A COMPARISON OF THE ANTIOXIDANT PROPERTIES OF SOME NATURAL ANTIOXIDANTS AND LIGNIN

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The antioxidant properties of some natural antioxidants and lignin have been measured by the volt-amperometric and chemiluminescence methods. The dynamics of the deoxidation of lignin are discussed. An ultraweak fluorescence of lignin solution has been detected.

The formation of lignin takes place by the oxidative hydrogenative polymerization of the structural elements of coniferyl alcohol [1]. This process of oxidative coupling reflects a gradual aging of the cell and its dying off in parallel with the lignification. The consideration of the chemical and biological role of lignin in plants usually terminates at this stage. It is considered that lignin substances subsequently act only as a reinforcement in improving the mechanical properties of woody tissue [2].

However, the participation of phenolic compounds in respiration has been demonstrated [3]. The concentration of lignin substances in the middle lamella, in the intercellular spaces, suggest that they may serve as a kind of protection from possible injuries to the plant on a disturbance of equilibrium of the redox processes in the cell. Phenols are capable of terminating the chain oxidation reactions [4] and of binding the superoxide anion-radical O_2^- [5] and thereby maintaining equilibrium of oxidative processes.

It may be assumed that the various components of wood will differ in their capacity for undergoing oxidation. In view of this, it is of interest to compare the inhibiting activity of certain known natural antioxidants present in plants and of lignin.

For this purpose, we have used independent methods — volt-amperometry and chemiluminescence. The volt-amperometric method permits the isolation of the components most readily oxidized from a mixture and the determination of their oxidation potentials. The measurement of the intensity of the chemiluminescnece of luminol (as indicator) during oxidation [3] gives information reflecting the total contribution of the components of the mixture which may act as inhibitors of radical processes and form antioxidants.

To compare the antioxidant properties we took carotenoids, the total fraction of pine lipids, the total fraction of the resins, α-tocopherol, and Pepper's lignin.

The results of oxidation at a rotating disk electrode show that the positive values of the oxidation potential of the substances investigated rise in the following sequence: Pepper's lignin, α-tocopherol, fraction of resinous substances, carotenoids, lipids.

Figure 1 gives the volt—ampere characteristics of the antioxidants considered. The results show that the resonance substances contain two components having oxidation potentials of 1.05 and 1.35 V.

The values of the antioxidant activity calculated from the dependence of the relative intensity of chemiluminescence of luminol on the concentration of added antioxidant (Fig. 2) decrease in the sequence carotenoids, Pepper's lignin, lipids, resinous substances, α-tocopherol:

Substance	Relative antioxidant activity	$E_{1/2}$ of oxidation, V
Carotenoids	1.00	+1.22
Pepper's lignin	0.72	+0.20

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Lipids	0.15	+1.27
Resins	0.09	+1.05
		+1.35
α-Tocopherol	0.06	+1.00

Thus, on the basis of the results obtained by two independent methods it has been established that the properties of lignin include it among the effective natural antioxidants.

At the present time it has been established that the oxidative processes in biosubstrates are accompanied by ultraweak fluorescence. This radiation gives information on the nature of the oxidative process taking place.

In the oxidation of lignin by O_2 and H_2O_2 in alkaline solutions we detected an ultraweak fluorescence the maximum intensity of which was in the interval from 2.5 to 5.0 mM (on the basis of a nominal lignin molecule of 180 nominal units).

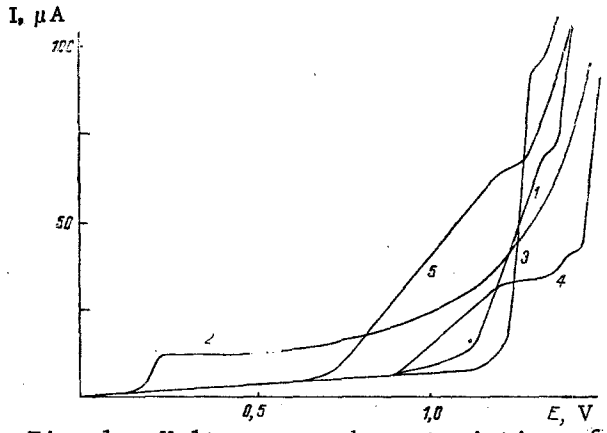

Fig. 1. Volt–ampere characteristics of the antioxidants studied: 1) carotenoids; 2) Pepper's lignin; 3) lipids; 4) resins; 5) α-tocopherol.

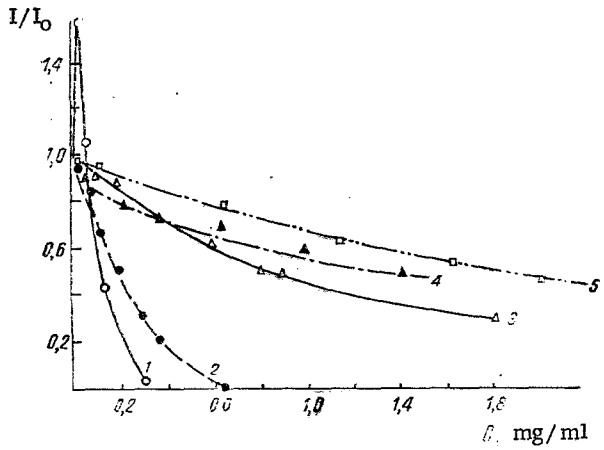

Fig. 2. Comparison of the antioxidant properties of various components of pine wood from the results of the chemiluminescence method (numbers on the curves the same as in Fig. 1).

