NITRATION OF LIGNIN ISOLATED FROM SPRUCE WOOD BY MECHANICAL GRINDING

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L. L. Sergeeva and N. N. Shorygina have previously carried out the nitration of standard compounds of lignin with nitric acid in the anhydrous media, ether and carbon tetrachloride [1-4]. The results of these investigations permitted certain conclusions concerning the behavior of hydrochloric-acid lignin in nitration under similar conditions. It was interesting to carry out the nitration of lignin in a nearly natural state under the same conditions. For this purpose, mechanically ground spruce lignin (MGL) was obtained by grinding in a ball vibration mill by Björkman's method [5].

We divided the MGL into two fractions: 1) insoluble at room temperature in butan-1-ol (93.94% of the weight of the MGL), and 2) soluble in butan-1-ol at this temperature (2.36% of the total MGL). The fraction insoluble in butan-1-ol was freed from accompanying carbohydrates and low-molecular-weight impurities by Freudenberg's method [6]. Both fractions were analyzed with respect to their elementary composition and their content of functional groups and carbohydrates. The total amount of hydroxy groups was determined by Verley's method and the number of phenolic OH groups by Adler and Hernestam's method [7]. The amount of carbonyl groups was found by reducing a sample with sodium borohydride in alcoholic solution by Marton, Adler, and Persson's method [8]. The analysis carried out showed that the fractions obtained had the following composition (%):

Fraction	C	H	O	CO	OH total	OH phen.	OCH_3	Sugar
1 (Insoluble in butan-1-ol)	62.38	6.17	31.45	3.60	10.54	2.91	15.03	2.89
2 (Soluble in butan-1-ol)	63.70	6.10	30.20	4.83	11.16	3.51	14.92	—

The sugar content in the 1st fraction (by Bertrand's method, calculated as $C_6H_{12}O_6$) after hydrolysis with 5% H_2SO_4 was 2.89%. After the introduction of a correction for the carbohydrate content, the mean composition of the C_9 structural elements for fraction 1 was calculated:

$$C_9H_{8.95}O_{2.74}(OCH_3)_{0.96} \qquad (I)$$

or, extracting functional groups,

$$C_9H_{7.87}O_{1.44}\ (OCH_3)_{0.96}\ (OH_{aliph.})_{0.73}\ (OH_{phen.})_{0.35}\ (O_{carbonyl})_{0.22}. \qquad (II)$$

The molecular weight found by the ebullioscopic method in a Ray apparatus in absolute dioxane, using a differential thermocouple consisting of 29 pairs of Chromel-Copel junctions connected in series, was 5200 [9]. For fraction 2:

$$C_9H_{8.52}O_{2.62}\ (OCH_3)_{0.87} \qquad (III)$$

or

$$C_9H_{7.32}O_{1.06}(OCH_3)_{0.87}(OH_{aliph.})_{0.82}(OH_{phen.})_{0.38}(O_{carbonyl})_{0.31} \qquad (IV)$$

The molecular weight and carbohydrate content of the second fraction were not determined because of the small amount of substance.

Fraction 1 was nitrated with a 4 N solution of nitric acid in ether at 18-20°C for 1 hr. The yield of nitrolignin was 88%. The orange-yellow product had the following composition, (%): C 56.13; H 5.10; OCH_3 10.71; COOH 5.80; N 2.18. The total nitrogen content was determined by the Dumas method; the nitrolignin contained no ester nitrogen (by the Schulze-Tiemann method).

On the basis of the analytical data, the calculated formula for C_9 was:

$$C_9H_{8.32}\,O_{2.94}\,(OCH_3)_{0.74}\ (O_{2[COOH]})_{0.28}(NO_2)_{0.33}. \qquad (V)$$

Formula (V) is comparatively close to the formula of the nitrolignin isolated by L. L. Sergeeva and N. N. Shorygina when they nitrated hydrochloric-acid lignin:

$$C_9H_{8.07}\,O_{3.15}(OCH_3)_{0.65}(O_{2[COOH]})_{0.21}\ (NO_{2[C-NO_2]})_{0.52}.$$

The nitration of the MGL with 4 N HNO_3 in dioxane was carried out under similar conditions. The nitrolignin obtained was separated into two fractions: A) insoluble in ethyl acetate, 78% of the initial lignin, and B) soluble in ethyl acetate, 32% of the initial lignin. In addition, on nitration about 20% of oxalic acid, calculated on the initial lignin, was obtained, together with a small amount of dinitroguaiacol and traces of formic acid. The fractions had the following composition (%):

Fraction	C	H	O	OCH_3	N	COOH
A	53.48	4.93	38.74	10.09	2.85	4.79
B	45.48	4.20	43.35	8.30	6.97	10.81

The content of hydroxyl and carbonyl groups could not be determined unambiguously. In an attempt to determine the amount of phenolic OH groups by periodate oxidation to m-nitrovanillic acid and 4, 6-dinitroguaiacol, (Adler and Hernestam's method) it was found that only 25-30% of methanol was formed. It is evident that the nitro group present in the nucleus stabilizes the molecule towards periodate oxidation.

