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SOLID-STATE ^{13}C CP/MAS NMR FOR ALKYL-O-ARYL BOND DETERMINATION IN LIGNIN PREPARATIONS

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Several lignin preparations (Freudenberg lignin, Björkman lignin, and Pepper lignin), technical lignins (soda, soda-AQ, Kraft, Kraft-AQ, and hydrolysis), dimeric lignin model compounds, and different polysaccharides (galactoglucomannan, arabinogalactan, xylan, and arabinan) were analyzed by means of solid-state ^{13}C CP/MAS NMR. Signals assignment in solid-state NMR lignin spectra was performed on the basis of the conducted studies and earlier published data. It was established that there exists strong linear correlation ($r = 0.985$) between Alkyl-O-Aryl inter-unit bond content in lignin and integral signals intensity in NMR spectra in the range of chemical shifts of 96–68 ppm. The integral signals intensity was measured in correlation with the reference integral signals in the range of chemical shifts of 162–102 ppm, typical for aromatic carbon atoms. To eliminate the effect, caused by carbohydrates contained in lignin, the correction factor of 0.67% of the area of integration per 1% of carbohydrates was determined. It was shown that the solid-state ^{13}C CP/MAS NMR method allowed to determine Alkyl-O-Aryl bond content in both soluble and insoluble lignin preparations, and also to determine methoxyl groups content in soluble preparations.

KEYWORDS. Solid-state ^{13}C CP/MAS NMR, lignins, Alkyl-O-Aryl bonds, methoxyl groups

INTRODUCTION

NMR spectroscopy, especially quantitative ^{13}C NMR spectroscopy in solution, has made significant progress in lignin structure understanding.^[1–4] Using this method, applying Heteronuclear Multiple Quantum Coherence (HMQC), the presence of previously unknown structural fragments in lignin, such as dibenzodioxocin,^[5] isochroman,^[6] and spirodienone^[7] was proved. It is well known, however, that not all of the lignin preparations are soluble in solvents used in NMR

spectroscopy, although the study of their structure is important not only in the case of native lignins, but also for investigating different lignin chemical transformations in various technological processes. These insoluble or/and partly soluble lignins include Freudenberg lignin, residual lignin, technical hydrolysis lignin, and some others.

Solid-state NMR spectroscopy created new possibilities for studying the structure of insoluble preparations. The advantage of this type of spectroscopy is the possibility to study

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wood components without their isolation from wood/plant matrices, i.e., in their native state. On the other hand, solid-state NMR spectroscopy has a relatively low resolving power compared to conventional NMR in solutions. Moreover, the signals of cellulose, hemicelluloses, and lignin in the spectra of wood considerably overlap. The principal reasons for low resolution in the ^{13}C spectra of powdered samples are the orientation-dependent ^{13}C - ^1H dipole-dipole and ^{13}C chemical shift anisotropic effect, and in high-resolution spectroscopy these adverse interactions are minimized due to the combined effects of ^1H dipolar decoupling and magic angle spinning (MAS). The ^{13}C - $\{^1\text{H}\}$ cross polarization (CP) is commonly employed to improve the sensitivity of the ^{13}C experiment.^[8]

Despite these limitations, solid-state NMR spectroscopy was used in studying lignin, including the one contained in wood. Earlier achievements in this area were summarized in other reviews,^[9,10] therefore here we mention the works published later. It was found out that in the solid-state ^{13}C CP/MAS NMR spectra of lignin, signals in the range of 155–110 ppm are attributed to aromatic carbon atoms, and the signal at 56 ppm is attributed to methoxyl groups.^[11–13] Hemmingson and Newman estimated lignin content in wood using the ratio of the integration area in the signals range of 159–141 ppm to the integration area of the entire spectrum.^[14] A couple of years later Manders described a method for estimating the ratio of syringyl/guaiacyl units in hardwood lignin.^[15]

