U. D. C. 547.458.84

The Heterogeneity of Natural Lignin

O.P.Grushnikov and N.N.Shorygina

The results of investigations of the molecular-weight distribution of lignin in the delignification of wood by various methods, modern ideas on the distribution of lignin in cell walls of plant tissues, and the presence of bonding between the lignin and the carbohydrate components are critically examined. The results obtained in the course of these investigations show that natural lignin evidently consists of two main components which differ in molecular weight, in their localisation in the cell walls of the plant tissues, in the nature and number of the bonds with the carbohydrate components of the wood, and also in the topochemical models at the time of delignification.
The present review is the first attempt to survey the results relating to the problem of the heterogeneity of natural lignin. There is a bibliography of 143 references.

CONTENTS

I. Introduction 657
II. Molecular weights of preparations of lignin isolated at successive stages of the delignification of wood 657
III. Distribution of lignin in the cell walls of plant tissues 661
IV. Some aspects of the heterogeneity of natural lignin 663
V. The heterogeneity of lignin in relation to its bonding with the other components of plant tissue 665

I. INTRODUCTION

The problem of the homogeneity or heterogeneity of natural (that is, not isolated from plant tissue) lignin is of both theoretical and practical interest. The establishment of homogeneity or heterogeneity of natural lignin is undoubtedly a necessary prerequisite for solving the problem of the nature and number of lignocarbohydrate bonds in plant tissues. As is well known, this problem is of considerable interest and is now attracting the attention of investigators both in the USSR and abroad. Furthermore, the importance of information about the homogeneity or heterogeneity of lignin is also apparent in the development of rational industrial processes for the delignification of vegetable raw materials.

At the same time the complexity of the solution of this problem is primarily due to the fact that wood chemistry does not yet have a method for isolating lignin from lignified plant tissues which does not involve the use of acids, alkalis, or mechanical action, which change natural lignin to a greater or lesser extent. However, despite this the intensive development of the physical chemistry of lignin which began in the middle of the 1950s and was linked with progress in the physical chemistry of polymers which had benefitted by a series of promising methods of investigation, led to the obtaining of a considerable number of results relating to this problem. These results concerned the mechanisms observable in the isolation of lignin from wood by various methods, the distribution of lignin in the cell walls of plant tissues, the nature of the bonds between lignin and carbohydrate constituents and various other questions. The generalisation of these results allows a representation of the present state of the problem of the heterogeneity of natural lignin to be compiled.

In conclusion we note that the results examined in the present review mainly relate to the lignin of coniferous species, which have been investigated most.

II. MOLECULAR WEIGHTS OF PREPARATIONS OF LIGNIN ISOLATED AT SUCCESSIVE STAGES OF THE DELIGNIFICATION OF WOOD

The results of the investigations [1-12] on the fractionation of lignosulphonates isolated as a result of the sulphite digestion of wood, made in the 1940s and 1950s, showed that these preparations had a considerable polydispersity. McCarthy and coworkers [13] made the first attempt to connect the change in molecular weight of the lignosulphonates isolated with the degree of delignification of the wood. They subjected wood flour from *Tsuga*, spruce, and maple to treatment with $NaHSO_3$–H_2SO_3 solutions at 135°C for various intervals of time. It was found that for coniferous woods (*Tsuga* and spruce) the molecular weights of the soluble lignosulphonates isolated as a result of brief (30 min) digestions amount to ~ 2000–4000 (diffusion method). On increasing the duration of the digestion the molecular weight of the lignosulphonates increases as the delignification of the wood proceeds, reaching the maximal value of ~ 11 000 at almost complete delignification (4 to 5 h digestion). Longer digestion reduced the molecular weight to a minimal value of ~ 5000 and, for *Tsuga*, then led to a further increase. The investigators associated these maximal and minimal values of the molecular weights of the lignosulphonates with the action of three processes which occur simultaneously during the sulphite digestion but at different rates: (a) the hydrolysis of hydrolysable bonds in the polymeric lignin; (b) the differential diffusion of soluble lignosulphonates from the wood tissue, with the finer fragments diffusing first; and (c) the irreversible autocondensation of the macromolecules of the lignosulphonates.

A serious drawback of the method used by these investigators is that the dissolution of the lignin is cumulative, so that each fraction contains not only the material dissolved during the given interval of the treatment but also products from the degradation of the lignosulphonates

which had been transferred to the solution in earlier stages of the digestion. The interpretation of these results is hindered by the fact that the main heterogeneous reaction is accompanied by a homogeneous reaction in which the lignosulphonate that has already dissolved undergoes various chemical changes.

Felicetta and McCarthy [14] attempted to improve the cumulative method considered above by "incremental" delignification, in which the wood is treated with the digestion solution in several successive stages. After each stage the residue is separated and subjected to further treatment. They delignified *Tsuga* wood by five successive treatments with $NaHSO_3$–H_2SO_3 solutions, each of which was continued for ~15 h at 130°C. An increased dissolution of the lignin was brought about by increasing the concentration of free SO_2 after each stage except the first (pH of digestion: 4.8, 4.8, 3.4, 2.5, and 2.1). The molecular weights of the lignosulphonates increased from 3400 to 30 000 (diffusion method) in the first four digestions. The final sulphite treatment led to the isolation of a lignosulphonate with molecular weight 20 500.

Table 1. Characteristics of the fractions of dioxan-lignin isolated as a result of four successive extractions of spruce wood [15].

Fraction	Yield, % of Klason lignin		Contents, %		M_w	D_A, $cm^2 s^{-1}$	E_{280}, litre $cm^{-1} g^{-1}$	dn/dc, $ml\ g^{-1}$	$\bar{v}$, $ml\ g^{-1}$
	not purified	purified	OCH_3	carbohydrates					
1	18.1	12.7	15.4	0.03	4300	$1.77 \cdot 10^{-6}$	19.8	0.113	0.703
2	25.0	17.0	15.5	0.02	7400	$1.22 \cdot 10^{-6}$	20.8	0.116	0.712
3	21.0	16.2	15.9	0.03	33000	$0.775 \cdot 10^{-6}$	22.1	0.124	0.693
4	17.6	12.9	15.5	0.03	85000	$0.55 \cdot 10^{-6}$	23.3	0.135	0.701

Rezanowich and coworkers [15] observed a similar tendency to an increase in the molecular weight of the lignin preparations with progress in the delignification of wood during four successive extractions of white spruce sawdust with aqueous dioxan containing a little hydrochloric acid, by Pepper's method [16]. The dioxan-lignin fractions obtained contained methoxyl groups and carbohydrate impurities which showed ultraviolet absorption at 280 mμm (E_{280}), an increase in the refractive index increment (dn/dc), characteristic viscosity, diffusion constant (D_A), partial specific volume ($\bar{v}$), and molecular weight (M_w), determined by sedimentation equilibrium in an ultracentrifuge (Table 1). These results show the homogeneity of the dioxan-lignin fractions in respect of the content of methoxyl groups and carbohydrate impurities, and also of the partial specific volumes. Nevertheless, with increase in the molecular weight of the fractions there is a small but definite increase in the ultraviolet light absorption and in dn/dc. Rezanowich and coworkers [15] consider that these changes in dn/dc and E_{280} are probably connected with differences in the chemical structure of the fractions, and these in turn may be due to the heterogeneity of the lignin in the wood. For example, the dioxan-lignin fractions which are extracted later can contain a greater proportion of biphenyl bonds which could explain the higher molecular weights and refractive index increment observable for these preparations. On the other hand, the chemical changes which accompany the changes in dn/dc and E_{280} can be the result of the prolonged acidolysis of the last fractions, which can also lead to an increase in their molecular weight, owing to polymerisation. Although their results cannot be interpreted with certainty, the investigators assume that slight structural heterogeneity of the dioxan-lignins extractible from spruce wood is superimposed on their polydispersity over a wide range of molecular weights.

Yean and coworkers [17] isolated five lignosulphonate fractions as a result of the successive extraction of spruce wood flour with an acid sulphite solution. The weight-mean molecular weights of the resulting preparations, determined by sedimentation equilibrium in the ultracentrifuge and by light scattering, ranged from 5000 to 130 000, increasing with quantity of lignin extracted from the wood. The ratio M_w/M_n, calculated on the basis of the weight distribution, was 3.1, which shows the appreciable polydispersity of the lignosulphonates isolated in sulphite digestion of spruce wood. This polydispersity is confirmed by the experimental separation of one of the fractions with molecular weight 95 200 by dialysis through membranes with successively increasing pore dimensions as a result of which nine subfractions with molecular weights from 37 000 to 1 270 000 were obtained.

