ENZYMATIC OXIDATION OF LIGNIN AND COMPOUNDS MODELING IT.

V. PRODUCTS OF PEROXIDASE OXIDATION OF α-GUAIACYLPROPANONE

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Using α-guaiacylpropanone as an example, it has been shown that, in the process of peroxidase oxidation of carbonyl-containing structures, biphenyl compounds having C—C bonds in the 5—5' positions are formed. On the basis of the results of physicochemical methods of analysis, the formation of biphenyl spatially hindered structures with screened radical centers is suggested.

In communication IV [1], it was shown that the peroxidase oxidation of α-guaiacylpropanone is accompanied by the formation of excited intermediates the deactivation of which leads to the emission of quanta of light with λ_{max} 560 and 630 nm. Excited carbonyl groups, either present in the α-position or belonging to structures of the p-quinoine type formed in the oxidation process, may be responsible for the radiative deactivation of the oxidation products of the substrate in the 560-nm region.

Since carbonyl groups in the excited triplet state possess an increased reactivity [2], it may be assumed that they can exert an influence on the formation of stable products of the peroxidase oxidation of α-guaiacylpropanone. The aim of the present work was to study the influence of an excited carbonyl group on the composition of the stable products of the peroxidase oxidation of α-guaiacylpropanone.

The oxidation of α-guaiacylpropanone by the peroxidase—H_2O_2 system leads to the formation of products with a higher molecular weight than the initial compound (Fig. 1). The separation of the oxidation products by gel-permeation chromatography showed the formation mainly of a dimeric compounds with a degree of conversion of ~90%.

To detect stable free radicals, we used the ESR spectrum of the products of the oxidation of the α-guaiacylpropanone. The concentration of paramagnetic centers amounted to 10^{14} spins/g and the rate constant of the destruction of the radicals to $5 \cdot 10^{-5}$ sec^{-1}. The ESR spectrum consisted of a singlet line with the parameters characteristic for a delocalized

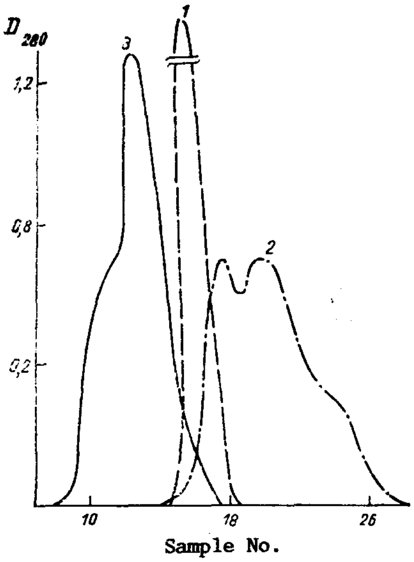

Fig. 1. Gel chromatogram of α-guaiacylpropanone (1) and the products of its oxidation by H_2O_2 (2) and by the peroxidase—H_2O_2 system (3).

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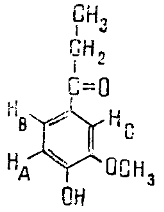
TABLE 1. Chemical Shifts of α-Guaiacylpropanone and the Dimerization Products

Compound	δ, ppm						SSCC*, Hz	
					aromatic protons			
	$-CH_3$	$-CH_2-$	OCH_3	$-OH$	H_A	H_B-H_C	$^3J\,(H_A, H_B)$	$^4J\,(H_B, H_C)$
α-Guaiacylpropanone	1,16	2,85	3,94	5,93	6,83	7,43	9,3	0,8
Product	1,08	2,84	3,85	—	—	7,41	...	...

electron. The absence of hyperfine splitting of the spectrum did not enable the radicals to be identified as phenoxyl radicals, but the disappearance of the hyperfine structure in the ESR spectrum and the singlet form of the signal can be explained from the point of view of a radical-dimer equilibrium, as has been suggested in [3]. As a result of the equilibrium, the forces of interaction of the unpaired electron with the protons of the surroundings are weakened, which may be the reason for the disappearance of the hyperfine structure.

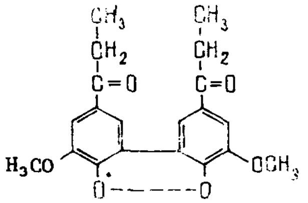
In order to study the change in the functional groups of α-guaiacylpropanone, the IR spectra were taken of the initial compounds and of the products of its peroxidase oxidation. The absorption spectrum of the oxidized substrate showed changes connected with the dimerization of the products. Thus, while in the initial spectrum absorption was observed in the 1515 cm^{-1} region in the form of a single intense band, in the oxidation product this band appeared in the form of a shoulder, which indicated a limitation of the skeletal vibrations of the aromatic nucleus. The formation of a biphenyl compound leads to the appearance of a rigid structure by virtue of a 5—5' bond, which is accompanied by the disappearance of the absorption band at 1000-1050 cm^{-1} (planar vibrations of an aromatic nucleus). On the other hand, an increase in the intensity of absorption in the 1150-1170 cm^{-1} region due to the formation of a C—O—C bond was observed. An indirect confirmation of the formation of a peroxide structure may be the appearance of an absorption band in the 1770-1790 cm^{-1} region. The IR spectra were interpreted on the basis of information in [4].

