II. A STUDY OF THE STRUCTURE OF THE LIGNIN OF *Sphagnum* MOSS BY REDUCTIVE DEGDRADTION WITH A SOLUTION OF METALLIC SODIUM IN LIQUID AMMONIA

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UDC 547.458.84+549.927.2

For a long time, the question of the presence of lignin in plants with a low degree of organization has remained open. Some authors have given experimental results which favor the idea of its presence [1], while others have questioned this point of view on the basis of the fact that the main function of lignin amounts to imparting a rigid structure to the cell wall and, consequently, lignin should appear in plants only in connection with the transition to epigeal form of life [2].

Thus, the particular question of the presence of lignin in a sphagnum mosses has acquired fundamental importance since the role and position of lignin in the development of plant organisms depend on the answer to it.

The presence of lignin in sphagnum moss has been shown by various authors [3-5], and at the same time the hypothesis has been put forward that the lignins of plants with a low degree of organization are based on structural fragments of the p-hydroxyphenylpropane type connected to one another mainly by carbon—carbon bonds.

The present work was undertaken as a development of investigations performed previously [4, 12] and has the aim of extending our ideas on the structure of the lignin of plants with a low degree of organization.

As the main method of investigation we selected the reductive degradation of the lignin by metallic sodium in liquid ammonia, which has proved extremely fruitful in the study of the structure of the lignin of plants with a low degree of organization.

The materials for investigation were a sphagnum moss of the *Sphagnum medium* type and a preparation of dioxane lignin isolated from it the comparative closeness of which to a MWL ["milled wood lignin"] preparation has been shown previously [7].

The reductive degradation of the samples was effected by the method of Shorygina et al. [9-11]. The qualitative determination of the composition of the phenols was performed by paper chromatography (Fig. 1 and Table 1), as described in the preceding paper [12].

We used gas—liquid chromatography for quantitative analysis.

In order to lower the boiling points of the phenols and to exclude the possibility of their pyrolysis during analysis, they were previously converted into the methyl ethers by methylation with dimethyl sulfate.

The analysis was performed on a LKhM-7A chromatograph using a flame-ionization detector and programming of the temperatures. We have described the conditions for the analysis previously [13].

In the identification of the substances detected by GLC we used the results of paper chromatography, on the basis of which standard ethers were synthesized. The identification

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S. M. Kirov Belorussian Technologic Institute. Institute of Physical Organic Chemistry, Academy of Sciences of the Belorussian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 77-83, January-February, 1975. Original article submitted May 17, 1973.

TABLE 1. Compositions and R_f Values of Phenols Obtained by the Decomposition of Sphagnum Moss and a Preparation of Dioxane Lignin by Metallic Sodium in Liquid Ammonia*

Names of the phenols	R_f values, cm			Colors of spots after revelation
	markers	sphagnum moss	dioxane lignin	
4-Hydroxyphenylpropane	0,68	0,67	0,67	Yellowish orange
1-(4-Hydroxyphenyl)propan-1-ol	0,30	0,28	0,29	Bright yellow
3-(4-Hydroxyphenyl)propan-1-ol	0,25	0,25	0,24	Pink
1-(4-Hydroxy-3-methoxyphenyl)propane	0,77	0,76	0,77	Light lilac
1-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	0,54	0,53	0,53	Orange
p-Hydroxybenzaldehyde + p-hydroxybenzyl alcohol	0,10	0,10	0,10	Pale orange
Vanillin + vanillyl alcohol	0,18	0,17	0,17	Pinkish orange
Unidentified substance	—	0,59	0,60	Lemon yellow

*The figures given were obtained on "crab" paper; similar R_f values were found on Leningrad slow paper.

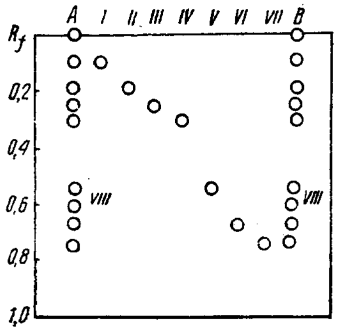

Fig. 1. Chromatogram of a mixture of the phenols obtained from the products of the decomposition of sphagnum moss (A) and of a preparation of dioxane lignin (B): I-VII) markers: p-hydroxybenzaldehyde; vanillin; 3-(4-hydroxyphenyl)-propan-1-ol; 1-(4-hydroxy)propan-1-ol; 1-(4-hydroxy-3-methoxyphenyl)propan-1-ol; 4-hydroxyphenylpropane; 1-(4-hydroxy-3-methoxyphenyl)propane.

proper was performed from the retention times and the introduction of standards.