The resolution of solid-state NMR lignin spectra can be improved by using dipolar dephasing (DD) technique, since DD-spectra in the field of aromatic carbon atoms consist of two ranges. Signals integration in the range of chemical shifts of 164–141 ppm (Range A) and 141–100 ppm (Range B) made it possible to determine the A / B ratio and to estimate the degree of lignin condensation.^[16–20]

Currently, ^{13}C CP/MAS NMR spectroscopy, in combination with other methods, is widely used to study the structure of lignin in isolated preparations^[21–23] as well as in wood after heat

treatment,^[24–27] biodegradation,^[28–31] and also for characterizing plant raw materials.^[32–36] Further this NMR method was developed for lignin quantitation in lignocellulosic biomass.^[37,38]

Alkyl-O-Aryl bonds play a fundamental role in describing the structure and chemical reactions of lignin. Determining the content of these bonds is an important analytical task and the method of quantitative ^{13}C NMR spectroscopy in solution has become the most widely used one in case of soluble lignin preparations.^[3,4] However, the ^{13}C CP/MAS NMR capabilities in this direction have not yet been fully investigated. This method is very attractive and has a great potential in exploring the structure of both soluble and insoluble samples.

In this work, we made an attempt to evaluate the capabilities of ^{13}C CP/MAS NMR to determine the content of Alkyl-O-Aryl bonds (sum of α -O-4 and β -O-4) and methoxyl groups in soluble and insoluble lignin preparations. Selected dimeric lignin model compounds were also investigated in addition to lignin samples, since the chemical shift values of model compounds obtained in solution for assigning those in solid-phase lignin spectra may lead to errors.^[8]

EXPERIMENTAL

Lignin preparations. Isolation of lignin preparations from spruce wood and their analysis were described previously.^[39] The analytical characteristics of the lignin preparations are presented in Table 1.

Lignin model compounds 1–7 were synthesized according to the procedures described by Zakis G. F.^[40] See characterization of compounds 1–7 in Supporting Information. The ^1H and ^{13}C NMR spectra of 1–7 in solutions in CDCl_3 were recorded with a Bruker AM-500 spectrometer equipped with a 5-mm broadband-observed probe head, at 500 MHz (^1H) and 125 MHz (^{13}C) at 25°C. The residual proton-solvent peaks of CDCl_3 (δ_{H} 7.26 ppm and δ_{C} 77.0 ppm) were used as references. Computer simulations of ^{13}C NMR spectra were done by ChemDraw 10.0. The elemental

TABLE 1. Contents of functional groups, bonds, and carbohydrates in preparations of spruce lignin.

Preparation	OMe, %	OH _{phen} , %	Bonds Alkyl-O-Aryl, n/100 PPU	Carbohydrates, %
Freudenberg lignin	15.11	2.00	71	6.5
Bjorkman lignin	15.78	2.97	61	5.3
Pepper lignin	15.08	4.52	44	3.7
Soda lignin	13.24	5.32	36	7.3
Soda-AQ lignin	12.90	6.43	24	7.2
Kraft lignin	13.27	6.48	23	7.1
Kraft-AQ lignin	12.96	6.63	22	7.0
Hydrolysis lignin	11.96	3.00	61	6.4

(CHN) analysis was carried out with a Euro Vector machine.