In order to obtain additional information on the structure of the products of the dimerization of α-guaiacylpropanone, an investigation was made by the NMR (PMR) method. NMR enables the number of protons to be calculated with the necessary definiteness and a high degree of accuracy. In the NMR spectrum of α-guaiacylpropanone the signals were observed of the protons of a $-CH_3$ group (1.16, triplet), of the protons of a $-CH_2-$ group (2.85, doublet), and of the protons of a methoxy group (3.94, singlet). A signal with a chemical shift of 5.93 was due to the presence of the proton of a hydroxy group, and shifts of 6.83 and 7.43 ppm were assigned to two doublets of aromatic protons, H_A and H_B—H_C, respectively.



Chemical shifts (δ, ppm) relative to tetramethylsilane (TMS) for α-guaiacylpropanone and the dimerization products are given in Table 1.

According to the results given in Table 1, the NMR spectrum of the products of the oxidation of α-guaiacylpropanone contained no signals of the H_A aromatic proton or of the proton of the hydroxy group —OH. The absence of the signal of the H_A proton indicated the formation of a biphenyl bond in the 5—5' position between the phenolic nuclei. The chemical shifts of the protons of the closest environment of the α-carbonyl group (H_B—H_C) had scarcely changed, which indicated that this group did not participate in an interaction with the formation of new bonds and underwent no change in the process of oxidation. The disappearance of the NMR signal in the protons of the hydroxy group may, in our opinion, be due to the following factors: 1) the formation of quinones; 2) the formation of a stable phenoxyl radical; or 3) the formation of a radical-dimeric structure.



The formation of a quinoid structure involves a change in the bonds in the aromatic nucleus, which should be reflected in the NMR spectrum in the form of a change in the chemical shifts of the aromatic protons and of the proton of the methoxy group. No such changes in δ were observed in the NMR spectrum. The formation of a stable phenoxyl radical was not confirmed by the results of ESR spectroscopy. The contradiction can possibly be solved by assuming the formation of a radical-dimer structure in which the delocalized electron migrates freely between two oxygen atoms.

The stability of the radical-dimeric product obtained from the oxidation of α-guaiacylpropanone (the rate constant of disappearance according to the ESR result, was $5 \cdot 10^{-5}$ sec^{-1}) can be explained by the formation of spatially hindered structure, as a result of which the radical proved to be screened by the methoxy group of the biphenyl system. The total number of biphenyl elements in lignin amounts to about 25%. It is quite probable that in condensed lignins the high number of paramagnetic centers is due precisely to the presence of biphenyl structures with screened radical centers.

EXPERIMENTAL

The gel filtration of the α-guaiacylpropanone and of the oxidation products obtained was performed on a chromatographic column (2 × 50 cm) filled with LH-20 gel. The eluent was dimethylformamide (DMFA). Fractions with a volume of 3 ml were selected with the aid of an automatic fraction collector. Changes in the concentrations of the solutions in the fractions were monitored on a Spectromon-195 spectrophotometer at 280 nm.

The ESR spectra were taken on a RE 1308 spectrometer. Diphenylpicrylhydrazyl (DPPH) was used as standard. A weighed sample (1.05 mg) was molded with KBr. The IR spectra were taken on a UR-20 spectrophotometer in the frequency range of 1900-900 cm^{-1} at a rate of scanning of 64 cm^{-1}/min. The NMR (PMR) spectra were recorded on a Tesla BS-478C spectrometer (80 MHz). The chemical shifts are given relative to tetramethylsilane (TMS). The samples were dissolved in CCl_4.

α-Guaiacylpropanone was subjected to oxidation by the peroxidase—H_2O_2 system at pH 5.6. A 3% solution of hydrogen peroxide was added to a mixture of the peroxidase and the substrate (1:100) until the formation of a precipitate ceased. The precipitate obtained was isolated from a mixture by centrifugation and it was washed on a glass filter with distilled water and dried in vacuum in a desiccator over P_2O_5. The sample obtained in this way was investigated by physicochemical methods of analysis.

CONCLUSIONS

The investigations performed show that the α-carbonyl groups of phenylpropane structural fragments of lignin do not take part in the processes involved in the dimerization of aromatic structures. In the process of peroxidase oxidation in an excess of H_2O_2 guaiacyl structures dimerize in the 5—5' positions of the phenol nuclei. The peroxidase oxidation by α-guaiacylpropanone forms biphenyl structures with screened radical centers.

LITERATURE CITED

1. V. A. Strel'skii and É. I. Chupka, Khim. Prir. Soedin., No. 1, 115 (1988).
2. J. A. Barltrop and J. D. Koyle, Excited States in Organic Chemistry, Wiley-Interscience, New York (1977).
3. E. S. Caldwell and C. Steelink, Biochim. Biophys. Acta, 184, 43 (1969).
4. O. N. Grushnikov and V. V. Elkin, Advances in and Problems of Lignin Chemistry [in Russian], Moscow (1973).