Figure 2 gives chromatograms of the methylated products of the decomposition of sphagnum moss (Fig. 2a) and of a preparation of DLA (Fig. 2b).

The chromatograms are identical and each contains nine peaks, not counting the solvent peak. The peaks correspond to the signals of the following substances: 1) 4-hydroxyphenylpropane; 2) vanillyl alcohol; 3) 1-(4-hydroxyphenyl) propan-1-ol; 4) p-hydroxybenzaldehyde; 5) 1-(4-hydroxy-3-methoxyphenyl)-propane; 6) p-hydroxybenzyl alcohol; 7) vanillin; 8) 1-(4-hydroxy-3-methoxyphenyl)propan-1-ol; 9) 3-(4-hydroxyphenyl)propan-1-ol.*

The determination of the percentages of the phenols in the mixture was performed by the area-normalization method using correction coefficients found previously by the chromatographic analysis of a model mixture [14].

Table 2 gives the quantitative composition of the phenolic fraction (PF) of the decomposition products of a preparation of DLA and of the sphagnum moss. The figures given in columns 6-9 were obtained by calculation taking into account the results of a chromatographic analysis of the methylated decomposition products (columns 3 and 4) and the yield of ethers formed in the methylation of model phenols with dimethyl sulfate (column 5).

The identity of the compositions of the phenolic fraction of the products of the decomposition of sphagnum moss and of the DLA preparation (Table 2, columns 6-9) shows, on the one hand, that the DLA preparation has a structure which is insignificantly different from that of protolignin and, on the other hand, that in the direct treatment of the moss in sodium in liquid ammonia the phenolic fraction was formed solely as the result of the decomposition of the lignin and not of any other substances present with it in the plant tissue.

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*On paper chromatography, the vanillyl and p-hydroxybenzyl alcohols were not detected since their R_f values are close to those of the corresponding aldehydes.

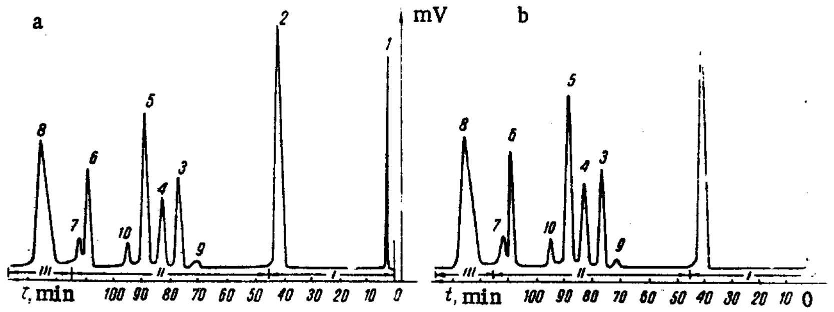


Fig. 2. Chromatogram of a mixture of methylated phenols at temperatures of 130°C (I), 130-200°C (1 deg/min) (II); 200°C (III), obtained from the products of the decomposition of sphagnum moss (a) and a preparation of DLA (b); 1) ethanol; 2) 4-methoxyphenylpropane; 3) 1-(4-methoxyphenyl)propan-1-ol; 4) p-methoxybenzaldehyde; 5) 1-(3,4-dimethoxyphenyl)propane; 6) 3,4-dimethoxybenzaldehyde; 7) 1-(3,4-dimethoxyphenyl)-propan-1-ol; 8) 3-(4-methoxyphenyl)propan-1-ol; 9) 3,4-dimethoxybenzyl alcohol; 10) 4-methoxybenzyl alcohol.

TABLE 2. Amounts of Phenols in the Phenolic Fraction of the Products of the Decomposition by Metallic Sodium in Liquid Ammonia of Sphagnum Moss and of a DLA Preparation

