STRUCTURE AND PROPERTIES OF LIGNIN AS AN ADSORBENT FOR MYCOTOXIN T-2

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The chemical structures of dioxane lignins isolated from several plant species were determined. Their adsorption capacity for mycotoxin T-2 was estimated. The surfaces and porous structures of the lignins were characterized. A correlation was found between the adsorption capacity and the chemical structures of the lignin preparations. It was shown that the preparation isolated from Juglans regia *wood had the highest adsorption parameters.*

Keywords: lignins, chemical structure, mycotoxin T-2, adsorption.

Lignins are promising natural compounds for designing new classes of polyfunctional biomedical preparations, e.g., antioxidants, onco- and geroprotectors, and enterosorbents [1]. It was found that technical lignins were excellent adsorbents for mycotoxin T-2, a metabolic product of *Fusarium* fungi [2, 3]. Deficiencies of technical lignin adsorbents are the variable composition of the preparations due to industrial processing parameters and undesirable impurities such as sulfur and ash. Therefore, it seemed interesting to evaluate slightly altered lignin preparations as adsorbents. Herein, the chemical structures of Pepper lignins isolated from various plant species were determined and their adsorption capacities for mycotoxin T-2 were evaluated.

The obtained dioxane-lignin preparations were beige or light-brown powders that were insoluble in H_2O and nonpolar solvents and soluble in polar organic solvents and basic aqueous solutions.

The results (Table 1) showed that preparation LJ, which was isolated from *Juglans regia* wood, had the highest adsorption coefficient Q_S of 81.4% for mycotoxin T-2. The value for preparation LT was slightly lower at 69.8%. Preparation LBt from *Betula verrucosa* wood had the lowest adsorption capacity. The adsorption coefficient is known to be due to both adsorbent surface properties, i.e., the specific surface area (SSA) for adsorption, and macromolecular structural features that affect chemisorption.

Table 1 also presents experimental data characterizing the lignin chemical structure. The elemental compositions of all preparations were practically the same. The C contents varied over a rather narrow range of 59.6–60.3%; O, 33.8–34.1%. The chemical structures of the lignins and the adsorbate were considered in interpreting results of adsorption measurements. Mycotoxin T-2 has a polycyclic structure with various functional groups including hydroxyl, ester, an ethylene double bond, and an oxirane ring (Fig. 1*a*).

Lignin macromolecules contained hydroxyl, methoxyl, carboxyl, and several other functional groups. A unique feature of natural lignins that differentiates them from cellulose and other biopolymers is the variability of the chemical structure, including the quantitative composition of functional groups, as a function of taxonomic origin.

TABLE 1. Chemical Characteristics and Adsorption Properties of Lignins for Mycotoxin T-2

Parameter	*Juglans regia*	*Triticum* sp.	*Brassica oleracea*	*Althaea officinalis*	*Betula verrucosa*	Polyphepan [3]**
Preparation	LJ	LT	LBr	LA	LBt	PP
Q, %	84.0	73.0	66.6	58.3	41.9	52.8
D_S, %	3.1	4.3	5.1	5.7	23.8	18.1
Q_S, %	81.4	69.8	63.25	55.0	31.9	34.7
OH_{ph},* %	4.2 ± 0.05	2.6 ± 0.04	3.6 ± 0.03	3.1 ± 0.06	1.9 ± 0.04	–
COOH*, %	4.4 ± 0.02	4.2 ± 0.05	3.0 ± 0.06	2.7 ± 0.04	2.0 ± 0.05	0.87
C*, %	57.6 ± 1.6	60.3 ± 1.9	59.6 ± 1.5	58.0 ± 1.4	60.0 ± 1.6	–
H*, %	5.6 ± 0.3	5.7 ± 0.5	6.6 ± 0.6	5.8 ± 0.6	5.9 ± 0.5	–
$MM \times 10^{-3}$	11.5	14.4	28.3	–	23.2	–

*Limits of absolute error X (P = 0.95), **preparation of enterosorbent based on hydrolyzed lignin (ZAO Saintek). C_9-formula for *Juglans regia* $[C_9H_{10.8}O_{3.5}(OCH_3)_{1.5}]$; *Triticum* sp. $[C_9H_{10.23}O_{3.62}(OCH_3)_{0.95}]$; *Brassica oleracea* $[C_9H_{9.60}O_{2.52}(OCH_3)_{1.38}]$; *Betula verrucosa* $[C_9H_{9.61}O_{3.1}(OCH_3)_{1.56}]$.
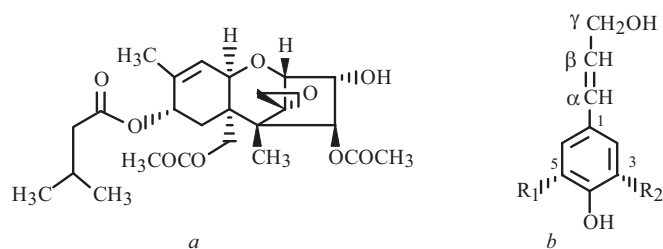

