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IR-SPECTROSCOPIC INVESTIGATION OF ASPEN WOOD BIOLIGNINS

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Aspen wood biolignins have been investigated by IR spectroscopy and a procedure has been developed for determining the amounts of α-, β- and γ-carbonyl groups in the lignins obtained from aspen wood after the action of the lignin-destroying fungus Phanerochaete sanguinea. *A large contribution of the C_α-oxidation reaction and the appearance in the biolignins of ester groups and also nonconjugated ketone and aldehyde groups have been revealed. The latter may, presumably, be a consequence of the oxidation of the C_γ atoms of the propane chain and the appearance of α-O-4 structural units in the lignin.*

The biodecomposition of lignin is a natural phenomenon capable of becoming the basis of technologies connected with the delignification of plant raw material [1]. The biodestruction of lignin is an oxidative process as a result of which new oxygen-containing, including carbonyl, groups appear in the lignin macromolecule. A determination of the type of carbonyl groups and their amounts will enable valuable information to be obtained about the directions of the processes of biodestruction.

A total quantitative determination of C=O groups in lignin is possible with the aid of spectral methods, including IR spectroscopy [2-5]. However, the identification and quantitative analysis of the various types of carbonyl groups by this method is extremely laborious because of the unresolved structure of the corresponding absorption bands.

Our aim was to develop a procedure for the differential quantitative determination of carbonyl groups in lignin by IR spectroscopy with a subsequent investigation of the lignins obtained from aspen wood after the action on it of the lignin-destroying fungus *Phanerochaete sanguinea*.

Aspen wood that had previously been extracted with ethyl alcohol to eliminate extractive substances was incubated with the fungus *Ph. sanguinea* for six months. From samples of the wood formed into groups having similar losses in weight we isolated the biolignin preparations (**2**)-(**8**) (Table 1). Lignin (**1**) was isolated from healthy aspen wood.

IR spectra in the 1800-1100 cm^{-1} region were obtained from solid lignin samples and from their solutions in DMSO-d_6, which are widely used in the investigation of the ^{13}C NMR spectra of lignins [6]. The νC=O absorption bands located at 1800-1650 cm^{-1} have more pronounced maxima than the weak inflections on the contour of the broad summary band in the spectra of solid samples. This has enabled us to measure the integral intensities of individual νC=O bands and to attempt a quantitative estimation of the changes in the amounts of the corresponding groupings in samples with different depths of biological attack, characterized by such a magnitude as the loss in weight of the wood (l.w.) as compared with the lignin of healthy wood (**1**).

The assignment of the νC=O bonds in the spectra of the lignins to carbonyl groups of definite types has been made previously in a number of studies on the basis of the spectra of model compounds and the spectra of lignins treated with sodium tetrahydroborate or hydroxide [3, 7, 8]. By using these results it may be concluded that: a) in the spectrum of the solution of the lignin (**1**) that we studied in DMSO-d_6 the νC=O bands have the usual position for native lignin; b) they may relate both to aldehydic and to ketonic carbonyl groups in the α- (1667 cm^{-1}) and β- and γ- (shoulder, ~1710 cm^{-1}) positions of the propane chain and also to the ester groups of $Ar-CH_2-C(O)OR$ fragments, where R = Alk (1763 cm^{-1}).

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TABLE 1. Relative Integral Intensities of the νC=O Absorption Bands (A_Σ/A_{1500}), (A_i/A_{1500}) in the IR Spectra of Solutions of the Lignin of Healthy Wood (**1**) and of the Biolignins (**2-8**) in DMSO-d_6 and the Amounts of C=O Groups in Percentages by Weight (ΣC=O) (from the results of chemical analysis). A_Σ, A_i, and A_{1500} are the Integral Intensities of Absorption Bands — Summary for 1800-1650 cm^{-1}, for the Wavenumber i (cm^{-1}), and for ν = 1500 cm^{-1}, Respectively.

