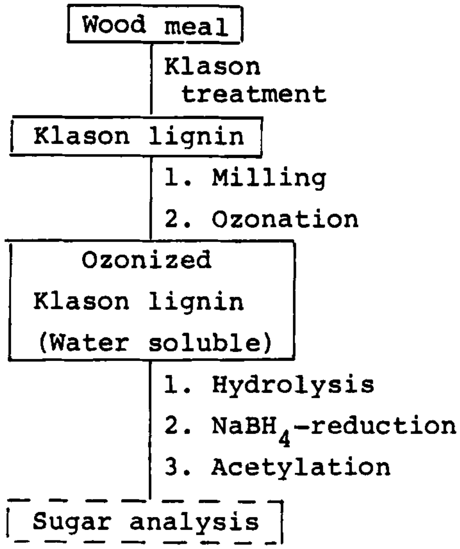

Fig. 1 Scheme for the determination of residual sugars in Klason lignin.

Ozonation of Klason Lignin

Klason lignin was finely crushed in a vibration ball mill. Finely-crushed Klason lignin (100 mg) was suspended in 25 mL of acetic acid containing water and methanol ($AcOH:H_2O:MeOH=16:3:1$, v/v) and treated with ozone (3% concentration in O_2, 0.5 L/min) for 25 and 45 min at 0°C with stirring.

Sugar Analysis

After removal of excess ozone and the solvent under reduced pressure, the residue was subjected to acid hydrolysis with 5 mL of 2N-trifluoroacetic acid (TFA) at 121°C for 1 h[5]. After removal of the TFA, the acid hydrolyzed residue was reduced with excess sodium borohydride overnight and acetylated with pyridine and acetic anhydride. Insoluble residue remaining after acetylation was removed by filtration, and the filtrate was concentrated, dissolved in chloroform, and washed with water. The chloroform layer was concentrated and subjected to GLC analysis.

> *Condition of GLC analysis:* EGA (0.2%)- EGS (0.2%)-Silicone GEXF-1150 (0.4%) on Gaschrom P, 2m. Column temp: 180°C, Injection temp.: 250°C, Carrier: N_2 detector: FID.

Bromine Oxidation of Ozonation Products

Ozonation products obtained from 100 mg of Klason lignin were reduced with 100 mg of $NaBH_4$ in water and then subjected to acid hydrolysis with 2N-TFA in the same way as described above. Acid-hydrolyzed samples were then oxidized with 20 µL of bromine in 20 mL of water containing 200 mg of barium benzoate at 0°C for 24 h. After treatment with sodium sulfite, the ozidized samples were acetylated with pyridine-acetic anhydride; insoluble residues and water soluble materials were removed as described above, and the samples were then subjected to GLC analysis.

RESULTS AND DISCUSSION

Finely crushed Klason lignin became almost completely soluble in water after about 45 min of ozone treatment. The sugar analysis of the whole products showed that neutral sugars are present in Klason lignin (Table 1). Without ozone treatment , finely crushed Klason lignin gave only trace amounts of neutral sugars on acid hydrolysis. Thus, most of the neutral sugars found in the ozonated products became accessible towards acid by ozone-induced destruction of lignin condensed structures.

As described above, it takes about 45 min to dissolve Klason lignin in water under the experimental conditions used. But the total amounts of neutral sugars found in Klason lignin ozonated for 45 min were a little lower than those found after ozonation for 25 min. This result can be attributed to the destruction of

TABLE 1 Residual Sugars in Klason Lignin

Species	Ozonation Time, min	Sugars, (%)				Total, %
		Xylose	Mannose	Galactose	Glucose	
Spruce	25	0.24	0.12	0.12	0.27	0.75
	45	0.20	0.11	0.10	0.22	0.63
Birch	25	0.55	0.10	0.10	0.20	0.95
	45	0.50	0.08	0.08	0.20	0.86

neutral sugars by attack by ozone and conversion into aldonic acids[6]. Therefore, the real amounts of neutral sugars in Klason lignin must be higher than those found in this experiment.

After hydrolysis of whole ozonated products from Klason lignin, xylose, mannose, galactose, glucose and trace amounts of arabinose were detected. Some of the glucose may be from cellulose, but the majority of the residual sugars originate from hemicelluloses. The high proportion of xylose in birch Klason lignin can be explained by birch's hemicellulose composition; the relative abundance of xylose in spruce Klason lignin, however, can not be explained by spruce's hemicellulose composition. These results seem to indicate that only 'selected' carbohydrates can participate in the formation of residual sugars in Klason lignin. Perhaps the participation of the sugars is related to an original lignin-carbohydrate complex. Yasuda et al. found that even at room temperature, lignin undergoes condensation reactions by treatment with concentrated sulfuric acid[2]. We suggest, therefore, that as lignin condensation reactions progress, some of the carbohydrate associated with lignin are incorporated into the condensed lignin and are not hydrolyzed further, because sulfuric acid can not penetrate into the three-dimensional network of lignin (Fig.2).