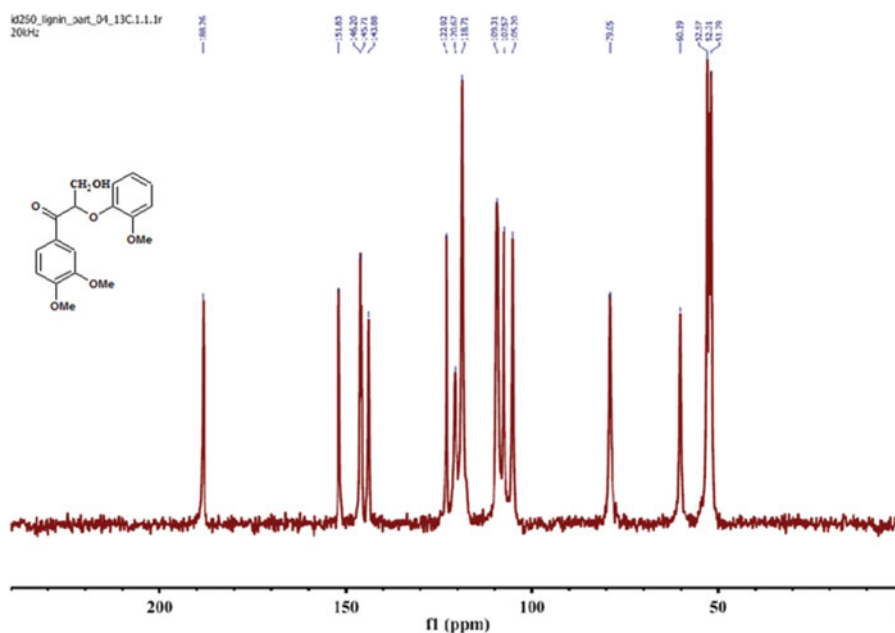
Polysaccharides (hemicelluloses). Galactoglucomannan was isolated from Norway spruce wood with an Accelerated Solvent Extractor (ASE) at 170°C during 17 min using water as solvent and precipitated in EtOH/H₂O (85/15 v/v) as described in [41]. Arabinogalactan (CAS: 9036-66-2) and birch xylan (CAS: 9014-63-5) were from Sigma-Aldrich Co. Arabinan (sugar beet) with sugar units composition (%) Ara (88): Gal (3): Rha (2): GalUA (7) was obtained from Megazyme International Ireland Ltd.

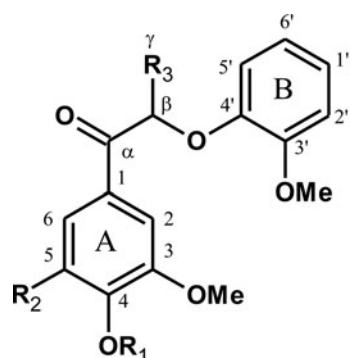
Solid-State ^{13}C CP/MAS NMR. Solid-state ^{13}C NMR spectra of 200 mg of the samples

were recorded at 25°C using a Bruker Avance III 400 WB spectrometer, equipped with a Bruker H-F/X 4 mm CP/MAS probe head, at 100.64 MHz for ^{13}C nuclei with TMS as an external reference. Cross-polarization technique (CP MAS) was applied, at contact pulse duration of 2 ms, relaxation time 3 s, with scans number of 12,000 at the MAS rate of 12.5 kHz.

RESULTS AND DISCUSSION

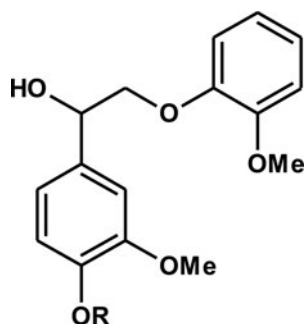
The ^{13}C CP/MAS NMR spectra of the model dimeric compounds **1–7** are shown in Figure 1 (for compound **5**) and S1–S6 in the Supporting Information (SI), and their bands assignment in Table S1 in SI.

**FIGURE 1.** Solid state ^{13}C CP/MAS NMR spectrum of lignin model compound **5**.



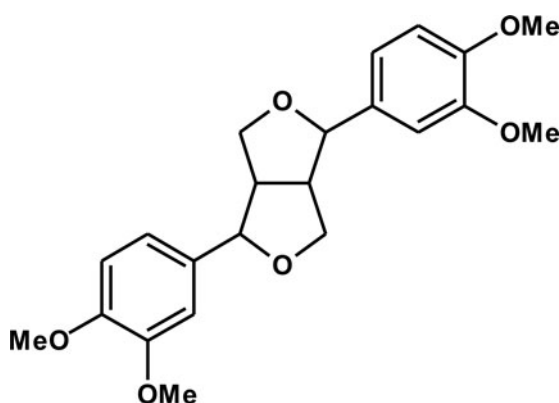
- 1** $R_1=H, R_2=H, R_3=Me$
2 $R_1=Me, R_2=H, R_3=H$
3 $R_1=Me, R_2=H, R_3=Me$ (ring A without 3-OMe)
4 $R_1=Me, R_2=H, R_3=Me$
5 $R_1=Me, R_2=H, R_3=CH_2OH$
6 $R_1=Me, R_2=OMe, R_3=H$
7 $R_1=Me, R_2=H, R_3=Me$ ($\alpha-C-OH$ instead $\alpha-C=O$)