Compound No.	l.w. %	A_i/A_{1500}				A_Σ/A_{1500}	ΣC=O C=O C(O)O	ΣC=O C=O
		1790–1780	1760–1730	1715–1705	1670–1660	1800–1650		
1	initial	0.02	0.16	0.17	0.27	0.62	4.3	3.5
2	0.0	0.05	0.41	0.44	0.58	1.48	5.1	4.1
3	2.8	0.25	1.27	0.79	0.85	3.16	10.7	8.1
4	6.4	0.29	1.44	0.74	1.14	3.61	11.2	8.3
5	9.6	0.33	1.39	0.80	1.14	3.66	10.0	8.9
6	13.0	0.44	1.39	1.04	1.18	4.05	–	–
7	17.0	0.53	1.47	1.13	1.12	4.25	9.5	8.0
8	23.0	0.42	1.61	0.82	1.14	3.99	11.1	8.2

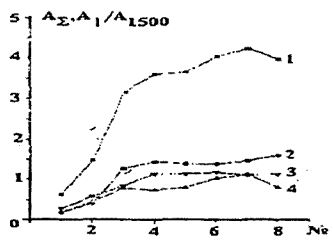

Fig. 1. Change in the relative integral intensity of the νC=O bands (A_Σ/A_{1500}), A_i/A_{1500} in the IR spectra of aspen lignins (**1**)-(**8**): 1) A_Σ/A_{1500}; 2) $A_{1760\text{-}1730}/A_{1500}$; 3) $A_{1715\text{-}1705}/A_{1500}$; 4) $A_{1670\text{-}1660}/A_{1500}$.

In the spectrum of a solution of biolignin (**2**) (l.w. 0%) bands of conjugated (α-) and nonconjugated (β-, γ-) carbonyl groups were observed at 1664 and 1707 cm^{-1}, respectively, but with a reversed ratio of their intensities as compared with lignin (**1**). In addition to these, an absorption band of the ester group of a Alk−CH_2−C(O)OR fragment appeared in the form of a shoulder at 1730 cm^{-1} [7].

Absorption in the region of νC=O vibrations rose sharply in the spectra of biolignins (**3**)-(**8**), isolated from wood with higher l.w. values Here, the band with a maximum at ~1670 cm^{-1} (α-carbonyl groups) appeared in the form of a shoulder on the low-frequency wing of a strong broad band with its main maxima at ~1740 and ~1710 cm^{-1} (ester and β-carbonyl groups), respectively. A shoulder at 1780-1790 cm^{-1} on its high-frequency wing in the spectra of biolignins (**3**)-(**8**) can apparently be explained by the Fermi resonance of the corresponding fundamental vibration with combination tones, as in the analogous absorption in the spectra of carbonyl-containing model compounds [9].

At the same time, it may be observed that in the spectra of biolignins (**2**)-(**8**), as compared with the spectrum of lignin (**1**), the intensity of the band of the C−O−C stretching vibrations of ester groups at 1230 cm^{-1} had increased, and this to a smaller degree in the spectrum of sample (**2**) and to a greater degree in the spectra of biolignins (**3**)-(**8**), i.e., the increase in the intensity of this band took place symbatically with a rise in the intensity of carbonyl absorption. In addition, in the spectra of all the biolignins there were practically no bands of the stretching vibrations of olefinic C=C groups, as were observed in the spectrum of the initial lignin at 1637, 1620 cm^{-1} in the form of a shoulder on the short-wave wing of the band (~1600 cm^{-1}) of the C=C stretching vibrations of the aromatic fragments of the molecules.

The main difference in the region of νC=O vibrations in the spectra of the biolignins (**2**)-(**8**) investigated consisted in a change in the ratio of the intensites of the bands observed. The characteristic nature of the νC=O stretching vibrations and the relatively isolated region of location of the corresponding bands in the spectrum provides the possibility of their use

for evaluating the relative levels of carbonyl groups in the biolignins. We have measured the integral intensities both of the total absorption in the 1800-1650 cm^{-1} region and of the individual $\nu C{=}O$ bands (using the procedure for their separation given in [10]) in the spectra of the lignins of healthy wood and that attacked by the fungus. The values obtained were normalized to the phenyl group contents of the lignins by dividing the numerical values of the integral intensities of the total and the individual $\nu C{=}O$ bands (A_Σ, A_i) by the integral intensity of the $\nu C{=}C$ band of aromatic rings (A_{1500}) (Table 1).

The validity of the recalculation of the results obtained to one aromatic ring of the biopolymer was shown by a functional analysis of lignins subjected to chemical and to microbial treatments [7]. In the quantitative spectroscopy of solid lignins satisfactory results are obtained by dividing the numerical values of the optical densities of the absorption bands of the corresponding functional groups by the optical density of the $\nu C{=}C$ band of the aromatic rings of the lignins (~ 1500 cm^{-1}) [11, 12].

