

## CHEMICAL STRUCTURE AND ANTIOXIDANT PROPERTIES OF LIGNINS FROM CONIFER, BROADLEAF, AND HERBACEOUS PLANTS

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*The chemical structures of Pepper lignins isolated from several coniferous, broadleaf, and herbaceous plants were elucidated. It was shown that the studied lignins had heterogeneous biopolymer compositions consisting of monomeric guaiacyl (G), syringyl (S), and p-coumaryl (H) units. The antioxidant activity of the lignins was determined. A correlation between the antioxidant activity and the structure of the lignins was established.*

**Keywords:** natural lignins, structure, antioxidant activity.

A unique feature of lignin biopolymers is the high variability and changeability of the chemical structure and properties that can depend on their biological origin [1]. It was found [2–4] that several types of lignins could successfully suppress free-radical oxidation by acting as natural antioxidants. Herein we present results from a comparison of the chemical structures of lignins isolated from several coniferous, broadleaf, and herbaceous plants and an estimate of their antioxidant activity (AOA).

Purified dioxanelignin samples were cream-colored x-ray-amorphous powders that were soluble in basic aqueous solutions and several polar solvents but were insoluble in H<sub>2</sub>O. Nevertheless, their water solubility was a necessary condition for effective manifestation of antioxidant properties. Our research [5] showed that any lignin samples that were soluble in organic solvents were capable of converting to a water-soluble state under certain conditions. Furthermore, water-soluble lignins exhibit clearly pronounced antioxidant properties despite a rather high molecular weight. The AOA parameters differed for lignins isolated from woody and herbaceous plants [6]. Therefore, it seemed interesting to establish the relationship between the structure and antioxidant properties of these biopolymers.

The results (Table 1) showed that dioxanelignin isolated from *Rhodiola rosea* L. (DL-RR) had the highest AOA with a parameter of 117.0 kC/100 g. The sample isolated from *Avena sativa* grain husks (DL-OO) had a slightly lower value of 81.8 kC/100 g. The AOA of the sample obtained from *Brassica oleracea* (DL-K) had the lowest value (24.1 kC/100 g). Both coulometry and spectroscopy gave similar values for the AOA.

The results showed that the antioxidant properties of the lignins actually depended on the taxonomic level of the plants and were apparently determined by the structural and chemical features of its macromolecules. Therefore, we next studied the chemical structures of the lignin samples in order to find the relationship between the chemical structure and the AOA.

Table 1 shows the elemental analyses and calculated so-called C<sub>9</sub>-formulas of the polymer monomeric phenylpropane unit. According to chemotaxonomic studies, lignins are assigned to one class or another depending on the relative contents of guaiacyl (G), syringyl (S), and *p*-coumaryl (H) units, which differ considerably in elemental composition and number of methoxyls.

The results showed that the sample with the maximum AOA, i.e., DL-RR, had the lowest carbon content whereas the sample with the maximum carbon content had a low AOA value. Figure 1a and 1b shows the AOA as a function of elemental composition. The points were significantly scattered, meaning that the AOA of the samples had a complicated dependence on the contents of O and H atoms. Mathematical data analysis that allowed quantitative characteristics of the goodness of the correlation to be established was used to find the relationship between the various quantities.

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TABLE 1. Antioxidant Activity and Chemical Properties of Lignins Isolated from Various Plant Species