Arlt and Schuerch [18] investigated four fractions of ethanol-lignin, isolated by successive extraction of spruce wood with 0.2 *N* HCl (gas) in an anhydrous mixture (4 : 1) of chloroform and ethanol at 60°C according to a method developed earlier [19]. Table 2 gives some results from this investigation; they relate to ether-insoluble fractions of the ethanol-lignin which had dissolved in chloroform, after their purification from carbohydrates.

Table 2. Characteristics of the fractions of ethanol-lignin isolated as a result of four successive extractions of spruce wood [18].

Fraction	Yield, % of Klason lignin*	Contents, %					Molecular weight (cryoscopic, in ethylene carbonate
		C	H	OCH_3	OC_2H_5	OH_{phen}	
1	33.3	61.24	6.66	12.7	10.00	2.88	528
2	18.4	62.44	6.62	14.3	8.28	2.86	729
3	9.9	62.96	6.34	14.8	6.97	2.72	827
4	8.9	60.74	6.02	14.8	7.55	3.23	1070

*Calculated by us from published results [18,19].

Successive treatments of lignified material with a mixture (1 : 4) of nitric acid (specific gravity 1.4) and 96% ethanol at the boiling point was proposed by Kürschner and Hoffer [20,21] as a method for the delignification of wood. On the basis of the results of an analytical investigation of the fractions obtained by the fractional precipitation of nitrolignin from the combined reaction solutions after several successive digestions of a single portion of spruce wood, Kürschner [22] concluded that the nitrolignin isolated in alcoholic nitration is a homogeneous product. This conclusion met with objections from Fuchs [23], who, by starting from the premise of the heterogeneity of the lignin in the wood, assumed that it could not be homogeneous in its derivative, nitrolignin. On the basis of the results of a chromatographic examination of the nitrolignin isolated from the combined reaction solutions after several successive digestions of a single portion of spruce wood under the conditions of the Kürschner method,

Chuksanova and coworkers[24] concluded that it was heterogeneous: the fractions obtained differed significantly in molecular weight. A more detailed investigation, made by Grushnikov and Shorygina[25], showed that the nitrolignins isolated after each of the successive digestions of spruce wood under the conditions of this method are similar as regards both elemental analyses and functional groups, and also spectroscopically. Nevertheless the molecular weight of nitrolignin isolated after the first nitration is about half that of the nitrolignins isolated after subsequent digestions[26]. Table 3 gives some of the results of this investigation.

Table 3. Characteristics of nitrolignins isolated as a result of successive nitric acid digestions of spruce wood[25,26].

Nitrolignin isolated after nitration	Yield, % of Komarov lignin	Contents, %		Contents, moles per C_6–C_3				Molecular weight (ebullioscopic, in absolute dioxan)
		C	H	NO_2	OCH_3	OC_2H_5	O_2 (COOH)	
First series of experiments								
1	21.0	51.88	5.14	0.79	0.88	0.27	0.25	1524
2	17.2	51.32	5.05	0.73	0.94	0.20	0.28	3054
3	12.0	51.92	4.82	0.74	0.86	0.19	0.29	3105
4	8.2	51.92	4.78	0.74	0.88	0.22	0.27	—
Second series of experiments								
1	26.3	51.26	5.00	0.66	0.92	0.17	0.30	1596
2	17.3	50.20	4.96	0.80	0.94	0.12	0.31	2783
3	10.9	50.38	5.07	0.68	0.89	0.20	0.32	3190
4	5.5	50.17	4.95	0.74	0.92	0.16	0.26	—

Each of the above methods of wood delignification suffers from a significant drawback, the possibility of irreversible changes occurring in the undissolved lignin during the repeated operations of filtration and washing needed to separate the residue from the digestion solutions. A special hazard is the sorption of molecular oxygen, which in repeat heating can facilitate an increase in the molecular weight of the preparations isolated, due to the polymerisation of the lignin.

These drawbacks are eliminated in continuous digestion, in which the wood is treated in a specially constructed autoclave in a continuous stream of digestion liquid at the temperature and pressure corresponding to the standard digestion conditions. Continuous digestion allows the dissolved material to be constantly removed from the autoclave without being subjected to the further action of the digestion solution and it also ensures the absence of contact between the wood being digested and the atmosphere, thus eliminating the possibility of secondary changes in the lignin.

Yean and Goring[27] undertook the acid sulphite digestion (135°C; total SO_2 concentration ~6%) of spruce sawdust by a continuous process. Six specimens of digestion liquid, representing the soluble material at successive stages of the wood delignification process, were withdrawn. The lignosulphonates isolated from these specimens were freed from low-molecular-weight impurities by dialysis and from non-dialysable polysaccharides by electrophoresis-convection. The purified lignosulphonates were analogous in the contents of sulphur and methoxy-groups and also in ultraviolet light absorption (E_{280}) and refractive index increment (dn/dc) (Table 4). However, the mean molecular weight (M_w) increased from 9700 to 143 000 (sedimentation equilibrium in an ultracentrifuge) as the wood was delignified. To investigate the possibility of the condensation of the lignin during treatment with acid hydrogen sulphite solution, two of the fractions isolated were subjected to repeat digestions under various conditions. In every instance degradation of the lignin was observed†. These results were thought by Yean and Goring to show that in acid sulphite digestion polymerisation of lignin either does not occur or is completed in the early stages of the digestion.

Table 4. Characteristics of the fractions of lignosulphonate isolated at various stages of acid sulphite digestion[27].

Fraction	Degree of delignification, %	Contents, %		M_w	E_{280}, litre $cm^{-1}\,g^{-1}$	dn/ds, ml g^{-1}
		OCH_3	S			
1	13.3	13.6	4.7	9700	12.1	0.189
2	34.2	13.4	4.8	22 900	12.4	0.191
3	49.7	12.5	4.4	39 000	12.6	0.181
4	65.1	14.0	4.7	75 400	12.9	0.192
5	79.0	13.8	4.9	100 000	13.2	0.197
6	92.5	14.0	5.7	143 000	13.9	0.198

The results of these investigations showed that ~40% of the lignosulphonate isolated from spruce wood as a result of acid sulphite digestion is capable of being dialysed and consequently has a molecular weight of < 5000. The remaining 60% has a molecular weight in the range from 9700 to 143 000. This molecular weight distribution enabled the investigators to postulate the existence of two types of lignin in spruce wood: low- and high-molecular-weight types and it was suggested that they were localised in different parts of the cell wall of the fibre. To check this assumption Yean and Goring[29] investigated lignosulphonates isolated from morphologically different parts of the wood structure. The differencs between sapwood and heartwood, earlywood and latewood, compressionwood, tensionwood, and ordinary wood were investigated. However, no significant differences in molecular weight were observed between the lignosulphonates isolated on the basis of which it could be concluded that the wide range of molecular weights observed earlier was unconnected with the morphological differences investigated. It was assumed that the results obtained could be explained on in molecular weight of the lignins localised in the material of the middle plate and in the secondary wall, or on the basis of the assumption that in the parts of the wood structure investigated lignins with different molecular weights are homogeneously mixed at the molecular level.

Yean and Goring[30] made a hydrogen sulphite digestion (153°C, pH 4) of spruce wood sawdust by a continuous process. Four specimens with approximately the same weight percentage of lignin were withdrawn during the digestion. The lignosulphonates isolated were purified by dialysis and moving boundary electrophoresis and were then analogous in the contents of methoxyl groups

†According to Reznikov's results[28] even relatively mild chemical action (acid treatment at ⩾ 100°C) of both the lignins isolated and the lignin in wood leads to the condensation of the macromolecules in the solid phase. In this connection Reznikov assumes that from the results on the molecular weights of preparations of lignin isolated from acid medium it is impossible to obtain even approximate ideas of the molecular weight of the lignin in the wood.

and sulphur, ultraviolet absorption (E_{280}), refractive index increment (dn/dc), and also infrared spectra (Table 5). Simultaneously the weighed mean molecular weight (M_w) increased from 10 000 (sedimentation equilibrium in an ultracentrifuge) for the first fraction to 77 000 for the final fraction.

Table 5. Characteristics of the fractions of lignosulphonate isolated at various stages of the hydrogen sulphite digestion of spruce wood[30].

Fraction	Degree of delignification, %	Contents, %		M_w	E_{280}, litre cm^{-1} g^{-1}	dn/dc, ml g^{-1}
		OCH_3	S			
1	8.3	11.8	6.2	10 000	13.3	0.185
2	26.6	12.8	5.6	13 600	12.1	0.181
3	43.1	12.9	5.2	31 000	12.8	0.191
4	54.9	12.6	5.5	77 000	14.1	0.198

A similar tendency for an increase in the molecular weights of lignin fractions during the course of the delignification of wood was also observed in continuous kraft digestion[31]. Some results are given in Table 6.

Table 6. Characteristics of the fraction of kraft lignin isolated at different stages of the kraft digestion of spruce wood[31].