The admission of oxygen into a lignin solution leads to a sharp flash of luminescence (Fig. 3b, section BC). Section AB corresponds to the steady-state level of fluorescence at 40°C. An excess of oxygen (point C) leads to the quenching of the ultraweak fluorescence of lignin to a definite level (section DE). Purging the solution with helium sharply decreases the intensity of fluorescence of lignin (section FG). Since the fluorescence practically disappears in an oxygen-free medium, it can be ascribed to chemiluminescence. The re-admission of oxygen into the system (point G) increases the intensity of the luminescence of lignin to a smaller extent than its initial admission. This is probably due to the partial "burning off" of the more readily oxidized structural fragments of the lignin on the first admission of oxygen.

A flash of luminescence is also observed on the oxidation of lignin by hydrogen peroxide (Fig. 3a). Section DE corresponds to the spontaneous quenching of the ultraweak fluorescence.

The free-radical nature of the oxidation reaction can be judged from the quenching of the the chemiluminescence by inhibitors of free-radical processes. One of such inhibitors is ascorbic acid [7]. The introduction of ascorbic acid (c = 1 g/liter) into a solution of lignin leads to a decrease in the intensity of fluorescence almost to the level of the background (Fig. 3a, section EF). It may be concluded from this that the oxidation of lignin by hydrogen peroxide takes place by a free-radical mechanism or, at least, has free-radical stages.

EXPERIMENTAL

The carotenoids and the total lipid fraction were isolated from pine wood and the Pepper's lignin was obtained by handbook methods [8] and [9], respectively. The resinous substances were isolated from pine gum and the α-tocopherol from a pharmacopeial preparation of vitamin E (it was identified by UV spectroscopy, thin-layer chromatography, and the qualitative reaction with Fe^{3+}).

Oxidation at a rotating disk electrode was effected in 0.1 N NaOH solution. The indicator electrode was type OSCh 7-4 graphite and the comparison electrode a saturated calomel half-cell. The volt—ampere curves were recorded on PPT-1 polarograph in the classical regime.

The chemiluminescence measurements were performed by a method described previously [10]. An FEU-119 photomultiplier was used as the light-receiver.

The ultraweak fluorescence of lignin was recorded on a KhLM-lts-01 apparatus.

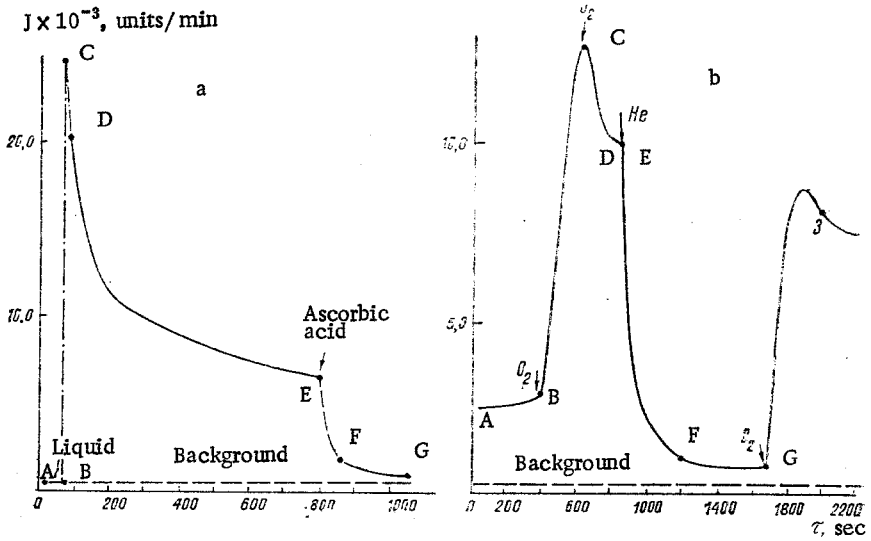

Fig. 3. Influence of hydrogen peroxide and ascorbic acid (a) and of oxygen (b) on the ultraweak fluorescence of lignin (t = 40°C, $C_{H_2O_2}$ = 1.5%, $C_{ascorbic\ acid}$ = 1.0 g/liter, C_{lignin} = 2.0 mM in 2 N NaOH solution).

SUMMARY

In its antioxidant properties and capacity for binding peroxide radicals, lignin is not merely not inferior but is actually superior to some natural antioxidants.

The oxidation of lignin in solutions by oxygen and hydrogen peroxide takes place by a free-radical mechanism and is accompanied by chemiluminescence.

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AMINOMETHYLATION OF DIOXANE LIGNINS OF HEALTHY AND WILT-DISEASED COTTON PLANTS OF THE VARIETY TASHKENT-1

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The products of the aminomethylation of dioxane lignins of healthy and wilt-diseased plants of variety Tashkent-1 according to the vegetation periods (ADLKhT-1-VII) have been studied. It has been established that the dioxane lignins of the wilt-diseased cotton-plant stems are less condensed.

The degree of condensation of lignin can be judged from the yield of vanillin and other aromatic aldehydes in the products of nitrobenzene oxidation, and also from the results of NMR spectroscopy [1, 2]. Mikawa et al. [3] have used the aminomethylation reaction to investigate the degree of condensation of thiolignin. By experiments with model compounds they established that, without exception, all the model compounds having a guaicacyl structure with a free phenolic hydroxy group take part in the reaction and give Mannich bases with the introduction of an aminomethyl group into position 5 of the aromatic ring.

Model compounds having substituents in position 5 of the aromatic ring do not take part in the reaction. A carboxy or a hydroxymethyl group in the para position to the phenolic hydroxy group is displaced by the aminomethyl group; the reaction does not take place when the phenolic hydroxy group is etherified or esterified and the other functional groups remain unchanged.

We have performed the aminomethylation of the dioxane lignins isolated previously [4] from healthy and wilt-diseased cotton plants of variety Tashkent-1 according to vegetation

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