Species	Sample	AOA, kC/100 g	OH <sub>phen</sub> , %	COOH, %	C, %	H, %	C <sub>9</sub> -formula
<i>Rhodiola rosea</i>	DL-RR	117.0	4.9	2.2	56.2	4.9	C <sub>9</sub> H <sub>7.4</sub> O <sub>4.3</sub> (OCH <sub>3</sub> ) <sub>1.0</sub>
Seed husks of <i>Avena sativa</i>	DL-OO	81.8	6.6	4.3	60.5	5.3	C <sub>9</sub> H <sub>7.39</sub> O <sub>3.21</sub> (OCH <sub>3</sub> ) <sub>1.05</sub>
<i>Serratula coronata</i>	DL-C	71.8	1.9	2.3	59.0	6.3	C <sub>9</sub> H <sub>9.1</sub> O <sub>3.0</sub> (OCH <sub>3</sub> ) <sub>1.4</sub>
<i>Avena sativa</i>	DL-O	69.4	4.3	2.7	59.2	5.30	C <sub>9</sub> H <sub>9.72</sub> O <sub>3.52</sub> (OCH <sub>3</sub> ) <sub>1.09</sub>
<i>Triticum</i> sp.	DL-P	61.3	2.6	4.2	60.3	5.66	C <sub>9</sub> H <sub>10.23</sub> O <sub>3.62</sub> (OCH <sub>3</sub> ) <sub>0.95</sub>
<i>Sorbus aucuparia</i>	DL-R	50.6	2.4	3.3	57.7	5.9	C <sub>9</sub> H <sub>11.4</sub> O <sub>3.5</sub> (OCH <sub>3</sub> ) <sub>1.6</sub>
<i>Larix sibirica</i>	DL-L	45.8	3.4	1.5	63.3	6.4	C <sub>9</sub> H <sub>9.23</sub> O <sub>2.6</sub> (OCH <sub>3</sub> ) <sub>0.94</sub>
<i>Festuca pratensis</i>	DL-OL	41.5	4.9	4.3	60.8	5.60	C <sub>9</sub> H <sub>10.0</sub> O <sub>3.2</sub> (OCH <sub>3</sub> ) <sub>0.91</sub>
<i>Robinia pseudoacacia</i>	DL-A	37.0	3.9	2.7	58.4	6.4	C <sub>9</sub> H <sub>9.16</sub> O <sub>3.16</sub> (OCH <sub>3</sub> ) <sub>1.35</sub>
<i>Brassica oleracea</i>	DL-K	24.1	3.6	3.0	59.6	6.6	C <sub>9</sub> H <sub>9.60</sub> O <sub>2.52</sub> (OCH <sub>3</sub> ) <sub>1.38</sub>

TABLE 2. Linear Correlation Coefficients and Mean-Square Error  $S_D$  for the Equations  $y = a + bx$  Relating Antioxidant Activity and Lignin Elemental Composition

Equation	$a$	$\Delta a$	$b$	$\Delta b$	$R$	$S_D$
AOA–H	274.7	58.5	–36.8	9.9	–0.80	17.2
AOA–O	–195.9	116.3	7.4	3.4	0.62	22.3