Fraction	Degree of delignification, %	Contents, %			M_w	E_{280}, litre cm^{-1} g^{-1}	dn/dc, ml g^{-1}
		carbohydrates	OCH_3	S			
1	5.5	22	12.1	2.5	1780	20.5	0.191
2	17.2	9	11.2	2.1	11 700	18.8	0.188
3	31.0	16	12.5	1.7	13 100	18.4	0.211
4	45.0	11	12.2	1.5	15 500	18.5	0.211
5	60.0	14	13.1	2.1	32 800	19.0	0.190
6	75.0	12	12.5	1.5	30 900	18.8	0.201
7	88.0	3	11.8	1.7	51 000	17.2	0.197

As the results show, seven fractions representing different stages of the delignification of spruce wood had analogous methoxy-group contents, ultraviolet absorption (E_{280}), and refractive index increment (dn/dc), but their weight mean molecular weights (M_w) increased from 1800 (sedimentation equilibrium in an ultracentrifuge) for the first fraction to 51 000 for the final fraction. Gel filtration on Sephadexes showed the considerable polydispersity of the fractions isolated.

The authors consider that the above results for acid sulphite[27], hydrogen sulphite[30], and kraft[31] digestions of spruce wood by continuous processes have shown that the different processes used for the dissolution of wood lignin do not alter its structural properties to any great extent, as is shown by the similarity in orders of magnitude of the methoxy-group content, ultraviolet absorption, and refractive index increment for lignosulphonates obtained as a result of acid sulphite and hydrogen sulphite digestions and also for kraft lignins (Table 7). In a comparison of lignosulphonates isolated as a result of the acid sulphite and hydrogen sulphite digestions their similar molecular weights at similar degrees of delignification of the wood is noteworthy. In comparison with the lignosulphonates, the kraft lignins have a lower molecular weight than the lignosulphonates, which is attributed to the more rapid degradation of the lignin in the kraft digestion. This regularity can be readily observed in Fig. 1, which shows the change in the molecular weight of the lignin preparations isolated during the course of the wood delignification.

Table 7. Mean value of the content of OCH_3 groups, absorption of ultraviolet light (E_{280}), and refractive index increment (dn/dc) for lignin derivatives isolated as a result of three different digestion processes.

Process	Content of OCH_3 groups, %	E_{280}, litre cm^{-1} g^{-1}	dn/dc, ml g^{-1}
Acid sulphite (Ref.27)	13.6 (±0.4)	12.8 (±0.5)	0.191 (±0.004)
Hydrogen sulphite (Ref.30)	12.5 (±0.4)	13.1 (±0.6)	0.189 (±0.006)
Kraft (Ref.31)	12.2 (±0.4)	18.7 (±1.6)	0.198 (±0.008)

Brown and coworkers[32,33] investigated the isolation of lignin from liquidambar and spruce woods with the aid of brown rot fungus. Lignin preparations were obtained by extracting the wood with dimethylformamide at successive stages of increased enzymatic removal of carbohydrates: 10.5, 27.1, 47.1, 59.5, 77.2, 80.5, 92.6, 97.7, and 100%.

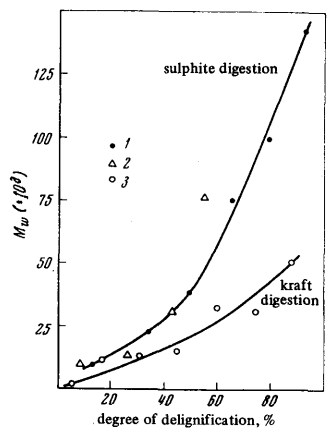


Figure 1. Variation of the molecular weights of the lignin preparations isolated by different methods with the degree of delignification of spruce wood[27,30,31]: *1*) acid sulphite digestion; *2*) hydrogen sulphite digestion; *3*) kraft digestion.

The separation of the resulting extracts on Sephadex G-50 led to the formation of three fractions (1) with molecular weight > 14 000; (2) with molecular weight from ~ 3000 to ~ 14 000; and (3) with molecular weight from 100 to ~ 3000. It was shown that as the proportion of carbohydrates removed increased, the relative quantity of lignin eluted from fraction 1 rose and the amount of lignin eluted from fraction 3 decreased. The relative

amount of lignin eluted from fraction 2 remained approximately constant at all stages of the carbohydrate removal. The observed increase in the ratio of high-molecular-weight to low-molecular-weight components as the carbohydrates are gradually removed was thought to show that wood lignin consists of at least two main components, which differ considerably in molecular weight and are localised in different parts of the cell walls of the fibres.

As regards the lignins isolated from plant tissue, their heterogeneity in molecular weight was shown by gel-filtration of lignin from mechanical grinding [34,35] and of alkali lignins [36], by fractionated dioxan-lignins [37,38] and by Brauns "native" lignin [39], by sulphite [40] and kraft [41] digestions of periodate lignin, and by many other investigations, consideration of which is not the task of the present review.

Hence the above results from studies of the molecular weight distribution of lignin during the delignification of wood by various methods (various digestion procedures, enzymatic isolation) has allowed us to pose the question of the existence of two types of natural lignin, which differ considerably in molecular weight. However, before examining the arguments for and against this hypothesis we must briefly characterise contemporary ideas about the distribution of lignin in the cell walls of plant tissue.

III. DISTRIBUTION OF LIGNIN IN THE CELL WALLS OF PLANT TISSUES

According to contemporary ideas concerning the anatomical structure of wood from coniferous species, the cell walls of moulded tracheids consist of two ontogenetically different structural parts: primary and secondary walls. The primary wall, which is the external sheath of the cell, plays an important part in the period of surface growth, whereas the secondary wall is formed after surface growth ceases. In turn the secondary wall consists of three layers with different optical properties (Figs. 2 and 3). In addition to the morphological subdivisions shown in Figs. 2 and 3, there are indications [46] of the existence of the so-called "warty layer", which covers the tertiary wall at the side of the lumen.

One of the first attempts to investigate the quantitative distribution of lignin in the cell wall was made by Ritter [47]. With the aim of removing the carbohydrate components, he treated 3 mm sections of wood with sulphuric acid according to the standard Klason method for determining lignin and found that middle lamella lignin and cell wall lignin could be distinguished in wood: the former retains the initial wood structure after removal of carbohydrates but the second is structureless. A mechanical separation of the lignin residue thus obtained showed that ~ 75% of the wood lignin is localised in the middle lamella, and ~ 25% in the cell wall and that the lignins localised in these two sites of the plant tissue differ significantly in the methoxy-group content (Table 8). Although the low methoxy-group content shows that the purity of the preparations is insufficient the fact that they are isolated under similar conditions and at the same time differ so much in the methoxy-group content is thought to show a significant difference between the middle lamella lignin and the cell-wall lignin.

On taking into account the volume occupied by the middle lamella in the wood tissue, Ritter was doubtful about the reliability of the values he had obtained which characterise the quantitative distribution of the lignin. The results obtained during this investigation enabled Ritter [48] to conclude that only half of all the wood lignin is localised in the middle lamella. However, in 1934 Ritter [49] confirmed his initial conclusion that the main mass of the lignin is localised in the middle lamella. Subsequently, this conclusion was confirmed in an investigation made by Harlow[50].

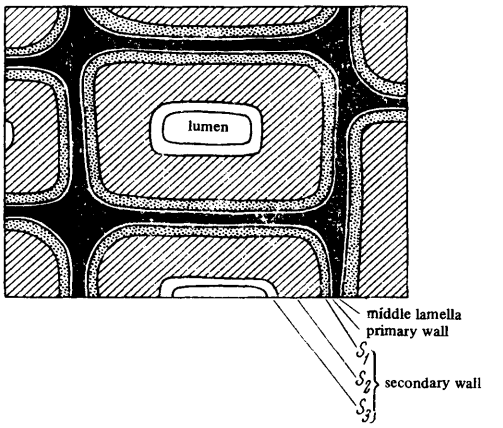


Figure 2. Transverse section of moulded tracheids of wood from coniferales species [42-44]. On the diagram are marked: the middle lamella or intercellular substance; the primary wall; the secondary wall, consisting of three layers: outer or external (S_1), middle or central (S_2), and internal (S_3); the lumen or cell cavity of the fibre.

The results obtained by Ritter and Harlow are evidently connected with the incomplete separation of the two types of lignin, since the mechanical method used for the separation is extremely imperfect, and also with the incomplete removal of carbohydrates, which is evident from the low concentration of methoxy-groups in the lignin preparations mentioned above.