Augustin *et al*. found that when woody material was subjected to the Klason treatment in the presence of C-14 labelled xylose

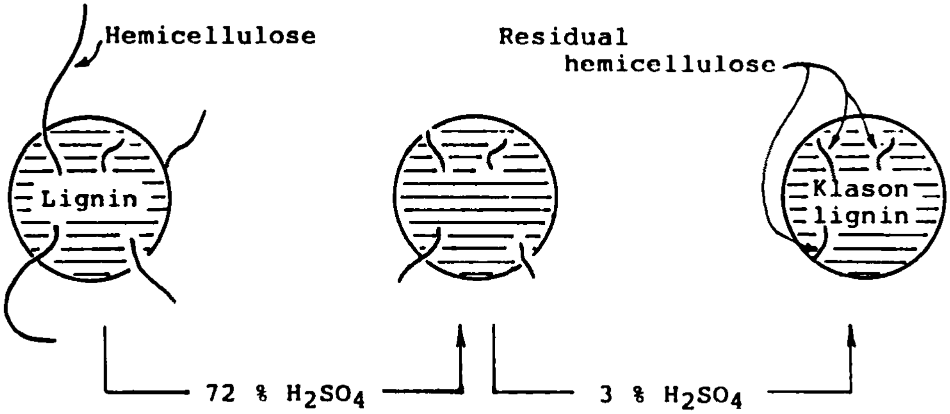

Fig. 2 Possible explanation for the presence of residual sugars in Klason lignin.

and glucose, radioactivity became incorporated into lignin[7]. In our experiments, however, monosaccharides could not be detected in ozonated products of Klason lignin. This indicates that the residual sugars in Klason lignin are present not as monosaccharides, but as oligo- or polysaccharides. Augustin's results may indicate that artifacts of sugars (e.g., furan derivatives) are incorporated into Klason lignin.

As an indication of molecular chain length of the residual sugars, the determination of reducing end groups was attempted according to the scheme shown in Fig. 3. In order to convert the reducing end groups into alditol end groups, ozonated products of Klason lignin were subjected to sodium borohydride reduction and then hydrolyzed by 2N-TFA. Reducing sugars newly formed during the acid hydrolysis were converted into aldonic acids by bromine treatment, and the resulting alditols were determined by GLC as their alditol acetates. Under the GLC conditions adopted, aldoses could interfere with the determination of alditols owing to their similar retention times, but aldonic acids did not pose this problem. Since some of the glycosidic linkages are cleaved by the

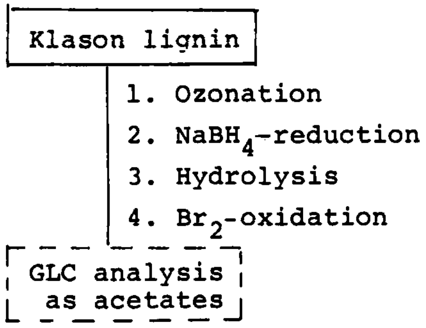

Fig. 3 Scheme for the determination of reducing groups.

ozone treatment to form reducing end groups, the amount of reducing end groups should be a little higher than that in non-ozonated Klason lignin. The amount of detected alditols was below about 10% of total residual sugars.

If there are no chemical linkages between lignin and carbohydrates in Klason lignin, this result indicates that carbohydrates in Klason lignin have a degree of polymerization higher than 10. If the linkages are of the phenylglycoside type, the same conclusion can be reached. This is due to the fact that the phenylglycoside can gain alditol end groups via production of reducing end groups by the ozone treatment followed by NaBH$_4$-reduction (Fig. 4-A). If the carbohydrates are linked glycosidically to lignin side-chains, however, they do not yield any alditol end groups by these successive treatments (Fig. 4-B). On the other hand, ether type linkages do not affect the determination of reducing end groups (Fig. 4-C), but the unit (a) can not be detected in the determination of total residual sugars because it is linked to a lignin fragment.

