

A STUDY OF THE MOLECULAR-WEIGHT DISTRIBUTION
OF BIOSYNTHETIC LIGNINS AND MILLED SPRUCE
AND REED LIGNIN

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In a preceding paper [1] we gave the results of a comparative study of the chemical composition and IR spectra of biosynthetic lignins isolated by the enzymatic dehydrogenation of coniferyl and p-coumaryl alcohols (DHP-1 and DHP-2) with the milled wood lignin (MWL) of Phragmatis communis and spruce.

In the present paper we consider the molecular weights (MWs) and polydispersities of these preparations. Biosynthetic dehydropolymers, which are of great importance in the study of natural lignin, were first obtained by K. Freudenberg from various precursors. However, they have scarcely been investigated up to the present time from the point of view of high-molecular-weight chemistry and have not been compared with natural lignin.

The molecular-weight distribution (MWD) of the lignin preparation was found from gel chromatograms when the samples were eluted through a column of Sephadex G-75 calibrated with respect to MW.

It has been shown previously [2] that in gel filtration through Sephadex G-75 with dimethyl sulfoxide as eluent, the distribution coefficient (K_{av}) of the MWL fractions of spruce is connected with their MWs by the following expression:

$$K_{av} = 2.9 - 0.65 \cdot \lg \bar{M}_w, \quad (1)$$

where \bar{M}_w is the weight-average molecular weight.

We used the same equation to find the MWs of fractions of lignin preparations, for which purpose the gel chromatograms were separated graphically into a number of sections for which we determined the weight fractions and distribution coefficients.

$$K_{av} = V_e - V_0 \cdot V_t - V_0. \quad (2)$$

Here, V_t , V_0 , and V_e are, respectively, the total volume of the gel, the volume of the solution between the gel granules, and the volume of the eluate before the appearance of the given fraction plus half the volume of the fraction, ml.

The MWs of the individual fractions were calculated by means of Eq. (1), and MWD curves were plotted by the usual method [3].

It can be seen from Fig. 1a that reed MWL possesses a relatively broad unimodal distribution with respect to MW which is similar in nature to that of spruce MWL (Fig. 1b) although it is shifted in comparison with the MWD of the latter in the direction of lower MWs. (The maximum of the MWD of spruce lignin corresponds to 3000-4000, and that of reed lignin to 2000-3000.) This shape of the MWD curve is typical for degraded polymers, giving in the limit a weight-average distribution of the type

$$\bar{M}_z : \bar{M}_w : \bar{M}_n = 3 : 2 : 1.$$

The reed MWL has not yet achieved this distribution (it has a MWD with $M_z : M_w : M_n = 2.5 : 1.6 : 1$), possibly as a consequence of a deficiency of the number of acts of mechanical degradation in the process of isolation.

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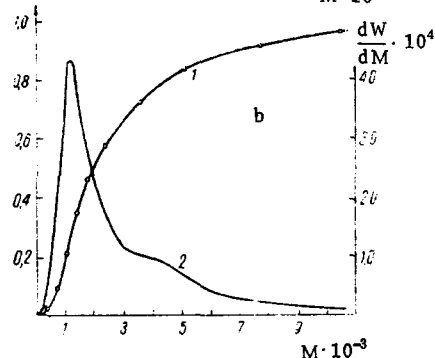
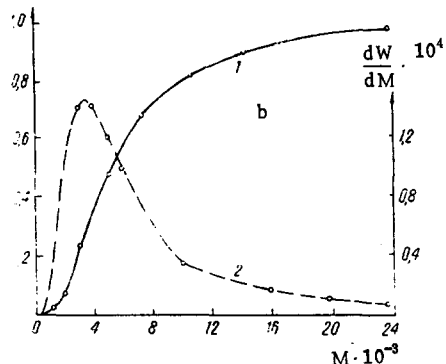
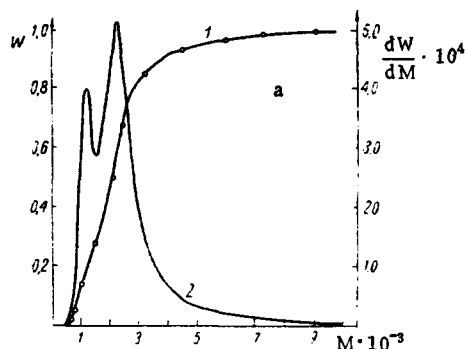
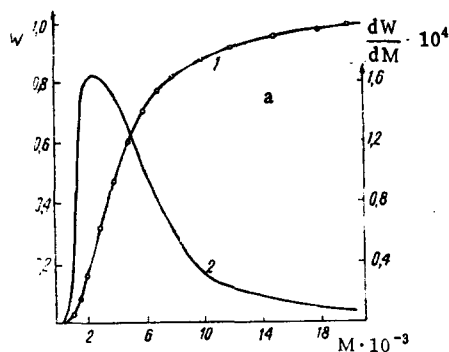