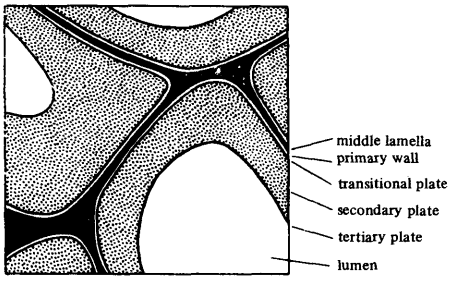


Figure 3. Transverse section of moulded tracheids of coniferales species [45].

Bailey [51,52] used a micromanipulator to separate 0.5 mg of the middle lamella from Douglas fir wood and found that the preparation isolated contained lignin 71, pentosans 14, cellulose 4%. Lange [53], using an ultraviolet microscope,

calculated that the weight concentration of lignin in the secondary cell wall amounted to 10–20%, and that in the middle lamella to 60–90%. The mean values obtained from 50 measurements were 16 and 73% respectively, and he suggested a gradual decrease in the lignin concentration from the middle lamella to the lumen. A series of investigations [54–56] showed that the distribution of lignin in the cell wall was more uniform than according to Lange's suggestion. The results of electron microscope investigations [57–59] of preparations of wood from which the carbohydrate components had been removed by various chemical treatments or by the action of enzymes showed that the distribution of lignin in the secondary cell wall is non-uniform; the lignin concentration is higher in the inner layer (S_3) close to the lumen and in the outer layer (S_1) adjacent to the primary wall.

Table 8. Distribution of lignin between the secondary cell wall and the material of the middle lamella [47].

Characteristic being studied	Red alder			Cuban white pine		
	secondary cell wall	material of middle lamella	total quantity	secondary cell wall	material of middle lamella	total quantity
Lignin, % of wood	7.3	19.5	26.8	6.9	23.4	30.3
Lignin, % of total lignin in wood	27.3	72.7	—	22.7	77.3	—
Methoxyl groups in Lignin, %	4.8	13.6	—	4.3	10.8	—

Investigations into the distribution of lignin in cell walls of plant tissues, published up to 1963, were examined in detail in the reviews of Northcote [60], and of Milyutina and Sergeeva [61].

The problem of the quantitative distribution of lignin in cell walls of plant tissues was critically examined by Berlyn and Mark [62], who emphasised that the results of Bailey and Lange are diametrically opposed to those of Ritter. Noting that the volume of the fractions of the middle lamella and primary-wall substances make up only 10–12% of the volume of the plant tissue, Berlyn and Mark calculated that even if this region consisted entirely of lignin it could not contain more than 40% of the total wood lignin. This surmise found experimental confirmation during an investigation by Goring and coworkers [63]. The method they employed was a combination of ultraviolet microscopy and densitometric analysis of negatives obtained by ultraviolet photomicrography. A special detailed examination of this method [64] showed that its use was well-founded, since when certain experimental conditions are satisfied the method enables the concentration of lignin in the various parts of the cell wall to be determined with sufficient accuracy. Furthermore, it is noteworthy that these investigators [63] calculated the concentration of lignin in the secondary wall by two methods: (1) on the basis of the relative ultraviolet absorption and the volumes of the fractions of different parts of the cell wall, and (2) directly from the absorption in the ultraviolet, using the Lambert–Beer law. The values obtained by the two methods showed good agreement, which also shows the reliability of their method.

Table 9 gives some results obtained by Goring and coworkers and shows that the middle lamella material is more lignified than that of the secondary wall, but despite this it contains only an insignificant proportion of the total lignin of spruce wood.

Table 9. Distribution of lignin in spruce tracheids [63].

Wood	Morphological differentiation*	Relative absorption of UV	Total fraction, %	Lignin, % of total lignin in wood	Lignin concn., g per g wood
Early	SW	1	87.4	72.1	0.225
	ML (r) (t)	2.21	8.7	15.8	0.497
	ML (cc)	3.77	3.9	12.1	0.848
Late	SW	1	93.7	81.7	0.222
	ML (r) (t)	2.7	4.1	9.7	0.60
	ML (cc)	4.5	2.2	8.6	1.0

*SW = secondary wood; ML middle lamella; r radial direction; t tangential direction; cc cell corner.

Scott and Goring [65] also used ultraviolet microscopy in conjunction with densitometric analysis of the ultraviolet photomicrographs obtained in investigating the concentration of lignin in the S_3 layer of wood of coniferous species.

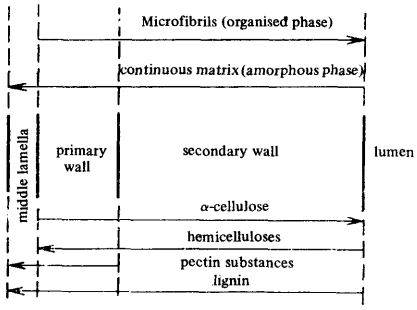


Figure 4. Distribution of the constituents of moulded cell walls. The arrows show the direction of the increase in the relative concentrations of the individual components [60].

Their results showed that in trunk wood a uniform distribution of the lignin in the secondary wall is usually observed. On the other hand, in the wood of the branches a high concentration of lignin in the S_3 layer is often observed.

Hence the above results show convincingly that the distribution of lignin in the cell walls of plant tissues is

non-uniform, and it is interesting to note that according to the results of several investigators [66-69] non-uniformity is also observed in the distribution of the carbohydrate components (hemicelluloses and cellulose) of plant tissue.

In ending the examination of the problems associated with the distribution of lignin in the cell walls of plant tissues, it is noteworthy that according to Northcote's views [60] the cell wall can be regarded as a two-phase system consisting of microfibrils immobilised in a continuous matrix. The microfibrils are concentrated close to the lumen and their density falls in the direction towards the outer parts of the cell, where the other phase prevails. Northcote proposed the following classification of the material of the cell wall:

1. Continuous matrix: (a) pectin substances; (b) lignin; (c) possibly, polysaccharides of the hemicellulose group.

2. Microfibrils: (a) polysaccharides containing glucose (cellulose); (b) polysaccharide (polysaccharides) containing xylose; (c) a polysaccharide (polysaccharides) containing mannose; (d) possibly, polysaccharides of the hemicellulose group.

The scheme presented in Fig. 4 is a representation of the distribution of the various components of the cell wall of plant tissue.

In conclusion, we must note that in comparison with coniferous woods the wood of deciduous species exhibits a more complex anatomical structure and also differs in the nature of the distribution of the lignin in the plant tissue [70].

IV. SOME ASPECTS OF THE HETEROGENEITY OF NATURAL LIGNIN

As stated above, the results obtained during experiments on the delignification of wood, allowed the problem of the existence of two types of natural lignin differing significantly in molecular weight to be settled. However, the solution of the problem of the homogeneity or heterogeneity of lignin not isolated from plant tissue is hindered by the impossibility of an unambiguous interpretation of the results examined in Section II. This is primarily because the use of acids or alkalis during the wood delignification processes considered above leads to more or less significant changes (on the one hand, hydrolytic; on the other, condensation) in the natural lignin, which change its structure significantly [28]. It is now impossible to speak with complete certainty about the absence of depolymerisation or condensation changes in the lignin even in its enzymatic isolation from the wood [33], which is the mildest method of those examined in Section II. Hence the heterogeneity, according to molecular weight, of the lignins isolated from wood by various methods may be due to the heterogeneity of the natural lignin or may be secondary, being the consequence of condensation or hydrolysis of the lignin during isolation.

In this connection other aspects of the problem of the heterogeneity of natural lignin which are of some interest and which can assist in presenting a more complete, even if more complex, picture of the contemporary state of this problem must be considered.

The non-uniformity of the distribution of lignin in plant tissue, considered in Section III, enabled certain investigators [27,30,33] to suggest the localisation of lignins with different molecular weights in different parts of the cell walls of wood tissue. The arguments of these investigators were based on a series of propositions.

According to contemporary ideas [71-74] the first traces of lignification are observed in the primary wall in the cell corners, then the process of accumulation and deposition of the lignin is extended to the middle lamella and throughout the entire primary wall, subsequently into the corners of the middle lamella of the cell, and finally, into the secondary wall (layers S_1, S_2, and S_3, successively, see Fig. 2). Starting from this, it is logical to suggest that the molecular weight of the lignin will be greatest at those parts of the cell wall, where the concentration of the lignin precursors is highest and consequently the degree of condensation polymerisation is increased. Such parts of the cell wall are the primary wall and the middle lamella.