Table S1 shows that the signals of aromatic carbon atoms of the model dimers are located in the range of 150.3–100.2 ppm and the signals shift of carbon atoms of the side chain depends essentially on the nearest surroundings. The $\alpha-C=O$ chemical shifts are in the range of 194.1–187.1 ppm, for $\alpha-C-OH$ it is 75.4 ppm. The carbon atom involved in the formation of $\beta-O-4$ bonds is of particular interest. The chemical shifts of $\beta-O-4$ are located in the range of 79.1–64.8 ppm. The $\gamma-CH_2OH$ chemical shift is 60.2 ppm and for $\gamma-CH_3$ it is 16.8–7.7 ppm. For the comparison purposes, we also provide the data on the signals of α , β , γ -carbon atoms shift position in the ^{13}C CP/MAS NMR spectra of the model dimers **8**, **9** and on pinoresinol **10** and phenylcoumarane **11** type structures.^[8]

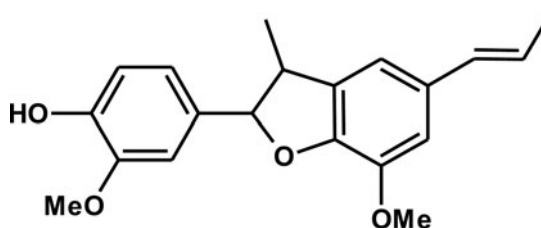


8 $R=H$, **9** $R=Me$

Comp. no.	Chemical shifts in solid state ^{13}C CP/MAS NMR		Ref.
	α	β	
8	74.2	75.6	8
9	72.8	74.5	8



Comp. no.	Chemical shifts in solid state ^{13}C CP/MAS NMR			Ref.
	α	β	γ	
10	85.1 88.5	52.8 52.8	67.6 67.6	8



Comp. no.	Chemical shifts in solid state ^{13}C CP/MAS NMR			Ref.
	α	β	γ	
11	95.2	43.0	16.3	8

The ^{13}C CP/MAS NMR spectra of lignin preparations are shown in Figure 2 and Figures S7–S13 in SI, and their bands assignment in Table 2. The bands assignment was based on

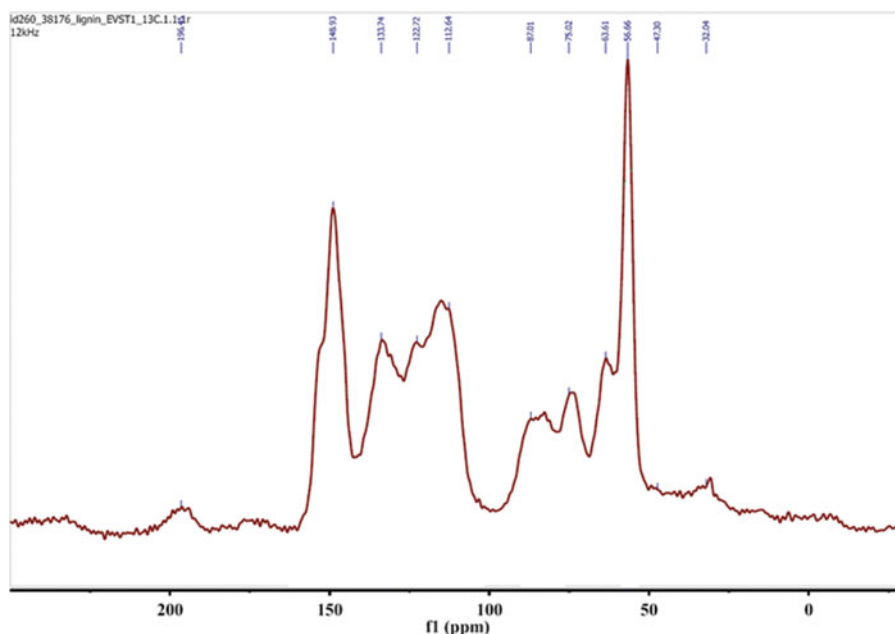


FIGURE 2. Solid state ^{13}C CP/MAS NMR spectrum of Bjorkman lignin.