Both fractions of the nitrolignins (A and B) obtained by nitration in dioxane contained a small amount of nitrate nitrogen.

The following formulas were calculated for C_9:

Fraction A (insoluble):

$$C_9H_{8.61}O_{3.22}(OCH_3)_{0.70}(O_{2[COOH]})_{0.23}(NO_{2[C-NO_2]})_{0.41}(NO_{2[O-NO_2]})_{0.023}. \qquad (VI)$$

Fraction B (soluble in ethyl acetate):

$$C_9H_{8.70}O_{2.48}(OCH_3)_{0.69}(O_{2[COOH]})_{0.61}(NO_{2[C-NO_2]})_{1.13}(NO_{2[O-NO_2]})_{0.13}. \qquad (VII)$$

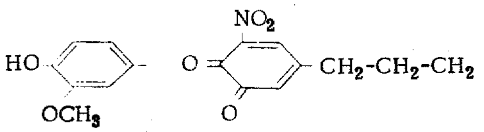
Fraction 1 of the MGL [insoluble in butanol (Formula II)] was also nitrated with anhydrous HNO_3 in CCl_4 suspension. Yield 98.5%. The nitrolignin had composition (%): C 39.84; H 3.28; OCH_3 6.27; N 9.30; NO_2 30.56; COOH 9.16. Calculated for C_9, this leads to the formula

$$C_9O_{3.0}H_{7.68}(OCH_3)_{0.58}(NO_{2[C-NO_2]})_{1.27}(NO_{2[O-NO_2]})_{0.63}(O_{2[COOH]})_{0.29}. \qquad (VIII)$$

On comparing the nitrolignins obtained by nitration with dilute HNO_3 in ether (V) (heterogeneous system) and in dioxane (VI) and (VII), and by HNO_3 in CCl_4 suspension (VIII), it can be seen that in the first case less nitrogen enters the lignin than in the second and third cases. When lignin is nitrated in ether one nitro group enters the aromatic nucleus for every three C_6—C_3 structural elements, while on nitration under the same conditions in dioxane, in which the lignin dissolves, considerably more nitrogen enters the lignin and the nitrolignin also contains a small amount of nitrate nitrogen. Formula (VIII) shows that with a large excess of HNO_3 (not passing into solution), the nitrolignin contains many nitrate groups. Attention is attracted by the practically identical fall in the content of OCH_3 groups in the lignin on nitration in ether, in dioxane, and in HNO_3 with an inert diluant (CCl_4). One third of the OCH_3 groups originally present in the MGL disappear. This amount almost exactly corresponds to the content of phenolic OH groups in the initial lignin. Similar results were obtained by L. L. Sergeeva and N. N. Shorygina for the nitration of hydrochloric-acid lignin.

The demethylation of lignin on nitration in the absence of water apparently takes place only because of the structural elements containing free phenolic hydroxyl groups.

L. L. Sergeeva and N. N. Shorygina [2] found that the nitration of 1-(4-hydroxy-3-methoxyphenyl)-propan-3-ol with 2 N nitric acid in absolute ether for several minutes gave a 40% yield of a crystalline nitroorthoquinone of the following structure:



In the nitrating medium, the bright red crystalline nitroquinone gradually loses its color, being converted into a brown powdery product. It is possible that in the nitration of lignin the structural elements of the guaiacyl type are demethylated and oxidized through the quinone stage, since, during nitration, both MGL and the mother liquor have the red color characteristic of an orthoquinone. Only when the nitrolignin is washed free from traces of acid with water does the product become orange-yellow. It is likely that the carboxyl groups [3] acquired by lignin on nitration arise partly through the oxidation of the quinone with rupture of the ring. About two thirds of the $C_6—C_3$ structural elements of lignin retain the aromatic ring on nitration in an anhydrous medium (in both ether and dioxane) as can be seen on considering the corresponding methoxyl contents.

The nitrolignin obtained by the nitration of MGL is inhomogeneous. The two nitrolignin fractions formed on nitration in dioxane differ markedly in their contents of nitro and carboxyl groups. The insoluble fractions (formula VI) contain less than one nitro group for every two C_9 structural elements and only traces of nitrate groups.

The fractions soluble in ethyl acetate (formula VII) have more than one nitro group per C_9 and about 1.5 $O—NO_2$ groups for every ten C_9 structures. The nitrolignin obtained by nitration in CCl_4 contains 12.7 nitro groups and 6.3 nitrate groups per ten lignin structural elements. Consequently, some of the elementary C_9 structures of MGL acquire one nitro group and some other C_9 units of lignin are not nitrated. Two nitro groups enter some structures. The presence in the nitrolignin of elementary units not containing nitro groups can be explained by the fact that the corresponding positions in these units are substituted (condensed structures). The presence of steric hindrance arising on the entry of the large NO_2 groups into the sterically complex lignin molecule is also possible.