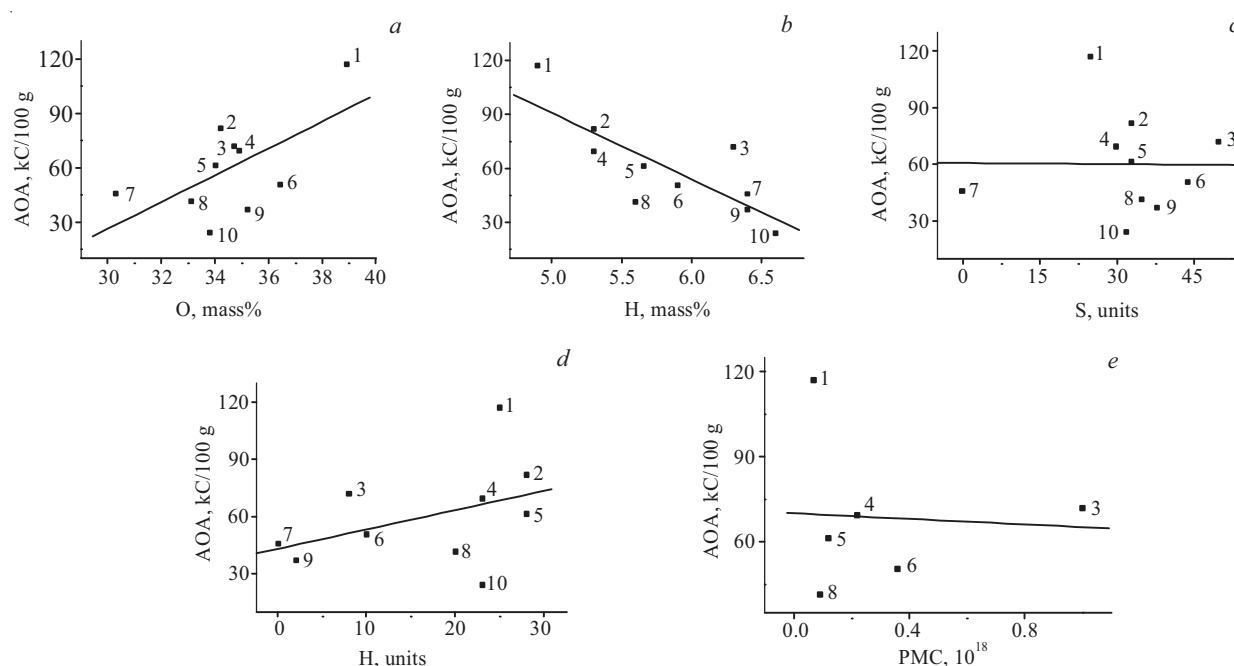


Fig. 1. AOA as a function of O (a) and H (b) atom contents and syringyl units ( $R = -0.01$ ) (c) and *p*-coumaryl ( $R = 0.41$ ) units (d) and the number of PMC in lignin samples (e).

First, we checked the statistical hypothesis of a linear relationship between random variables with a normal distribution. Least-squares methods found the parameters of the regression line (regression equation), the standard deviations of these parameters, and the approximation error and calculated the sample correlation coefficient. The mathematical analysis established that the relationship between the content of H atoms and the AOA of the lignin samples could be written as the linear equation  $y = 274.7 - 36.8x$ ,

where  $y$  is the AOA and  $x$ , the H content (mass%).

Table 2 presents the parameters of the regression equation  $y = ax + b$ , correlation coefficient  $R$ , and the standard deviation  $S_D$ .

TABLE 3. EPR Spectral Parameters and PMC Concentrations in Lignins

Sample	$g$ -Factor	$\Delta H$ , mT	PMC concentration, $c \times 10^{18}$ , spin/g	Length of conjugated C–C chains
DL-RR	2.0039	0.43	0.07	13
DL-C	2.0043	0.63	1.0	–
DL-O	2.0044	0.78	0.22	12
DL-P	2.0042	0.81	0.12	11
DL-R	2.0043	0.88	0.36	10
DL-OL	2.0042	0.63	0.09	18

These data indicated that the parameters were correlated. In particular, increasing the content of O atoms in the sample had a positive effect on their AOA.

We used  $^{13}\text{C}$  NMR spectroscopy as the characteristic feature of the chemical structure of the various lignins. The number of basic structural units was calculated using the integrated intensity of aromatic  $\text{C}_{\text{ar}}$  resonances in the range 100–160 ppm and a quantitative determination of the number of  $\text{OCH}_3$  groups (Table 1). This indicated that the biopolymer samples had heterogeneous compositions consisting of monomeric guaiacyl (G), syringyl (S), and  $p$ -coumaryl (H) units. The ratio of H, G, and S structures for lignin DL-C was estimated as 8:42:50; for DL-RR, 25:50:25. For comparison, lignin from *Larix sibirica* had a homogeneous composition because it was constructed exclusively of guaiacyl units (H:G:S, 0:100:0). The fraction of  $p$ -coumaryl units in lignin DL-A from *Robinia pseudoacacia* was low (H:G:S, 2:60:38) and slightly greater in mountain ash lignin DL-R (10:46:44). Grain lignins were also GSH-lignins (H:G:S ratio for DL-P from wheat, 28:39:33; from oats DL-O, 23:47:30; for oat grain husks, 28:39:33; for rye lignin, 0.53:1.0:0.69; for barley lignin, 0.79:1.0:0.98; and for fescue grass DL-OL, 20:45:35). It was assumed that the compositional heterogeneity of the polymer structural units did not substantially affect the antioxidant properties of the lignins. However, the number of syringyl units and AOA were not related ( $R = -0.01$ ). Moreover, the number of  $p$ -coumaryl units had a positive effect ( $R = 0.41$ ) on the AOA of the samples (Fig. 1c and 1d).

All natural lignins are known to exhibit paramagnetic properties due to the presence of stable free radicals in them. Research showed that lignin EPR signals were practically isotropic singlets with  $g$ -factors in the range 2.0039–2.0043 (Table 3). EPR spectra could also be characterized by the signal intensity and the shape and width of the spectral line  $\Delta H$ .