At the same time, according to Cowling's results [75], obtained in the enzymatic isolation of lignin with brown rot fungus, the micro-organisms initially occupy the lumens of the cells of the wood tissue. It is logical to assume that in this connection the enzymatic "digestion" of carbohydrates takes place gradually in the direction of the middle lamella and consequently the lignin in the earlier stages of the enzymatic decomposition of the wood is extracted from the secondary layer and in the later stages from the middle lamella. Such a sequence of enzymatic action is shown by several investigators [45,76,77]. On this basis, Brown and coworkers [33] assumed that the low-molecular-weight lignin extracted in the initial stages of the enzymatic removal of carbohydrates is extracted from the middle lamella. The stated distribution of lignins with different molecular weights in the cell walls will favour a gradient in the size of the spaces between the microfibrils and the distances between the cellulose macromolecules in the amorphous regions from the central plate substance through the secondary wall to the lumen [53]. The electron microscope studies mentioned above [57-59] showed that the greatest density of lignin is observed in the middle lamella, the primary wall, and the S_3 layer. In the S_2 layer of the secondary wall the lignin exists as a branched three-dimensional lattice which immobilises the carbohydrate chains, which exhibit crystal structures. Furthermore all the investigators noted an obvious orientation of the lignin in the S_1 layer.

Finally, as a result of the investigation of the path of penetration of various liquids into wood and of the influence on this process of morphological factors, Wardrop and Davies [78] found that after penetration into the lumens of the cells the reagents used diffuse through the cell walls so that the middle lamella and especially the regions of the cell angles of the middle lamellas are the last parts which react with these agents. In the digestion of wood the path of penetration of the digestion solutions stayed the same but the rate of passage was greatly increased. These results give a basis for suggesting that low-molecular-weight lignin isolated at different stages of the delignification of wood by different methods (see Section II) is localised in the secondary wall, but the high-molecular-weight lignin is localised in the middle lamella.

A special investigation of the topochemistry of the delignification of spruce wood during industrial digestions showed the following regularities [79]. In the early stages of kraft and acid sulphite digestions the lignin is preferentially removed from the secondary cell wall. After ~50% delignification the lignin of the middle lamella is rapidly dissolved, leaving the residual lignin in the secondary wall. In the neutral sulphite digestion the removal of lignin from the secondary cell wall and middle lamella proceeds with approximately the same rates up to 50% delignification. Approximately analogous topochemical models of delignification were observed in kraft and neutral sulphite diges-

tions of birch wood [74]; in the early stages of the digestions preferential delignification of the secondary walls of the fibres and vessels. It is interesting to note that the sequence of the delignification of the various morphological parts of the cell wall of both spruce [79] and birch [74] wood in the order of the digestion processes used is: SW > > ML(cc) > ML(r)(t) (for symbols see Table 9), opposite to that examined above for the deposition of lignin during the lignification of plant tissue.

According to some results [74,79], the above morphological differences in the rate of delignification are not connected with the diffusion of the active reagent into the cell wall or with the diffusion of lignin macromolecules from the cell walls, but are due evidently to the different stability of the lignins to the actions of the digestion solutions. Were the morphological structure of the cell the main factor affecting the rate of delignification, it could be expected that the sudden breakdown of the wood structure (for example, as a result of the compression treatment of the wood at high temperature) leads to a considerable change in the rate of delignification. However, as the investigations of Yean and coworkers [80] have shown, preliminary treatment of spruce and birch sawdusts at pressures up to 35 155 kgf cm^{-2} and temperatures up to 200°C hardly altered the rate of delignification. Analogous results were obtained by Kleinert [81], who showed that preliminary compression of the wood had no effect on the absorption of SO_2 nor on the delignification in sulphite and kraft digestions. These results show that since the changes in the morphological structure of the cells have no significant effect on the rate of delignification of wood, the rate-determining factor for the dissolution of lignin in the various morphological parts of the cell wall is not its accessibility but the chemical structure of the lignins in these regions, which also determines their different degree of stability to the action of digestion solutions.

The mathematical model for the degradation of a three-dimensional gel developed by Szabo and Goring [82], applied to the delignification of spruce wood in kraft and acid sulphite digestions is thought [82] to confirm this assumption. In their calculations Szabo and Goring started from the assumption that the degradation of a three-dimensional gel is a reversible process in relation to trifunctional polycondensation, the theory of which was propounded by Flory [83]. According to this theory, the polycondensation of monomers which contain more than two functional groups leads to the formation of a three-dimensional lattice polymer. A characteristic feature of the three-dimensional polycondensation is the sudden formation of an infinite lattice observable at the point of gel formation. After this a high-molecular-weight three-dimensional lattice polymer (the so-called "gel-component") and a low-molecular-weight soluble product (the so-called sol-component) are present in the system, but a sharp separation into these components is not observed: the particles of the sol enclose the infinite lattice of the gel. With further condensation the amount and molecular weight of the sol component decrease as a result of the selective addition of its coarser and more complex particles to the infinite lattice of the gel-component, According to the model developed by Szabo and Goring, in the degradation of the three-dimensional gel initially low-molecular weight fragments are formed, which are distinct from the infinite lattice of the gel owing to the breaking of the intramolecular transverse bonds. In the later stages of the degradation the gel will "break up" and give coarser fragments of soluble lignin. In this model Szabo and Goring started from the assumption of the presence of two structurally different gels, one of which corresponds to the secondary wall lignin, the other to the lignin of the central lamella. The quantities predicted on the basis of the model developed by Szabo and Goring agree well with the experimentally obtained topochemical model of the delignification of spruce wood [79] and also with the molecular weight distribution of lignin in its isolation in acid sulphite [27] and kraft [31] digestions of spruce wood.

An analogous condensation was proposed by Elton [84] on the basis of a kinetic investigation of the delignification of wood of the loblolly pine during neutral sulphite digestion. As a result of the investigations it was found that 8–11% of the lignin is removed at an appreciably greater rate than the remaining lignin. This difference in the rate of delignification in the initial and subsequent periods of digestion was explained primarily on the basis of the molecular weight distribution of the lignin in the wood, and also on the basis of the diffusion of the reaction product and the physical availability of the lignin in the plant tissue. Elton assumed that the low-molecular-weight lignin dissolves slowly after sulphidation, and the rate of delignification in the initial digestion period is determined by the rate of sulphitation. On the other hand, for the dissolution of the remaining high-molecular-weight lignin its hydrolysis to finer fragments is necessary, which also determines the rate of delignification in the subsequent period of digestion.

In ending our examination of the problems associated with the topochemical delignification of wood during industrial digestions mention must be made of the work of Jayme and Torgersen [85], who investigated the topochemical delignification in sulphite and sulphate digestions. Their ultraviolet microscopical investigation into the partial delignification of spruce wood showed that in the sulphite digestion (loss of 27.5% of the initial lignin) the concentration of lignin in the secondary cell wall hardly altered and almost all the dissolved lignin was removed from the middle lamella. The delignification began with the material of the middle lamella and proceeded in the direction of the lumen. In sulphate digestion (removal of 29.8% of the initial lignin) a considerable proportion of the lignin was removed from both the secondary wall and from the middle lamella. The delignification in this instance began simultaneously from the middle lamella and the lumen.

In the present review we have not spent time on an examination of the occasionally contradictory results of the early investigations on the topochemistry of the delignification of wood [78,86–97]. However, a comparison of only two late investigations in this field (Goring and coworkers [79] and Jayme and Torgersen [85]) who used the same method but arrived at different conclusions shows that this problem requires further experiments to be made.

In conclusion, it is interesting to trace the evolution of Goring's opinions on the heterogeneity of natural lignin. On the basis of investigations of the molecular weight distribution of the lignins isolated during acid sulphite [27] and bisulphite [30] digestions, he suggested the presence in spruce wood of two types of lignin: low-molecular and high-molecular-weight, localised in different parts of the cell wall. Later experiments [79] on the topochemistry of the delignification of spruce wood, which showed that in the early stages of sulphite and kraft digestions there is preferential delignification of the secondary wall, allowed Goring [98] to suggest that the low-molecular-weight lignin is localised in the secondary wall. Finally, the theory of the degradation of a three-dimensional gel, developed by Szabo and Goring [82] led them to the conclusion that low-

molecular-weight lignin, which, dissolves in the early stages of the digestion, is formed as the result of the degradation of both lignin gel localised in the secondary wall and also the lignin gel of the middle lamella. They assume that instead of low- and high-molecular weight lignins we must speak of the structurally different lignin of the secondary wall and the lignin of the middle lamella.

V. HETEROGENEITY OF LIGNIN IN RELATION TO ITS BONDING WITH THE OTHER COMPONENTS OF PLANT TISSUE

In conclusion yet another aspect of the problem of the heterogeneity of natural lignin must be mentioned, one which is connected with the existence of lignocarbohydrate bonds in plant tissues [99].

In 1958 Aaltio [100,101] published the results of an extensive investigation of the type of bonding of lignin with other components of plant tissue. He undertook a series of nine butanol digestions of aspen wood. Fine sawdust, previously extracted with acetone was digested with neutral buffered butanol–water mixture for 4 h at 158°C. Each successive digestion was carried out on the residue from the previous one. The solubility of the wood was traced by analysis of the insoluble residues and digestion solutions. Table 10 gives some of the results of the investigations.