the results from the model compounds study and the published data.^[2,8]

From Table 2, one can see that signals of aromatic carbon atoms in solid-state NMR spectra of coniferous lignins are located in the range of 150–110 ppm. Side chain α -carbon atoms signals, depending on the environment, appear either at 79–70 ppm (α -OH, β -O-4), or at 88.5–85.1 ppm in pinoresinol type structures, or at 95.2 ppm in phenylcoumarane type structures. For β -carbon atoms, the chemical shift range was 79–70 ppm (α -OH, β -O-4), (α -CO, β -O-4), the signals were also detected at 52.8 ppm and 43.0 ppm in pinoresinol and phenylcoumarane structures, respectively. For γ -carbon atoms, the corresponding shift range was 65–60 ppm (α -OH, γ -OH, β -O-4), the signals were detected at 67.6 ppm in pinoresinol structures and 16.8–8.2 ppm (γ -CH₃).

Since all the lignin preparations usually contain carbohydrates (Table 1), heteropolysaccharides, i.e., hexosans (galactoglucomannan, arabinogalactan) and pentosans (xylan, arabinan) were also studied with the ^{13}C CP/MAS NMR method. The obtained results are shown in Figures S14–S17 in SI; the spectra bands

assignment—in Table S2 in SI. Previously published data were used for their bands assignments.^[42,43] According to the aim of this work, the most interesting signals in solid-state spectra of polysaccharides were in the chemical shift range of 150–110 ppm, since this range is also typical for aromatic carbon signals of the corresponding Alkyl-O-Aryl (α -O-4 and β -O-4) bonds in lignin structures.

It can be noted that the signal at 95.2 ppm in phenylcoumarane type structures corresponds to α -O-4 bonds. The α -O- γ bonds in pinoresinol type structures, the bands of which appear in spectra at 88.5–85.1 ppm, also belong to the ether bonds. For the β -O-4 bonds, the range of chemical shifts was 79–70 ppm. Thus, the entire signals range of different Alkyl-O-Aryl type inter-units connections in the solid-phase spectra of lignin is in the range of 95.2–70 ppm.

Solid-state NMR spectra of polysaccharides have no signals in the 150–110 ppm range (Figures S14–S17, Table S2 in SI), which creates a potential for using this range of chemical shifts as a reference for Alkyl-O-Aryl bonds determination in lignins. As for the range of 95.2–70 ppm, the situation is more

TABLE 2. Assignment of signals in solid state ^{13}C NMR spectra of spruce lignin preparations.