Fig. 1. Structural formula of mycotoxin T-2 (*a*); general formula of lignin monomeric unit (*b*); $R_1 = R_2 = H$, *p*-coumaroyl alcohol (H-un); $R_1 = H$, $R_2 = OCH_3$, coniferyl alcohol (G-un); $R_1 = R_2 = OCH_3$, sinapyl alcohol (S-un).
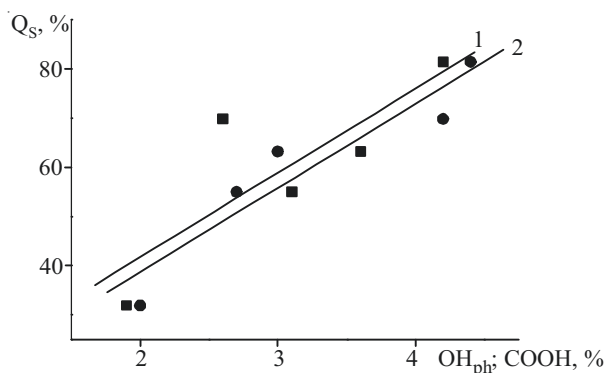

Fig. 2. Adsorption capacity Q_S as a function of number of OH_{ph} (1) and COOH (2) groups in lignin preparations from Table 1.

Preparation LJ had the greatest number of reactive functional groups, i.e., phenols and carboxylic acids. This preparation contained twice the number of these acidic groups as LBt wood lignin.

Mathematical analysis was used to reveal relationships among various variables. This enabled the tightness of the correlation relationship to be characterized quantitatively. The statistical hypothesis that the various normally distributed random variables were linearly dependent was checked. Regression equation parameters were found. Standard deviations, mean-square deviations S_D, and correlation coefficients R of those parameters were calculated.

An analysis of the experimental results showed that the number of ionizable groups and the adsorption capacity Q and Q_S of the preparations for mycotoxin T-2 were closely correlated (Fig. 2). In particular, it was found that the contents of carboxylic acids and the adsorption capacity of the lignins were related by the equation $y = 4.5 + 17.0x$, where $y = Q_S$; x, content of COOH groups (%). Table 2 presents the regression equation ($y = a + bx$) parameters, correlation coefficients R, and standard deviations S_D. Calculations showed that the linear correlation coefficient R between Q_S and the number of COOH groups in the lignin samples was 0.94. The correlation coefficient R for the pair Q_S–OH_{ph} was 0.82.

TABLE 2. Linear Correlation Coefficients and Mean-Square Deviations S_D for Equations $y = a + bx$ Relating Adsorption Capacity Q_S and Lignin Characteristics

Ratio	a	Δa	b	Δb	R	S_D
Q_S–OH_{ph}	7.4	22.0	17.1	6.9	0.82	12.3
Q_S–COOH	4.5	12.3	17.0	3.6	0.94	7.4
Q_S–H_{un}	4.6	17.5	2.6	0.8	0.92	9.9
Q_S–MM	93.1	31.4	−1.6	1.5	−0.60	20.7
Q_S–SSA by BET	61.4	25.0	−0.07	1.5	−0.03	21.4
Q_S–SSA of meso- and macropores	62.2	21.8	−0.15	1.47	−0.06	21.4

TABLE 3. Number of Guaiacyl (G), Syringyl (S), and *p*-Coumaroyl (H) Structural Units per 100 PPU

Preparation	H	G	S	Preparation	H	G	S
LJ	25	34	41	LBr	23	45	32
LT	28	39	33	LBt	11	40	49

Thus, it was logical to assume that mycotoxin was adsorbed via interactions of mycotoxin and lignin adsorbent functional groups, including most probably ester and ether covalent bonds.

Lignins are assigned to one chemotaxonomic class or another depending on the relative contents of guaiacyl (G), syringyl (S), and *p*-coumaroyl (H) phenylpropane units (PPU) with differing degrees of methoxylation (Fig. 1*b*). The so-called average C_9-formulas of the lignins (Table 1) indicated that they had considerably different compositions (of monomers). This could also have been a reason for the different adsorption capacities of the lignins.

The numbers of monomeric H, G, and S units were counted using ^{13}C NMR spectroscopy. Spectra of slightly altered lignins were sets of large numbers of resonances. This indicated that the chemical structures of these biopolymers were exceedingly complicated. Resonances of CH, CH_2, and CH_3 C atoms appeared in the range 5–45 ppm.