Table 10. Some results obtained in successive digestions of aspen wood with buffered aqueous butanol [100].

Digestion No.	Ratio of dissolved lignin to dissolved pentosane-uronids	OCH_3 groups in dissolved lignin, %		Digestion No.	Ratio of dissolved lignin to dissolved pentosane-uronids	OCH_3 groups in dissolved lignin, %	
		found	calculated			found	calculated
1	1.05	21.4	21.7	6	0.59	—	15.3
2	0.75	—	21.3	7	0.55	15.0	15.0
3	0.77	20.1	20.2	8	0.54	—	15.0
4	0.73	—	21.4	9	0.55	14.9	12.0
5	0.74	17.9	17.9				

The results led Aaltio to the conclusion that aspen wood consists partly of homogeneous ligno-carbohydrate complexes. According to his interpretation, during the first five digestions there is a gradual action of the digestion solution on the middle lamella, which leads to the separation of a highly methoxylated lignin and pentosan uronides in the ratio ~ 0.75. After dissolution of all the middle lamella defibrisation of the material takes place and in subsequent digestions the secondary cell wall is subjected to the action of the digestion solution. This leads to the separation of lowly methoxylated lignin and pentosan uronides again in a constant ratio (~ 0.55), which, however, differs from the ratio found for the dissolution of the middle lamella. It is noteworthy that according to Aaltio, the greater part of the lignin in the complex isolated from the middle lamella is in the free state and does not have chemical bonds with the carbohydrate components. This assumption of Aaltio agrees with the conclusions of other investigators.

Thus, according to Björkman's ideas [102], the lignin in the growing plant tissues can be divided into two fractions in respect of its bonding with hemicelluloses. One fraction has a very small number of bonds with hemicelluloses or none at all and in all probability is localised in the middle lamella. This fraction is isolated under mild conditions and corresponds to the lignin of mechanical grinding obtained by Björkman's method. The other fraction is firmly linked with hemicelluloses and can be isolated by more severe methods. This fraction corresponds with the lignocarbohydrate complex obtainable by the Björkman method. The ratio between these fractions is ~1:1. On the basis of experiments on the fractionation of hydroxyethylated wood (previously ground in a ball mill) dissolved in dimethylformamide, Brownell and West [103] also concluded that approximately three-quarters of all the lignin in wood is chemically bound with carbohydrates. About one-quarter of the lignin can be easily separated from the carbohydrates. They assume that this fraction is not linked with the carbohydrates by a hydrogen bond although it is quite possible to assume that these bonds are broken at the time the wood is ground in the ball mill. As a result of an investigation involving oxidation of wood with hydrogen peroxide, Berlin [104] concluded that the lignin localised in the middle lamella is of higher molecular weight and is chemically unconnected with the polysaccharides. Tishchenko [105] concluded, on the basis of results on the stepwise ozonation of spruce wood in ethyl acetate, that in spruce there are both lignin unconnected chemically with carbohydrates (not less than 43% of all the lignin) and lignin thus connected.

It is interesting to note that in 1934 Boyarkin [106] made a microchemical examination of the sclerin-chemical fibres using two widely known colour reactions: phloroglucinol and the Mäule reaction. As a result of this, he found the presence of different microchemical reactions at different elements of the lignified tissues. Thus a characteristic reaction with phloroglucinol + conc. H_2SO_4 is shown only by the primary walls of neighbouring cells with intercell substance between them, whereas the Mäule reaction is characteristic only for the secondary cell wall‡. These results enabled him to suggest the existence in the lignified tissue of two components of lignin, designated respectively as "F" and "M". Later Bardinskaya [112] found that alkaline extraction of lignified material alters the microchemical reaction significantly: after extraction with alkali (0.5 *N* NaOH) the vessels which had retained their form were not stained by phloroglucinol + H_2SO_4. Evidently this is because the alkali extracts that part of the lignin which is responsible for the phloroglucinol reaction of the lignified fibres. On the basis of this a scheme for the isolation of both components of the lignin was proposed; it involved the alkaline extraction of the plant material, previously extracted with alcohol–benzene mixture (isolation of component "F") and then the acid hydrolysis of the residue (isolation of component "M"). The fractions obtained were quite different in elementary and functional composition (Table 11).

According to Bardinskaya [116], fraction "F" is the most "mobile" part of the lignin, less firmly connected with the other components of the cell wall. The remaining part of the lignin (fraction "M") is evidently more strongly

‡It has been repeatedly suggested [107–111] that this difference in the colours in the microreactions on the lignin is because some of the histochemical reactions (including that of phloroglucinol) are characteristic for the guacyl residues of the lignin and the second group (including the Mäule reaction) for the syringyl residues.

bound to the polysaccharides components of the cell wall and therefore is not extracted by alkali under the conditions employed. Bardinskaya assumes that the two fractions also differ in molecular weight. In addition it has been suggested [117,118] that there is the possibility of the existence of different paths of biosynthesis for the two components of the lignin. Later Furst [119] investigated differential conduction systems and the dynamics of the accumulation of lignin in the elements of the xylem at various stages of the development of leaves of certain types of onion from the moment of germination of the seed up to 4–6 month seedlings. He showed that the "F" component is formed earlier than "M" lignin, and if the "F" component starts to be deposited at the side of the middle lamella and primary wall, then the component "M" is mainly deposited on the inner (at the side of the cavity) envelopes of the leaf tracheids.

Table 11. Characteristics of the lignin fractions isolated from sugar beet stalks [114,115].

Fraction	Yield of lignin, % of initial raw material	Present, %				Yield of aromatic aldehydes after nitrobenzene oxidation of the lignin, % of lignin
		C	H	N	OCH_3	
Lignin "F"	0.59	57.32	6.62	3.71	14.05	6.0
Lignin "M"	7.25	58.32	6.02	0.9	18.46	4.8

The hypothesis of the existence of two types of lignin received confirmation as a result of Lüdtke's chemical investigation [120] of the best fibres of various plants. According to his results, these lignins are distinguished in localisation in the cell wall of the plant. The major fraction of the lignin is localised in the middle lamella; the second fraction, which makes up 10–30% of all the lignin, is localised in the primary and secondary cell walls and is chemically bound with carbohydrate material. These fractions differ somewhat in elemental composition and content of OCH_3 groups.

In conclusion, mention must be made of the results of an investigation by Matsukura and Sakakibara, published recently [121]. They isolated from the wood of the spruce (*Picea jezoensis*), ground in a vibromill, the mechanically ground lignin (MGL) and lignin-carbohydrate complex (LHC) (by extraction with dimethylsulphoxide) according to Björkman's method. The MCL and LHC and the residue were subjected to ethanolysis and also to oxidation by $KMnO_4$ or nitrobenzene in alkaline medium. The quantity of degradation products was found by gas chromatography.

The oxidation of LHC by permanganate led to the production of a very small quantity of degradation products: 1.78% (reckoning from the lignin in the LHC) veratric acid, 0.46% isohemipinic, and 0.25% dehydrodiveratric acid. As a result of the ethanolysis of LHC, monomeric products were obtain in good yield: guacylacetone, methyl vanyloyl ketone, and 2-ethoxy-1-guacylpropan-1-one. From these results it was concluded that bonds of the arylglycerol β-aryl ethers type predominate in the lignin present in the LHC.

The oxidation of the wood residue with permanganate led to the production of the same acids in good yields: 9.88, 3.46, and 3.43% on the lignin; at the same time as a result of ethanolysis only insignificant amounts of the monomeric "Hibbert ketones" were obtained. These results are thought to show the presence in the wood residue of a large quantity of condensed chains of lignin. The results of alkaline nitrobenzene oxidation of all three specimens showed small but distinguishable differences between them.

Matsukura and Sakakibara assume that the above results convincingly show the heterogeneity of the chemical structure of the lignin. According to the results obtained the LMP isolated by the Björkman method exhibits a structure intermediate between the other two preparations and in this connection they think that it cannot be regarded as a model for all the lignin of wood.

---o0o---

The investigations of the last 10–15 years which we have considered above enable us to acknowledge to the first approximation the presence in plant tissue of two types of lignin, which differ in molecular weight, in localisation in the cell wall, in bonding with other components of the plant tissue, and also in the topochemical behaviour in the different processes for the digestion of wood.