№	Names of the phenols	Amount of the ethers in the PF, %		Yield of ethers, %	Amounts of phenols in the PF			
		DLA	moss		DLA		moss	
					%	moles	%	moles
1	2	3	4	5	6	7	8	9
1	4-Hydroxyphenylpropane	27,3	27,6	70,0	25,1	0,185	26,4	0,194
2	1-(4-Hydroxyphenyl)propan-1-ol	8,8	8,8	63,0	7,1	0,047	6,0	0.040
3	3-(4-Hydroxyphenyl)propan-1-ol	25,5	25,7	90,0	21,1	0,138	21,7	0,143
4	1-(4-Hydroxy-3-methoxyphenyl)-propane	16,2	15,7	58,0	20,9	0,126	20,8	0,125
5	1-(4-Hydroxy-3-methoxyphenyl)-propan-1-ol	2,5	3,0	92,0	2,2	0,012	2,4	0,013
6	p-Hydroxybenzaldehyde	7,6	7,0	60,0	8,1	0,066	7,4	0,061
7	Vanillin	8,8	8,7	52,0	12,8	0,084	12,6	0,083
8	p-Hydroxybenzyl alcohol	2,4	2,4	95,0	2,5	0,020	2,5	0,020
9	Vanillyl alcohol	1,0	1,2	93,0	1,1	0.007	1,3	0,007

Knowing the yield of phenolic fraction from the moss (0.89%) and from the isolated lignin (10.2%), it was not difficult to determine the amount of lignin in the moss. We previously [7] estimated it as 6.5%, while an accurate value from the results of the present work is 8.7%.

The work of Shorygina et al. [6, 10] has convincingly shown that in the reductive degradation with metallic sodium in liquid ammonia of lignin from conifer wood the cleavage of the β-alkyl aryl ether bond takes place, and among the phenolic degradation products 1-(4-hydroxy-3-methoxyphenyl)propane and 1- and 3-(4-hydroxy-3-methoxyphenyl)propan-1-ols, and also the analogous phenols of the p-hydroxyphenylpropane series, have been found [15]. The very same phenols have been obtained from the lignin of sphagnum moss which unambigously establishes the lignin nature of the DNA preparation and, which is most important, confirms the genetic relationship of the lignin of lower and higher plant organisms.

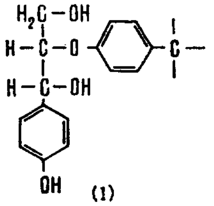
Since more than 50% of the phenols present in the phenolic fraction of the products of the decomposition of sphagnum moss lignin belongs to compounds of the p-hydroxyphenylpropane series, the hypothesis according to which the lignin of lower plants is based on a structural unit of the p-hydroxyphenylpropane type is confirmed.

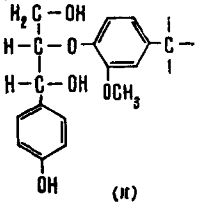
In addition, there are sufficient grounds for assuming that the "noncondensed" structures

TABLE 3. Comparative Amounts of p-Hydroxyphenols in the Phenolic Fractions of the Products of the Reductive Decomposition of Lignin and of 1-(4-Hydroxyphenyl)-2-(2'-methoxyphenoxy)propane-1,3-diol

Names of the p-hydroxyphenols	Yields (mole)	
	lignin	dimer
4-Hydroxyphenylpropane	0,194	0,241
1-(4-Hydroxyphenyl)propan-1-ol	0,040	0,059
3-(4-Hydroxyphenyl)propan-1-ol	0,143	0,100

of the lignin are represented by groups of the β-aryl alkyl ethers (I) and (II).


(I)


(II)

In favor of this are, in the first place, the fact that phenols containing a hydroxy group in the β position of a propane chain are not found among the decomposition products and, in the second place, a comparison of the yield in moles of phenols obtained in the decomposition of lignin and of a dimeric model of the grouping (II). The corresponding figures are given in Table 3, from which it can be seen that identical phenols are obtained in the two cases, and this in extremely close ratios.

The higher yield of 3-(4-hydroxyphenyl)propan-1-ol is probably due to the fact that the sphagnum moss lignin contains a considerable amount of p-coumaraldehyde groupings the reduction of which must lead to 3-(4-hydroxyphenyl)propan-1-ol. The capacity of a carbonyl group conjugated with an aromatic ring reduced by metallic sodium in liquid ammonia has been shown by Semechkina and Shorygina [10] and has been confirmed by ourselves, as will be shown below.

In a comparison of the yield of phenols of the p-hydroxyphenylpropane and guaiacylpropane types (Table 2, columns 7 and 9), it was estimated that in the sphagnum moss lignin the structural fragments (I) and (II) are present in a ratio of 2:1, respectively.

As follows from Fig. 2 and Table 2, in addition to phenolic alcohols the phenolic fraction contains p-hydroxybenzaldehyde (7.4%) and vanillin (12.6%). It was difficult to find a rational explanation for their presence in the reaction products, since if it is assumed that these aldehydes are present in the lignin, they should be converted into the corresponding alcohols under the reaction conditions.