A large number of resonances was observed in this range for all preparations except LBt. This indicated that the chemical structure of the side aliphatic chains was highly variable. The methoxyl C atoms gave a strong resonance with chemical shift ~56.0 ppm. Resonances at 100–160 ppm were due to various types of aromatic structural units, e.g., 100–117 ppm, resonances of tertiary aromatic C atoms (C-2 and C-5 in uncondensed guaiacyl units or C-2 and C-6 in syringyl units); 117–125 ppm, resonances of tertiary aromatic C atoms C-2 and C-6 in *p*-coumaroyl units and C-6 in guaiacyl units; 125–142 ppm, resonances of aromatic quaternary C atoms (C-1 and C-5); 142–160 ppm, resonances of esterified aromatic C atoms. Calculations of the amounts of main structural units using integrated intensities of resonances for aromatic C_{ar} atoms in the range 100–160 ppm and quantitative determination of the OCH_3 contents indicated that the studied polymers consisted of macromolecules with different numbers of guaiacyl, syringyl, and *p*-coumaroyl structural units (Table 3).

The various structural units were present in comparable amounts in LT and LBr samples. Lignin of *B. verrucosa* LBt was dominated by guaiacyl and syringyl structural units with only one of ten units being the H-type. A close correlation was found (Fig. 3*A*) between the adsorption capacity and number of *p*-coumaroyl structural units ($R = 0.92$ for the pair Q_S–H_{un}). Table 2 presents correlations indicating convincingly that the chemical structure of the lignins played a very important role in the adsorption of mycotoxin T-2.

The key parameters for characterizing the surface and porous structure of adsorbents were the SSA and pore size and volume. Table 4 presents the results for these.

The results showed that preparation LBr had the best characteristics with respect to SSA. The total SSA calculated using the Langmuir equation was 95.3 m^2/g; by the BET method, 26.1 m^2/g. Because the total SSA was formed by meso- and macropores, it was fully expected that preparation LBr would exceed the other samples with respect to both total pore volume and SSA. Nevertheless, the adsorption capacities Q and Q_S of this sample for mycotoxin T-2 were noticeably less than those of LJ and LT. Lignin LJ, which was isolated from *J. regia* wood, had the smallest SSA. However, the adsorption capacities Q and Q_S of LJ were much greater than the analogous parameters of all other preparations. An analysis of the correlations (Table 2, Fig. 3*B*) indicated that neither the SSA nor the lignin porous structure had a noticeable effect on the adsorption parameter ($R \ll 0.1$).

TABLE 4. Surface Properties of Lignin Samples

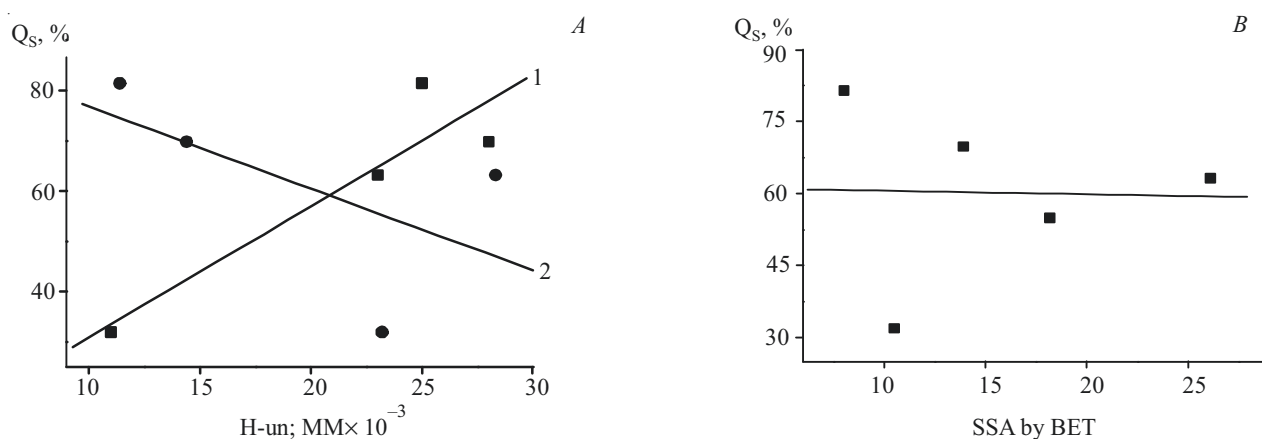| Parameter	LJ	LT	LBr	LA	LBt
SSA by BET, m^2/g	8.0	13.9	26.1	18.2	10.5
SSA by Langmuir, m^2/g	11.1	61.4	95.3	68.4	38.0
SSA of meso- and macropores, m^2/g	4.3	14.4	23.3	16.1	8.6
SSA of mesopores, m^2/g	2.2	9.9	14.2	10.2	6.9
SSA of micropores, m^2/g	3.7	0.0	2.8	2.1	1.9
Total pore volume by BET, cm^3/g	0.01	0.05	0.06	0.05	0.03
Mesopore volume, cm^3/g	0.01	0.03	0.03	0.02	0.02
Average pore width, nm	1.54	1.76	1.53	1.62	1.63