The use of this approach enabled us to reduce the measurement of the integral intensities of the bands ($A = 1/Cl\int_{\nu_1}^{\nu_2} I_o/I \, d\nu$) to a definite area bounded by their contour, since the relative values A_Σ/A_{1500} and A_i/A_{1500} do not contain the parameters C and l (concentration of the molecules and thickness of the absorbing layer, respectively).

Thus, it was found that the total relative intensity (A_Σ/A_{1500}) of the $\nu C{=}O$ bands in the biolignins, as compared with the initial lignin (**1**), had already increased in sample (**2**) (l.w. 0%), increased further in lignins (**2**) and (**3**), and then, beginning from lignin (**4**) (l.w. 6.4%) remained on the same level, within the limits of experimental error (Fig. 1).

The separation of the total $\nu C{=}O$ absorption band in the spectra of the biolignins studied and of the initial lignin (**1**) into its components was achieved for four of these components, having maxima at 1790-1780, 1760-1730, 1715-1705, and 1670-1660 cm^{-1} (Table 1), with the inclusion in the general contour of the $\nu C{=}C$ bands at 1600 and 1500 cm^{-1}. The most intense band in the spectrum of (**1**) was the band of conjugated carbonyl groups at 1667 cm^{-1} (Fig. 1, Table 1). In the spectrum of biolignin (**2**), the relative intensities of the bands at 1664, 1707, and 1730 cm^{-1} were roughly the same, while in the spectra of (**3**)-(**8**) the band of ester groups at 1730-1740 cm^{-1} became predominant. A general tendency to rise as the result of the biotreatment of the lignin was observed for all the $\nu C{=}O$ bands

The extinction coefficients of the ester bands and also of the conjugated and nonconjugated aldehyde and ketone groups of the lignin macromolecules may differ substantially from one another [13]. Consequently, in order to correspond to the relative (per phenyl ring) content of carbonyl groups in the molecules the resulting numerical values of A_i/A_{1500} require calibration by an independent method of determining C=O groups quantitatively. For this purpose we used the results of chemical functional analysis for the aldehyde, ketone, ester, and acidic carbonyl groups of the lignins under investigation (Table 1). The correlation between the total content of C=O groups as a percentage by weight ($\Sigma C{=}O$) and the relative integral intensity of the $\nu C{=}O$ absorption (A_Σ/A_{1500}) in the 1800-1650 cm^{-1} interval for seven measurements is expressed by the equation

$$A_\Sigma/A_{1500} = -0.89 \pm 0.69 + 0.44 \pm 0.07\ (\Sigma C{=}O),\ R=0.92,\ S_o=0.5.$$

Furthermore, the values of the total integral intensity of the $\nu C{=}O$ bands at ~ 1710 and ~ 1670 cm^{-1} of α- and β-carbonyl groups and the percentage levels of aldehydic and ketonic C=O groups are in good linear relationship. In this case, the regression equation has the form:

$$A_{\Sigma 1710,1670}/A_{1500} = -0.28 \pm 0.32 + 0.27 \pm 0.04 \Sigma C{=}O.$$
$$R = 0.93,\ S_o = 0.2$$

The correlations established (equations (1) and (2)) will serve as a basis for converting spectral results into percentages by weight and will ensure the possibility of their future independent use.

The results obtained with the aid of IR spectroscopy show that biological action on lignin is accompanied by oxidation reactions and that with increasing l.w. of the wood the oxidative processes intensify. Moreover, the results obtained supplement our information on possible pathways of the biotransformation of lignin under the action of the fungus *Ph. sanguinea* [14]. Thus the great contribution of C_α-oxidation and the appearance of ester groups in biolignins have been confirmed.

EXPERIMENTAL

The IR spectra of the lignins (tablets with KBr) and of their solutions in DMSO-d_6 were obtained on a SPECORD M82 spectrophotometer. Aspen wood was incubated with the fungus *Phanerochaete sanguinea* by the procedure of [6, 14]. The lignins were isolated from healthy wood (**1**) and from biologically degraded wood (**2**)-(**8**) as described in [15]. The carbonyl group content was determined by the oximation method, and the carboxy groups by a chemosorption method as laid down in [2].

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