Fig. 1

Fig. 2

Fig. 1. MWD curves of reed (a) and spruce (b) milled lignin. 1 and 2 are the integral and differential curves, respectively.

Fig. 2. MWD curves of DHP-1 (a) and DHP-2 (b). 1 and 2 are the integral and differential curves, respectively.

In contrast to the natural lignins, the MWD curves of the biosynthetic lignins (Fig. 2) possess two maxima. (In DHP-2, the second maximum does not appear so clearly as in DHP-1.) The multimodal distribution shows the heterophase nature of polymerization. Multimodality arises as the result of the co-existence of several polymerization mechanisms [4]. The first peak with a MW of 1100-1250 corresponds to approximately seven phenylpropane structural units in both DHPs, and the second peak to 14 units in DHP-1 and 26 in DHP-2.

The low MW of the "dehydropolymers" (Table 1) shows a partial disturbance of the growth of the live chains and can be explained:

- 1) by the low concentration of monomers in solution (the initial alcohols were strongly diluted in the process of biosynthesis: from 0.2 to 0.033%);
- 2) by the precipitation of the dehydropolymer in the form of a solid phase; and
- 3) by the termination of the growing chain on impurities (on the initiator, of which more than half with respect to the amount of monomer is consumed in the synthesis). An excess of hydrogen peroxide will also be a cause of multiple initiation (including that of the solid-phase polymer), which, in its turn, must lead to an increase in the content of free radicals in the biosynthetic lignins.

Thus, in two-phase polymerization [4] the complete weight-distribution function has two maxima if the radicals are destroyed by disproportionation, and three if they undergo recombination. The bimodality of the MWD of the biosynthetic lignins could indicate that chain termination by disproportionation of the radicals dominates over their recombination reactions. However, according to Freudenberg, the formation of lignin molecules on biosynthesis *in vitro* is considerably more complex and includes several "growth principles." Freudenberg put forward these growth principles mainly on the basis of a study of the intermediates - oligolignols - isolated from the reaction mixture in the incomplete synthesis of lignin. The main growth principle is the recombination of the radicals arising on dehydrogenation; the second in importance is the addition of hydroxyl-containing structures to quinone methides. This process leads to the appearance

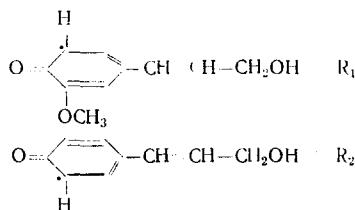
TABLE 1. MWs and Polydispersities of Lignin Preparations Found by the Statistical Treatment of the MWD Curves

Preparations	\bar{M}_w	\bar{M}_n	\bar{M}_w/\bar{M}_n
Spruce MWL	7,050	4,120	1.71
Reed MWL	5,350	3,300	1.62
DHP-1 (from coniferyl alcohol)	2,280	1,730	1.32
DHP-2 (from coumaryl alcohol)	2,930	1,550	1.88

of branching in the chain. Aryl benzyl ethers are capable of polymerization and of the formation of poly(aryl benzyl ethers) [6]. Polymerization through unsaturated side chains of the monomers of the styrene type is also possible to a small extent [7]. According to Freudenberg, the existence of several growth principles makes polymer lignins unique among the known natural and synthetic polymers.