The lignin contained stable phenoxy free radicals because of the lignin structural organization that was formed by dehydrogenation during free-radical polymerization. According to several hypotheses, the presence phenoxy radicals provided plant lignins with AOA. Substituents on the benzene ring that caused steric hindrance and also chains of conjugated C–C double bonds could enhance the stability of the phenoxy radical. The mechanism of action of the lignin as an antioxidant could be refined by considering that natural lignin contains unpaired electrons that are localized primarily on the phenoxy O atom and interact with the lignin macromolecule and a certain free radical to induce oxidation of some organic compounds or others. The lignin donates an electron and thereby reduces the free radical to a stable inactive product. Is there a relationship between the number of paramagnetic centers (PMC) and the AOA? At this time, the question is open although, as the calculations showed, a substantial dependence between the AOA and the number of DL-OO PMC was not observed.

A comparison of the lignins with known antioxidants found that the AOAs of lignins DL-OO (81.8 kC/100 g), DL-O (69.4), and DL-P (61.3) were greater than that of rutin (61). The DL-RR sample had greater AOA than mitofen (92.6) and ascorbic acid (109.6).

Quercetin typically has a very high AOA (127.7 kC/100 g), is known to be one of most effective antioxidants, and is widely used in medical practice. In contrast with quercetin and other recognized antioxidants, lignins are high-molecular-weight compounds. It is possible that polymeric antioxidants will find applications and, considering that several samples (e.g., lignin isolated from *R. rosea*) are practically equivalent to known antioxidants, that new pharmacological antioxidant drugs based on natural lignins could potentially be developed.

## EXPERIMENTAL

Samples of slightly altered lignins were isolated using the dioxane method [7] by treating ground defatted plant raw material with refluxing aqueous dioxane (9:1) in the presence of HCl (0.7%). The starting lignin-containing raw materials were ripe stalks of *A. sativa* L., *Triticum* sp., *Festuca pratensis* Huds. (Sysol'skaya Variety Test Station, Vizinga, Komi Republic),

and *Brassica*; wood of *Sorbus aucuparia*, *Larix sibirica*, and *R. pseudoacacia*; roots of *R. rosea*; and stalks of *Serratula coronata* L. (Inst. Biol., Komi Sci. Cent., Ural Br., RAS, Syktyvkar, Komi Rep.).

Samples of dioxanelignins were purified by reprecipitation from dioxane into an aqueous solution of  $\text{Na}_2\text{SO}_4$  and  $\text{Et}_2\text{O}$  and were lyophilized. Functional groups were determined by standard methods used in lignin chemistry [8].

Elemental analysis was performed on a Hewlett–Packard analyzer (USA).  $^{13}\text{C}$  NMR spectra were recorded in pulsed mode on a Bruker AM-300 spectrometer at operating frequency 75.5 MHz. The spectral width was 18,000 Hz; pulse length, 2  $\mu\text{s}$ ; time between pulses, 5 s. The solvent was  $\text{DMSO-d}_6$  containing *Cr tris*(acetylacetonate) (0.02 M) (relaxant). The lignin solution concentration was 15%. The number of scans was 20,000–40,000. Quantitative calculations of  $^{13}\text{C}$  NMR spectra used the literature procedures [9].

EPR spectra of the compounds were studied on an SE/X-2547 radiospectrometer (RadioPAN, Poland) in the X-band with 100 kHz HF modulation at room temperature and microwave power 2.2 mW using an RX 102 rectangular resonator in TE 102 mode. The free-radical concentration was estimated using standard diphenylpicrylhydrazyl with  $(7.0 \pm 0.7) \times 10^{16}$  and  $(2.7 \pm 0.3) \times 10^{18}$  spins/g. The uncertainty of the PMC concentration was 15%.

AOA was assessed by Coulombic titration of electrically generated Br compounds [10]. We used an Ekspert-006 Coulombic analyzer (Ekoniks-Ekspert Ltd., Moscow) with glassy carbon electrodes. The cathode and anode compartments were separated by a semi-permeable membrane. The working electrodes were Pt. The amount of charge in Coulombs that was consumed in the titration was calculated using the formula  $Q = (100 \times I \times t) / V_{\text{al}}$  where I is the current (A); t, the time to reach the titration end point (s);  $V_{\text{al}}$ , the aliquot volume (mL). The AOA measurement units were kC/100 g of tested compound. The lignin aqueous solution concentration was 2%.

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