Fig. 3. Adsorption capacity Q_S as a function of p-coumaroyl units (H-un) (1) and lignin molecular mass (2) (A) and BET SSA (m^2/g) of lignin preparations from Table 4 (B).

Previous results [3] suggested that the adsorption parameters for mycotoxin T-2 by technical lignins could also depend on the preparation molecular mass (MM). Table 1 presents results from MM determinations of the studied lignin samples. A comparison of their adsorption and macromolecular characteristics indicated that Q_S and MM were definitely related (Fig. 3A, line 2, $R = -0.60$). It could be assumed that this was related to the different accessibility of functional groups localized in macromolecular globules of different sizes.

Thus, the adsorption capacity for mycotoxin T-2 depended mainly on the lignin preparation chemical structure. The preparation isolated from *J. regia* wood had the highest adsorption parameters. A comparison of mycotoxin adsorption, surface and porous structures, and chemical structures of the different lignins showed that chemisorption was the most important mechanism whereas physical effects were insignificant.

EXPERIMENTAL

Lignins were obtained from wood of *J. regia* and *B. verrucosa*, stems of *Triticum* sp., *Althaea officinalis*, and *Brassica oleracea*. Air-dried raw material was mechanically ground and extracted with H_2O and an EtOH–benzene mixture by the known method in order to prepare the biomass for subsequent lignin extraction. Lignin preparations were isolated by the Pepper dioxane method [4], i.e., defatted plant material was refluxed in aqueous dioxane (9:1) containing HCl (0.7%). Functional groups (phenolic hydroxyls OH_{ph}, carboxylic COOH, methoxyl OCH_3) were determined by standard methods used in lignin chemistry [5].

Dioxane lignin preparations were analyzed for C and H contents using a Hewlett Packard analyzer (USA). ^{13}C NMR spectra were recorded in DMSO-d_6 containing Cr(acac)$_3$ (0.02 M) on a Bruker AM-300 at 75.5 MHz, spectral width 18,000 Hz, pulse length 2 μs, and 5 s between pulses. The lignin solution concentration was 15%. The number of scans was 20,000–40,000. The Nuts program and published procedures were used for quantitative calculations using the ^{13}C NMR spectra [6].

Mycotoxin T-2 was adsorbed from a solution of concentration 50 μg with a 1:1000 toxin-to-adsorbent ratio at pH 2.0 and 37°C. Then, the solution was centrifuged. Toxin was back-extracted from the supernatant by $CHCl_3$ (3 × 20 mL). The $CHCl_3$ extracts were combined and evaporated to dryness in a rotary evaporator. The residual amounts of mycotoxin T-2 in the dry residue were determined quantitatively by TLC-bioautography using a culture of *Candida pseudotropicalis* strain 44 PK [7, 8]. The mycotoxin adsorption coefficient at pH 2 (Q, %) and its desorption coefficient at pH 8 (D_S) were determined. The quantity Q was the average adsorption coefficient expressed in percent of total amount of mycotoxin used in the experiment. The number of tightly adsorbed mycotoxins (Q_S, %) was calculated by difference from the adsorption Q and desorption D_S parameters.

The molecular masses (MM) of LT, LBr, and LBt were determined using a MOM-3180 analytical ultracentrifuge (Hungary) and sedimentation–diffusion analysis [9]. MM were calculated using experimental diffusion coefficients D_0, sedimentation rate S, and characteristic viscosity $[\eta]$ of sample fractions and the equations $M_{SD} = SRT/(1 - \bar{v} \rho_0)D_0$ and $M_{D\eta} = A_0^3([D]^3[\eta])$, where A_0 is the Tsvetkov–Klenin hydrodynamic invariant. The MM of the LJ sample was determined using the Mark–Kuhn–Houwink equation $[\eta] = 2.3 \times 10^{-2} M^{0.64}$.

SSA was determined and lignin porous structure was studied using an automated ASAP 2020MP system (Micromeritics, USA) designed to measure adsorption capacities using gas volumes. The instrumental error of the measurements was 0.12–0.15%. Lignins samples were studied by low-temperature N_2 adsorption (77 K).

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