Without rejecting Freudenberg's opinion on the question of the formation of lignin, we nevertheless consider that a more profound comparative study of natural and biosynthetic lignins by the methods of high-molecular-weight chemistry will be necessary in the future. At this stage, the question of the different MWD_S of milled wood lignins and biosynthetic lignins remains open.

On comparing the MWDs of synthesized preparations of lignin, it can be seen that the second maximum in DHP-2 is shifted towards higher MWs (4000 as compared with 2500 for DHP-1). Since the conditions for obtaining the preparations were completely identical, this difference is probably due to the nature of the initial alcohol. In actual fact, if a radical mechanism of the growth of the lignin polymolecule is taken as a basis, as proposed by Freudenberg for the conditions considered [5], it is obvious that on dehydrogenation by peroxidase p-coumaryl alcohol has a greater possibility for the formation of aromatic radicals than coniferyl alcohol through position 3 of the aromatic nucleus, which is not occupied by a methoxy group. Thus, it follows indirectly from an analysis of the MWDs of the "dehydropolymers" that aromatic radicals of types R₁ and R₂:



play a fundamental role in the growth of the polymer.

The high degree of branching of DHP-2 with respect to C-C bonds is also shown by the low yield of ether-soluble phenols (15% as compared with 25% for DHP-1) in the cleavage of the preparations with metallic sodium in liquid ammonia [1]. The yields of vanillin (from DHP-1) and of p-hydroxybenzaldehyde (from DHP-2) from the oxidation of the preparations with nitrobenzene in an alkaline medium do not contradict this conclusion either. DHP-2, having a maximum on the distribution curve at 4000 and having $\bar{M}_w = 2930$, on oxidation with nitrobenzene gave 10.0% of p-hydroxybenzaldehyde, while the yield of vanillin from DHP-1, with a lower molecular weight, was 13.7%. This indicates a greater degree of condensation of the DHP-2 and confirms the leading role of aromatic radicals of types R₁ and R₂ in the chain propagation reactions.

EXPERIMENTAL

Procedure for Gel Chromatography. Volumes of 0.1-0.2 ml of a 0.5% solution of the sample (DHP-1, DHP-2, or spruce or reed MWL) in dimethyl sulfoxide were eluted by DMSO through a column of Sephadex G-75 (d 0.9 cm, h 45 cm) at the rate of 6-8 ml/h at room temperature (16-20°C). Approximately 1-ml fractions were collected by means of a KhKKV automatic collector, and the concentration of lignin in them was measured spectrophotometrically at λ 280 nm.

To calculate K_{av} in an individual experiment we determined V_0 . For this purpose, a solution of Dextran Blue in DMSO was passed through the column. (Dextran Blue is a narrow fraction of the polymer with a molecular weight of $\sim 2,000,000$ and is excluded completely by all types of Sephadex.) The position of the Dextran Blue maximum with respect to the axis of abscissas was taken as V_0 . In our case V_0 was 8 ml and V_t 30 ml, and K_{av} was calculated by means of formula (2) [8].

SUMMARY

1. Molecular-weight distribution (MWD) curves of biosynthetic lignins and of spruce and reed milled wood lignins (MWL) have been obtained, and their molecular weights have been calculated. The MWD curves of the dehydropolymers are bimodal, unlike the unimodal MWD curves of the natural lignins.

2. An analysis of the MWDs has shown that the process of preparation of the biosynthetic lignins consists of a heterophase suspension polymerization taking place by a radical mechanism, the growth of the live radicals not being the result of a single mechanism.

3. From a comparison of the MWDs of the "dehydropolymers," the results of their cleavage by sodium in liquid ammonia and of their oxidation by alkaline nitrobenzene it follows that aromatic radicals of types R_1 and R_2 play a principal part in the chain propagation reactions, leading to an increase in the mean MWs of the biosynthetic lignins.

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