Freudenberg lignin	Bjorkman lignin	Pepper lignin	Soda lignin	Soda-AQ lignin	Kraft lignin	Kraft-AQ lignin	Hydrolysis lignin	Assignment of signals, δ , ppm
196.0	196.5	209.1	196.0	196.0	196.0	196.0	196.9	210–190 –CHO >C = O (α)
176.4	175.0	176.4	176.9	176.0	177.2	177.0	174.3	183–172 >C = O (γ -lactone in β - β) –CO ₂ H (α) H,G –CO ₂ H (γ) cinnamic acid
150.0	148.9	152.9 148.0	147.9	148.2	148.0	147.9	146.8	150–147 G4e (with α -OH, β -O-4) G3 (with α -OH, β -O-4) G3 (with 1-CHO) G3 (with 1-CO, β -O-4) G3 (in 5-5') G3 (with α -O-ether in β - β)
134.5	133.7	133.2	132.4	134.5	133.0	134.6	130.4	139–131 G1 (with α -OH, β -O-4) G1 (with α -O-ether in β - β) G1 (with α -Ar) G1 (with α -H, 5-5')
125.0	122.7	123.7	123.7	127.4	124.2	129.2	124.1	130–124 G1 (with 1-CHO, 1-COMe or CH:CHCO ₂ H) G1 (with 1-CO, β -O-4) G1 (with 1-CHO, 5-5') G5 (with α -H, 5-5')
117.1	112.6	115.7 113.2	114.1	113.7	115.9	113.3	114.7	116–110 G5 (with 1-CO) G5 (with α -OH, β -O-4) G5 (with α -O-ether in β - β) G2 (with α -Ar) G2 (with 1-CO ₂ H, 1-COMe or CH:CHCO ₂ H) G2 (with α -H, 5-5')
87.4	87.0	87.0 80.5	—	—	—	—	83.7	90–80 G α (with α -O-ether in β - β) G β (with α -OH, β -C, β -O-4)
77.7 74.1	75.0	73.1	75.9	75.4	77.8	74.9	75.3 72.7 71.8	79–70 G β (with α -OH, β -O-4) G α (with α -OH, β -O-4) G β (with α -CO, β -O-4)
65.0	63.6	63.0	64.4	64.3	63.6	63.0	65.3	65–60 G γ (with α -OH, γ -OH, β -O-4)
56.7	56.7	56.0	56.6	57.0	56.9	56.5	55.9	56–52 OMe
32.0	32.0	42.5	35.0	37.6	37.0	35.0	38.0 30.4 18.7	20–40 CH ₃ and CH ₂ in saturated aliphatic chains

complicated, as hemicelluloses signals (83.52–71.95 ppm) are located in the same range.

During the integration of the signals in order to determine Alkyl-O-Aryl lignin bonds, not only the position of the signals in the spectrum, but their width was also taken into

account, since it was much wider than those in the corresponding NMR spectra recorded in solution. The integration ranges were additionally optimized so that the obtained results were close to the experimental data obtained by independent methods, i.e., using lignins with

the known content of functional groups and bonds (Table 1).

Based on the experimental data obtained with the authentic model compounds, with different lignins and hemicelluloses preparations as described above, the following integration intervals in solid-state NMR spectra of lignin were chosen: 162–102 ppm as a reference, 96–68 ppm for Alkyl-O-Aryl bonds, and 58–54 ppm for methoxyl groups.

It should be noted, however, that all the lignin preparations contain from 3.7 to 7.3% carbohydrates (Table 1). Even if it can be expected that the effect of the relatively low carbohydrates content on the determination of lignin bonds will not be too great, this influence should be evaluated and eliminated. That was why the solid-state NMR spectra of the series of sulfate lignin samples containing an increasing amount of galactoglucomannan were recorded and the dependence of the integrated intensities ratio of peak clusters in the ranges of 96–68 ppm and 162–102 ppm of Kraft lignin vs. the galactoglucomannan content was established.

The results are shown in Figure S18 in SI. The slope of the straight line shows that 1% of carbohydrates in lignin corresponds to 0.67% of the bands cluster area in the range of 96–68 ppm of the lignin spectra. Taking this value into account, the amount of I_{96-68} for each lignin preparation was corrected according to the carbohydrate content data from Table 1 in all the subsequent measurements and calculations.

The next step was to reveal the correlation between the ratio $I_{96-68} / I_{162-102}$ and the content of Alkyl-O-Aryl bonds (Figure 3). It can be