The results of these investigation confirm the hypotheses, repeatedly expressed earlier, concerning the heterogeneity of natural lignin. Here we must first note Klason's hypothesis [122–124] concerning the existence in spruce wood of α- and β-lignins. He formulated this hypothesis on the basis of experiments on the precipitation with 2-naphthylamine hydrochloride of the lignosulphonic acid obtainable in the sulphite digestion of wood. Later Holmberg and Wintzell [125] and also Hibbert and coworkers [126] reported the presence of two fractions in alkali lignin which could be isolated from black alkali after the soda digestion of spruce wood. Proof of the heterogeneity of natural lignin is also provided by the results of the fractionation of a series of preparations that have been isolated: formic acid lignin isolated from birch wood [127], ethanol-lignin from wood [128–130], acetic acid [131] and butanol-lignins [132–135], isolated from various wood species; lignin isolated by hydrogenolysis of maple wood [130,136], lignins extracted from oat and wheat straw by methylcellolsolve + HCl [137]; lignins isolated from bamboo [138] and aspen [139,140] woods by a hydrotropic method, and also enzymatically isolated lignins [141]. Finally, mention must be made of the hypothesis of Purves [142], who assumed that the molecular weight and the ratio of the "free" and "combined" lignins in the plants can vary not only with the type of plant but also with growth and the growing conditions. These investigations have been partly considered in Lebedev's review [143].

As regards the practical recommendations [74,79] which can be made from the existence of different types of natural lignin, clearly of most importance would be the finding of a delignifying agent which exhibits specific action towards the middle lamella, which probably consists mainly of lignin. This would lead to the obtaining of a fibrous mass in very high yield which could then either be used as such or be subjected to further delignification for obtaining a desired type of fibre.

REFERENCES

1. H. Erdtman, Svensk papperstidn., 45, 374 (1942).
2. G. Racky, Cellulosechem., 20, 22 (1942).

3. W. Lautsch and G. Piazolo, Cellulosechem., 22, 48 (1944).
4. K. Schwabe and E. Hahn, Holzforschung, 1, 42, 79 (1947).
5. F. M. Ernsberger and W. G. France, J. Phys. Colloid. Chem., 52, 267 (1948).
6. E. D. Olleman, D. E. Pennington, and D. M. Ritter, J. Colloid Sci., 3, 185 (1948).
7. A. E. Markham, Q. P. Peniston, and J. L. McCarthy, J. Amer. Chem. Soc., 71, 3599 (1949).
8. B. Ivarsson, Svensk papperstidn., 54, 1 (1951).
9. T. A. Loomis and R. E. Beyer, J. Pharmacol. Exptl. Therap., 109, 21 (1953).
10. J. L. Gardon and S. G. Mason, Canad. J. Chem., 33, 1477 (1955).
11. J. Moacanin, V. F. Felicetta, W. Haller, and J. L. McCarthy, J. Amer. Chem. Soc., 77, 3470 (1955).
12. V. F. Felicetta, A. Ahola, and J. L. McCarthy, J. Amer. Chem. Soc., 78, 1899 (1956).
13. E. Nokihara, M. J. Tuttle, V. F. Felicetta, and J. L. McCarthy, J. Amer. Chem. Soc., 79, 4495 (1957).
14. V. F. Felicetta and J. L. McCarthy, J. Amer. Chem. Soc., 79, 4499 (1957).
15. A. Rezanowich, W. Q. Yean, and D. A. I. Goring, Svensk papperstidn., 66, 141 (1963).
16. J. M. Pepper, P. E. T. Baylis, and E. Adler, Canad. J. Chem., 37, 1241 (1959).
17. W. Q. Yean, A. Rezanowich, and D. A. I. Goring, Chim. Biochim. Lignine, Cellulose, Hemicelluloses. Actes Symp. Intern., Grenoble, France, 1964, p. 327.
18. H. G. Arlt, Jnr., and C. Schuerch, Tappi, 41, 481 (1958).
19. H. G. Arlt, Jnr., K. Sarkanen, and C. Schuerch, J. Amer. Chem. Soc., 78, 1904 (1956).
20. K. Kürschner and A. Hoffer, Techn. Chem. Papier Zellstoff-Fabr., 26, 125 (1929).
21. K. Kürschner and A. Hoffer, Chemiker-Ztg., 55, 161 (1931).
22. K. Kürschner, Cellulosechem., 12, 281 (1931).
23. W. Fuchs, Zellstoff-Faser, 32, 86 (1935).
24. A. A. Chuksanova, O. P. Grushnikov, and N. N. Shorygina, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, 1810 (1961).
25. O. P. Grushnikov and N. N. Shorygina, Izv. Akad. Nauk SSSR, Ser. Khim., 1774 (1967).
26. O. P. Grushnikov and N. N. Shorygina, N. P. Mikhailov, Izv. Akad. Nauk SSSR, Ser. Khim., 2447 (1969).
27. W. Q. Yean and D. A. I. Goring, Pulp Paper Mag. Can., 65, T127 (1964).
28. V. M. Reznikov, Khim. Drevesiny, 4, 5 (1969).
29. W. Q. Yean and D. A. I. Goring, Svensk papperstidn., 68, 787 (1965).
30. W. Q. Yean and D. A. I. Goring, Svensk papperstidn., 71, 739 (1968).
31. J. G. McNaughton, W. Q. Yean, and D. A. I. Goring, Tappi, 50, 548 (1967).
32. W. Brown, S. I. Falkehag, and E. B. Cowling, Nature, 214, 410 (1967).
33. W. Brown, E. B. Cowling, and S. I. Falkehag, Svensk papperstidn., 71, 811 (1968).
34. K. H. Ekman and J. J. Lindberg, Paperi ja Puu, 48, 241 (1966).
35. A. D. Alekseev, V. M. Reznikov, B. D. Bogomolov, and O. M. Sokolov, Khim. Drevesiny, 4, 49 (1969).
36. J. J. Lindberg, K. Penttinen, and C. Majani, Suomen Kemistilehti, B38, 95 (1965).
37. R. L. Karpovskaya, V. L. Levdikova, N. M. Dorzet, and V. M. Reznikov, Zhur. Prikl. Khim., 37, 1318 (1964).
38. E. I. Chupka, G. L. Burkov, A. V. Obolenskaya, and V. M. Nikitin, Khim. Drevesiny, 3, 69 (1969).
39. C. L. Hess, Tappi, 35, 312 (1952).
40. D. A. I. Goring and A. Rezanowich, Canad. J. Chem., 36, 1653 (1958).
41. P. R. Gupta and D. A. I. Goring, Canad. J. Chem., 38, 248 (1960).
42. T. Kerr and I. W. Bailey, J. Arnold Arboretum, 15, 327 (1934).
43. I. W. Bailey and T. Kerr, J. Arnold Arboretum, 16, 273 (1935).
44. A. D. J. Meeuse, Rec. trav. botaniques Neerlandais, 38, 18 (1942).
45. H. Meier, Holz Roh-Werkstoff, 13, 323 (1955).
46. W. Liese, J. Polymer Sci., C2, 213 (1963).
47. G. J. Ritter, Ind. Eng. Chem., 17, 1194 (1925).
48. G. J. Ritter, Ind. Eng. Chem., 20, 941 (1928).
49. G. J. Ritter, Raper Ind., 16, 178 (1934).
50. W. M. Harlow, Paper Trade J., 109, No. 18, 38 (1939).
51. A. J. Bailey, Ind. Eng. Chem., Anal. Ed., 8, 52 (1936).
52. A. J. Bailey, Paper Ind., 18, 379 (1936).
53. P. W. Lange, Svensk papperstidn., 57, 525 (1954).
54. H. P. Frey, Holz Roh-Werkstoff., 17, 313 (1959).
55. F. Ruch and H. Hengartner, Beih. Z. Schweiz. Forstver., 30, 75 (1960).
56. A. J. Stamm and H. T. Sanders, Tappi, 49, 397 (1966).
57. G. Jayme and D. Fengel, Holzforschung., 15, 97 (1961).
58. I. B. Sachs, I. T. Clark, and J. C. Pew, J. Polymer Sci., C2, 203 (1963).
59. W. A. Côté, Jnr., T. E. Timell, and R. A. Zabel, Holz. Roh-Werkstoff., 24, 432 (1966).
60. D. H. Northcote, Biol. Rev., 33, 53 (1958).
61. S. V. Milyutina and V. N. Sergeeva, in collected papers "Khimicheskaya Pererabotka i Zashchita Drevesiny" (The Chemical Treatment and Protection of Wood), Izd. Akad. Latv. SSR, Riga, 1964, pp. 115–132.
62. G. P. Berlyn and R. E. Mark, Forest Prod. J., 15, 140 (1965).
63. B. J. Fergus, A. R. Procter, J. A. N. Scott, and D. A. I. Goring, Wood Sci. and Technol., 3, 117 (1969).
64. J. A. N. Scott, A. R. Procter, B. J. Fergus, and D. A. I. Goring, Wood Sci. and Technol., 3, 73 (1969).
65. J. A. N. Scott and D. A. I. Goring, Cellulose Chem. Technol., 4, 83 (1970).
66. S. Asunmaa and P. W. Lange, Svensk papperstidn., 55, 217 (1952).
67. S. Asunmaa and P. W. Lange, Svensk papperstidn., 57, 501 (1954).
68. H. Meier and K. C. B. Wilkie, Holzforschung., 13, 177 (1959).
69. H. Meier, J. Polymer Sci., 51, 11 (1961).
70. B. J. Fergus and D. A. I. Goring, Holzforschung., 24, 118 (1970).
71. V. I. Sharkov and E. S. Tsvetkova, Sbornik Trud. VNII Gidroliz. i Shul'fit.-Spirt. Prom., 3, 69 (1960).
72. A. B. Wardrop, Tappi, 40, 225 (1957).
73. T. M. Saleh, L. Leney, and K. V. Sarkanen, Holzforschung., 21, 116 (1967).
74. B. J. Fergus and D. A. I. Goring, Pulp Paper Mag. Can., 70, T314 (1969).