In view of this, a model experiment was performed in which p-hydroxybenzaldehyde and vanillin were treated with metallic sodium in liquid ammonia under the conditions that we used, except that the time of the reaction was increased to seven days.

From the reaction products we recovered 20.6% of unchanged vanillin and 9.0% of unchanged p-hydroxybenzaldehyde, i.e., almost twice the reaction time proved to be insufficient for the complete reduction of the aldehyde groups.

The reductive degradation of lignin by metallic sodium in liquid ammonia takes place under such mild conditions that the hypothesis of a secondary origin of these aldehydes must be excluded. An alternative to this may be the recognition of the fact that p-hydroxybenzaldehyde and vanillin are structural fragments of the lignin attached to the macromolecule through an alkyl—aryl or aryl—aryl ether bond.

EXPERIMENTAL

Characteristics of the Materials Investigated. The sphagnum moss was collected at the end of the vegetation period, carefully freed from mechanical impurities, dried to the air-dry state, and then ground to a flour and sieved through a 20-mesh sieve. The group composition of the moss was, %: ash 4.32; ether-soluble matter 2.57; matter soluble in ethanol–benzene (1:2) 5.64; hot-water soluble matter 10.05; readily hydrolyzable substances (2% HCl) 42.58; and unhydrolyzable residue (72% H_2SO_4) 11.80%.*

A preparation of dioxane lignin was isolated from the sphagnum moss by brief acidolysis in an atmosphere of nitrogen [8]. The starting material was previously treated in an extractor with ether, ethanol–benzene (1:2), and hot water. Then it was dried in a vacuum desiccator over P_2O_5 to a moisture content of 2-3%. Chemical characteristics of the specimen: elementary composition – C 62.97%, H 6.39%, O 30.49% (by difference); functional composition: methoxyls [16] 2.22%, carbonyls [17] 6.15%, total hydroxy groups [18] 8.50%, phenolic hydroxy groups [19] 1.16%, benzyl alcohol hydroxyls (free and esterified) [20] – 3.31%.

The yield of the DLA preparation amounted to 1.3% reckoned on the organic matter of the moss, or about 16% reckoned on the lignin of the moss.

Decomposition of the Moss and of the DLA Preparation with Metallic Sodium in Liquid Ammonia. The reductive degradation of the specimens was performed in a reaction vessel with liquid ammonia to which a weighed sample of the dioxane lignin or of the moss had been added.

Small pieces of metallic sodium in an amount of 75% of the weight of the lignin taken and 100% of the weight of the moss taken was added in two or three portions to the resulting mixture. After the complete disappearance of the blue color (from four to five days) and the elimination of the ammonia, moist ether was added to the reaction vessel and then, in a current of nitrogen, water and dilute sulfuric acid to an acid reaction. After neutralization with bicarbonate, the phenols were separated from the acids by extraction with ether. The yield of phenols from the moss was 0.89%, calculated on the organic matter of the absolutely dry moss, and from the lignin 10.2%, calculated on the lignin.

SUMMARY

1. The decomposition of sphagnum moss and of an isolated preparation of lignin (DLA) by means of metallic sodium in liquid ammonia has been performed, and a phenolic fraction has been isolated with a yield of 0.89% from the moss and 10.2% from the lignin.

2. The following phenols were isolated from the phenolic fraction and their yields were determined by paper and gas–liquid chromatography: 4-hydroxyphenylpropane, 1- and 3-(4-hydroxyphenyl)propan-1-ols, 1-(4-hydroxy-3-methoxyphenyl)propane, 1-(4-hydroxy-3-methoxyphenyl)-propan-1-ol, p-hydroxybenzaldehyde, vanillin, and p-hydroxybenzyl and vanillyl alcohols.

3. The quantitative figures on the composition of the phenolic fraction of the decomposition products give grounds for assuming that the "uncondensed" structural fragments of sphagnum moss lignin are composed to the extent of two thirds of structural units of the p-hydroxyphenyl type joined to one another by β-alkyl aryl ether bonds.

4. Weighty evidence is given in favor of the assumption that sphagnum moss lignin contains as monomeric structural fragments vanillin and p-hydroxybenzaldehyde, these being attached to the polymer chain by alkyl–aryl or aryl–aryl ether bonds.

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*All the figures were calculated on the organic matter of the absolutely dry mass.

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