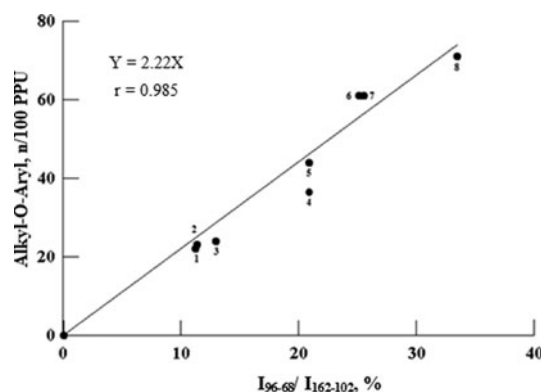
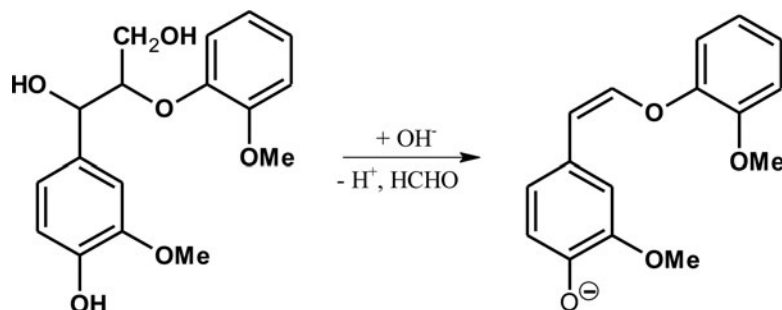


FIGURE 3. Correlation between the integrated intensities ratio of the peak clusters in the ranges of 96–68 ppm and 162–102 ppm in the solid-phase NMR spectra of the lignin preparations vs. the Alkyl-O-Aryl bonds content: 1. Kraft-AQ; 2. Kraft; 3. soda-AQ; 4. soda; 5. Pepper lignin; 6. hydrolysis lignin; 7. Bjorkman lignin; 8. Freudenberg lignin.

noted that for the studied lignin preparations there is linear correlation, going to the origin of the graph, between these two variables ($r = 0.985$). The exception here was the experimental point corresponding to soda lignin. This point was excluded when constructing the discussed dependence and calculating the correlation coefficient.

If for all the other lignin preparations the standard deviation in Alkyl-O-Aryl bonds determination was of $\pm 2\text{--}4/100$ PPU (phenylpropane unit), for soda lignin the deviation from the average value was $\sim 11/100$ PPU. In our opinion, this was due to the fact that soda lignin contained higher quantity of enol ether structures.^[44] Under the alkaline wood cooking conditions, they are formed through a series of intermediate stages from structural lignin fragments containing $\beta\text{--O--}4$.



It is evident that in the NMR spectrum of enol ether structure the signal of the carbon atom in the β -position of the side chain will be shifted toward the chemical shifts of aromatic carbon atoms. The computer simulation of NMR spectra showed that the C_β carbon signal in the starting dimer has a chemical shift at 86.6 ppm, and in the enol ether structure—at 143.2 ppm (in the neutral form), therefore this signal is outside the integration zone of Alkyl-O-Aryl bonds in solid-phase lignin spectra (96–68 ppm). Thus, the ^{13}C CP/MAS NMR method allows determining the content of Alkyl-O-Aryl bonds in lignin preparations isolated from wood by various methods, including technical ones, both soluble and insoluble, with high precision. Based on the obtained results it can be seen, that in respect of Alkyl-O-Aryl bonds content per PPU Freudenberg lignin is the closest to the native one (in wood) (71 and 79/100 PPU, respectively). The content of the above-mentioned bonds in native lignin and discussion of inter-unit bonds degradation depending on lignin isolation conditions is described earlier in details.^[39]

Along with the inter-unit Alkyl-O-Aryl bonds, methoxyl groups are also important for the characterization of isolated lignin preparations. As for the studied lignin preparations, the narrow resonance signal of these groups appeared at 57–56 ppm. Only to a small extent it overlapped with signals of carbohydrates located at 60–59 ppm (Figures S14–S17, Table S2 in SI).