As discussed above, it depends on the nature of lignin-carbohydrate linkages whether or not alditol end groups can be produced according to the scheme shown in Fig. 3. Thus, no clear information can be obtained on DP values of residual carbohydrates asso-

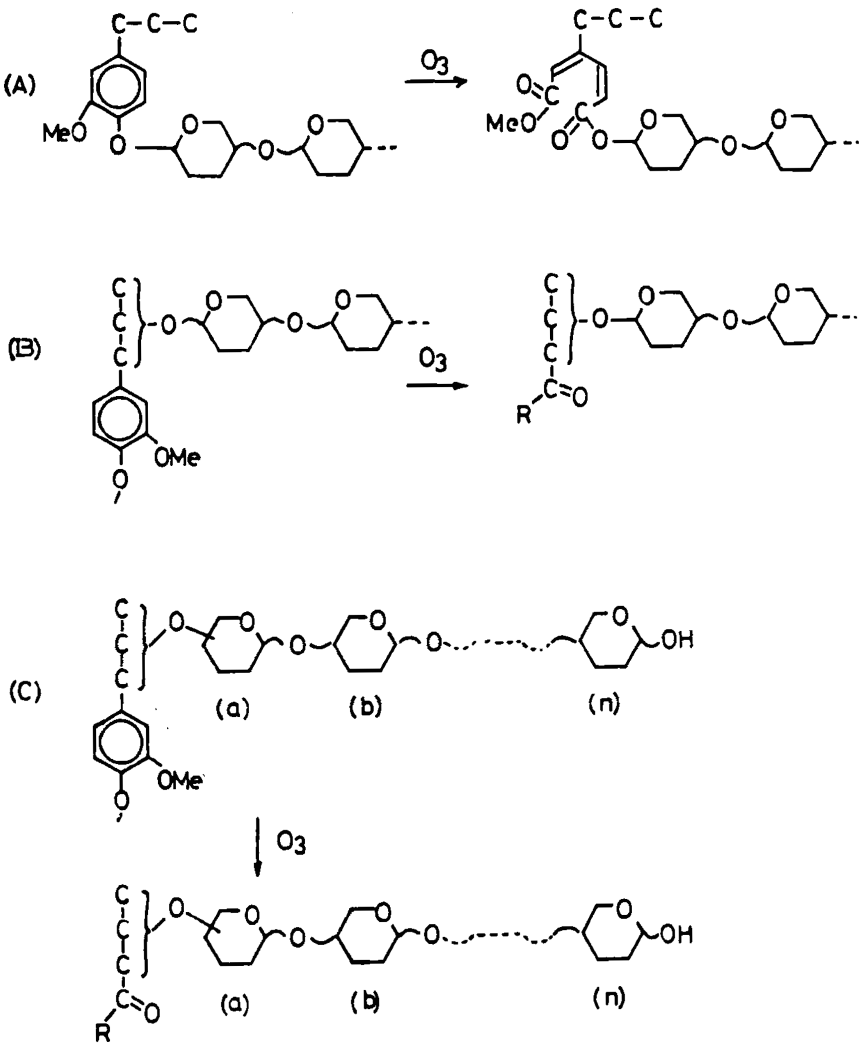

Fig. 4 Behavior of residual sugars towards ozonation.

Table 2 Residual sugars in spruce Klason lignin*

Conditions				Sugar content, %
72% H_2SO_4	2 h	3% H_2SO_4	4 h	0.70
	4 h		2 h	0.75
	48 h		2 h	1.10

* Ozonated for 25 min

ciated with Klason lignin. This study shows, however, that carbohydrates in Klason lignin are mainly oligosaccharides originating from hemicelluloses. The stabilization of originally acid-unstable hemicellulose may be attributed to intimate association of lignin and carbohydrates. Thus, there is some part of the carbohydrates which is not attacked by sulfuric acid during the Klason treatment.

Residual sugar content increased with increasing treatment time in 72% sulfuric acid (Table 2). Further experiments are required to explain this result.

CONCLUSION

(1) After ozonation and then acid hydrolysis, spruce and birch Klason lignins were found to contain 0.75% and 0.95% neutral sugars, respectively. These values indicate the minimum contents of residual sugars, because the ozone partly destroys the neutral sugars.

(2) Most of the residual sugars originate from hemicellulose. The abundance of xylose in spruce and birch Klason lignins may be due to the abundance of xylose residues in the polysaccharides associated with the lignin.

ACKNOWLEDGMENT

The authors thank Prof. N. Terashima, Nagoya University, for valuable discussions and Dr. Martin MacLeod, PAPRICAN, for linguistic revision of the final manuscript.

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