75. E. B. Cowling, U. S. Dept. Agr., Tech. Bull., No. 1258, 1961.
76. M. P. Levi, J. Inst. Wood Sci., No. 12, 56 (1964).
77. W. W. Wilcox, Forest Prod. J., 15, 255 (1965).
78. A. B. Wardrop and G. W. Davies, Holzforschung., 15, 129 (1961).
79. A. R. Procter, W. Q. Yean, and D. A. I. Goring, Pulp Paper Mag. Can., 68, T-445 (1967).
80. W. Q. Yean, T. Higgs, G. Suranyi, and D. A. I. Goring, Pulp Paper Mag. Can., 67, T-570 (1966).
81. T. N. Kleinert, Holzforsch. Holzverwert., 17, 98 (1965).
82. A. Szabo and D. A. I. Goring, Tappi, 51, 440 (1968).
83. P. J. Flory, "Principles of Polymer Chemistry", Cornell University Press, Ithaca, New York, 1953, Ch. IX.
84. E. F. Elton, Tappi, 46, 404 (1963).
85. G. Jayme and H. F. Torgersen, Holzforschung, 21, 110 (1967).
86. E. Hägglund, Svensk papperstidn., 39, 95 (1936).
87. A. L. M. Bixler, Paper Trade J., 107, No. 15, 29 (1938).
88. P. W. Lange, Svensk papperstidn., 50, 130 (1947).
89. M. G. Eliashberg and M. N. Tsypkina, Materialy TsNII Tsellyuloz. i Bum. Prom., No. 41, p. 135 (1956).
90. M. G. Eliashberg and M. N. Tsypkina, Nauch. Trud. Ts. NII Tsellyuloz. i Bum. Prom., 1956, No. 41, p. 135.
91. H. Meier and S. Yllner, Svensk papperstidn., 59, 395 (1956).
92. D. E. Marth, Tappi, 42, 301 (1959).
93. A. B. Wardrop, Svensk papperstidn., 66, 231 (1963).
94. G. W. Davies, Appita, 19, 95 (1966).
95. V. Jacopian and G. Casperson, Faserforsch. Textiltech., 17, 267 (1966).
96. G. Casperson, V. Jacopian, and B. Philipp, Svensk papperstidn., 70, 702 (1967).
97. L. T. Purinya, P. N. Odintsov, P. P. Erin'sh, and V. I. Smilga, Khim. Drevesiny, 5, 23 (1970).
98. D. A. I. Goring, Chem. Soc. (London), Spec. Publ., No. 23, 115 (1968).
99. O. P. Grushnikov and N. N. Shorygina, Uspekhi Khim., 39, 1459 (1970) [Russ. Chem. Rev., No. 8 (1970)].
100. E. Aaltio, Ann. Acad. Sci. Fennicae, Ser. A, II, No. 88, 58 (1958).
101. E. Aaltio and R. H. Roschier, Paperi ja Puu, 36, 157 (1954).
102. A. Björkman, Svensk papperstidn., 60, 329 (1957).
103. H. H. Brownell and K. L. West, Pulp Paper Mag. Can., 62, T374 (1961).
104. A. A. Berlin, in "Trudy 4-i Konf. po Vysokomol. Soed." (Proceedings of the Fourth Conference on High-Molecular-Weight Compounds), Izd. Akad. Nauk SSSR, Moscow–Leningrad, 1948, p. 172.
105. D. V. Tishchenko, Zhur. Prikl. Khim., 32, 686 (1959).
106. A. N. Boyarkin, Trudy Inst. Nov. Lubyan. Syr'ya, No. 1, 75 (1934).
107. F. E. Brauns, "The Chemistry of Lignin", Academic Press, New York, 1952, Ch. IV.
108. R. H. J. Creighton, R. D. Gibbs, and H. Hibbert, J. Amer. Chem. Soc., 66, 32 (1944).
109. E. Adler, K. J. Björkqvist, and S. Häggroth, Acta chem. scand., 2, 93 (1948).
110. M. Lüdtke, Holzforschung., 15, 161 (1961).
111. L. M. Srivastava, Tappi, 49, 173 (1966).
112. M. S. Bardinskaya, Dokl. Akad. Nauk SSSR, 73, 133 (1950).
113. M. S. Bardinskaya, Dokl. Akad. Nauk SSSR, 76, 435 (1951).
114. M. S. Bardinskaya, Izv. Akad. Nauk SSSR, Ser. Biol., No. 2, 111 (1952).
115. M. S. Bardinskaya, Uspekh. Sovr. Biol., 35, 189 (1953).
116. M. S. Bardinskaya, "Rastitel'nye Kletochnye Stenki i Ikh Obrazovanie" (Plant Cell Walls and Their Formation), Izd. Nauka, Moscow, 1964, pp. 58–65.
117. M. S. Bardinskaya, Fiziolog. Rast., 6, 336 (1959).
118. E. I. Barskaya, Fiziolog. Rast., 9, 210 (1962).
119. G. G. Furst, in "Fiziologiya Immuniteta Rastenii" (Physiology of Plant Immunity), Izd. Nauka, Moscow, 1968, pp. 91–100.
120. M. Lüdtke, Holzforschung, 15, 141 (1961).
121. M. Matsukura and A. Sakakibara, J. Japan Wood Res. Soc., 15, 35 (1969).
122. P. Klason, Ber., 53B, 706 (1920).
123. P. Klason, Ber., 53B, 1864 (1920).
124. P. Klason, Cellulosechem., 13, 113 (1932).
125. B. Holmberg and T. Wintzell, Ber., 54B, 2417 (1921).
126. H. B. Marshall, F. Brauns, and H. Hibbert, Canad. J. Res., 13B, 103 (1935).
127. M. Lieff, G. F. Wright, and H. Hibbert, J. Amer. Chem. Soc., 61, 1477 (1939).
128. R. F. Patterson, K. A. West, E. L. Lovell, W. L. Hawkins, and H. Hibbert, J. Amer. Chem. Soc., 63, 2065 (1941).
129. E. L. Lovell and H. Hibbert, J. Amer. Chem. Soc., 63, 2070 (1941).
130. C. Schuerch Jnr., J. Amer. Chem. Soc., 72, 3838 (1950).
131. H. Pauly, A. Foulon, O. Hansen, O. Haberstroh, H. Bailom, and J. Sextl, Ber., 67B, 1177 (1934).
132. A. Bailey, Paper Trade J., 111, No. 7, 27 (1940).
133. A. Bailey, Paper Ind. and Paper World, 30, 1196 (1948).
134. A. Bailey, Paper Ind. and Paper World, 32, 395 (1950).
135. A. Bailey, J. Amer. Chem. Soc., 73, 2325 (1951).
136. C. P. Brewer, L. M. Cooke, and H. Hibbert, J. Amer. Chem. Soc., 70, 57 (1948).
137. L. Marion, Canad. J. Res., 6, 521 (1932).
138. H. Lau, Paper Ind. and Paper World, 23, 247 (1941).
139. V. S. Gromov and P. N. Odintsov, Trudy Inst. Lesokhoz. Problem, Akad. Nauk Latv. SSR, 12, 79 (1957).
140. V. S. Gromov and P. N. Odintsov, Trudy Inst. Lesokhoz. Problem, Akad. Nauk Latv. SSR, 12, 91 (1957).
141. W. J. Schubert and F. F. Nord, Proc. Natl. Acad. Sci. U. S., 41, 122 (1955).
142. C. B. Purves, in Emil Ott, "Cellulose and Cellulose Derivatives", Interscience Publ., Inc., New York, 1943, p. 101.
143. K. K. Lebedev, Sbornik Trud. TsNIPI Lesokhim. Prom., 1965, No. 16, p. 65.

Zelinskii Institute of Organic Chemistry,
Academy of Sciences of the USSR, Moscow