In order to determine methoxyl groups, the ratio $I_{58-54} / I_{162-102}$ was used. Possible effects of carbohydrates on the integration in the chemical shift range of 58–54 ppm can be estimated if one knows the contribution of the signals in the range of 60–59 ppm in solid-phase NMR spectra of individual polysaccharides. The signals integration in this chemical shifts range revealed, that the ratio of I_{60-59} to I of the whole spectrum for galactoglucomannan, arabinogalactan, xylan, and arabinan was 2.86%, 1.61%, 1.49%, and 2.02%, respectively. Since the carbohydrates content in lignin preparations does not exceed 7.3% (Table 1), it can

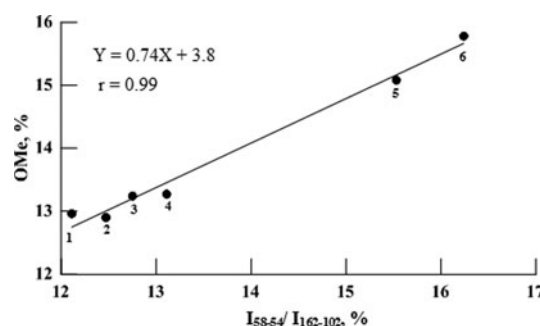


FIGURE 4. Correlation between the integrated intensities ratio of the peak clusters in the ranges of 58–54 ppm and 162–102 ppm in the solid-phase NMR spectra of the lignin preparations vs. the methoxyl groups content: 1. Kraft-AQ; 2. soda-AQ; 3. Kraft; 4. soda; 5. Pepper lignin; 6. Bjorkman lignin.

be assumed that the contribution of carbohydrates to the signals integral in the range of 58–54 ppm in lignin spectra will be insignificant.

The correlation between the ratio $I_{58-54} / I_{162-102}$ and the content of methoxyl groups is shown in Figure 4. It can be seen that between these values there is a linear correlation ($r = 0.99$). The standard deviation of methoxyl groups determination according to this method was $\pm 0.4\%$ on the average. Thus, the above-mentioned metrological characteristics proved, the ^{13}C CP/MAS NMR method is an accurate tool for the analysis of methoxyl groups in different lignin preparations.

It is important to note, that two points in Figure 4, corresponding to Freudenberg lignin and hydrolysis lignin, did not “fit” into the linear correlation, thus, left out from the correlation coefficient calculation. It is also relevant to point out, that these two lignins, unlike the others, are insoluble in most of solvents. For such lignin preparations, which suffer the lack of solubility, for example, for Klason lignin, the position of the signal of methoxyl groups depends substantially on the spatial difficulties around these groups and the conditions for recording solid-state NMR spectra.^[45]

Due to that, we carried out additional studies by recording the solid-state NMR spectrum of Klason lignin from spruce containing 7.8% of methoxyl groups. The corresponding ratio of $I_{58-54} / I_{162-102}$ was 12.8%, i.e., this lignin

preparation also “drops out” from the dependence presented in Figure 4. Additional studies are needed to establish the exact cause of this phenomenon. New perspectives for solving of these problems give the use of advanced solid-state NMR spectroscopy.^[46]

CONCLUSION

Studies of different lignin preparations, model compounds, and polysaccharides show a great potential of the ^{13}C CP/MAS NMR method for lignocellulosics characterization. In addition to the applications described earlier, this NMR method becomes a reliable analytical tool in lignin chemistry including studying reactions involving Alkyl-O-Aryl inter-unit bonds. It was proved that Alkyl-O-Aryl bond destruction occurs in lignin during alkaline cooking of wood at different conditions to a greater or lesser degree. Based on these bonds content it was shown that Freudenberg lignin is the closest to natural lignin (in wood). The undoubted advantage of the method is the possibility to analyze both soluble and insoluble sample preparations.

The ^{13}C CP/MAS NMR method is also a reliable tool for accurate determination of methoxyl groups in soluble lignin preparations.

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SUPPORTING INFORMATION

Supporting information contains figures of solid state ^{13}C CP/MAS NMR spectrum of lignin model compounds, lignins, hemicelluloses, and tables with the assignment of signals in solid state ^{13}C NMR spectra of the model compounds and hemicelluloses, and characterization of lignin model compounds. Supplemental data for this article can be accessed on the publisher's website at <http://dx.doi.org/10.1080/02773813.2017.1393436>.

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