The nitrate groups are apparently formed through the OH groups present on the α (with respect to the aromatic nucleus) carbon atoms of the side chains.

On nitrating 1-(3, 4-dimethoxyphenyl)-propan-1-ol with nitric acid in CCl_4, L. L. Sergeeva and N. N. Shorygina obtained the nitrate of 1-(6-nitro-3, 4-dimethoxyphenyl)-propan-1-ol [2]. However, the number of nitrate groups entering lignin when it is nitrated in ether and dioxane is very small. Only the nitrolignin formed on nitration in CCl_4 contains a large number of $O—NO_2$ groups, which corresponds to the results of the nitration of standard compounds. The number of carboxy groups in the insoluble fraction of nitrolignin is 0.23 per C_9 structural element (formula (VI)). The fraction of nitrolignin soluble in ethyl acetate contains a considerably larger number of COOH groups, namely 0.61 per C_9 (Formula VII).

To confirm and supplement the chemical data, the IR spectra of the products obtained were investigated. It was found that the IR spectrum of the MGL fraction (formula II) insoluble in butanol well exhibited the following absorption bands: 1272 ($C_{ar}-O-C$), 3447 (OH), and 1668 cm^{-1} (C=O). The presence of a benzene ring is shown in the spectrum by bands at 1591 and 1512 cm^{-1} (Fig. 1, a).

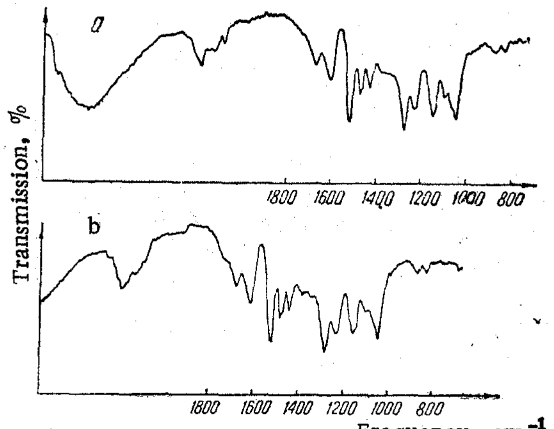


Fig. 1

The spectrum of the fraction of the MGL sample No. 2 (formula IV) soluble in butanol is basically similar to the spectrum of the insoluble fraction. It contains the same absorption bands. In the spectrum of the insoluble fraction, only a low-intensity band at 1718 cm^{-1} apparently corresponding to RCOH is somewhat stronger than the band in the spectrum of the soluble fraction (Fig. 1, b).

The IR spectrum of the nitrolignin obtained by nitration of the insoluble fraction in ether, sample No. 3, (formula V), contains bands characteristic for the following functional groups: 3442 (OH), 1728 (C=O), 1600 and 1581 (benzene ring), 1328 ($C-NO_2$), 1276 cm^{-1} ($C_{ar}-O-C$) (Fig. 2).

In the IR spectrum of the fraction insoluble in ethyl acetate, sample No. 4 (formula VI) obtained by the nitration of the MGL in dioxane, bands at 3425, 1278, 1596, and 1721 cm^{-1} correspond to OH groups, $C_{ar}—O—C$, a benzene ring, and C=O. The intense band at 1342 cm^{-1} is due to the nitro group $C-NO_2$. No absorption bands belonging to the nitrate group $O-NO_2$ are found in the spectrum, although these groups have been found chemically in small amount (Fig. 3)

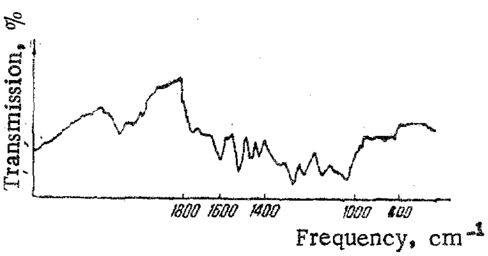


Fig. 2

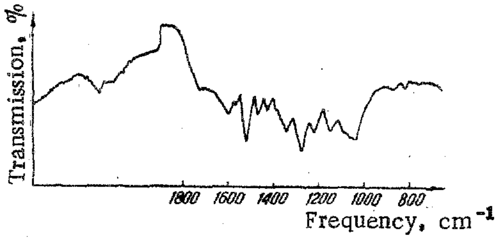


Fig. 3

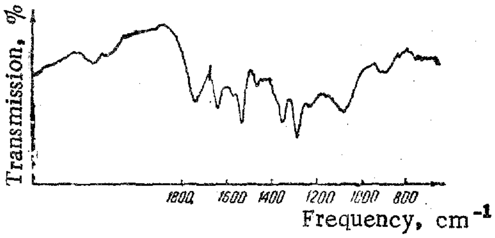


Fig. 4

The presence of strong bands at 3435, 1737, 1635, 1346, and 1282 cm^{-1} is characteristic for the IR spectrum of the fraction soluble in ethyl acetate (Formula VII) of the product of the nitration of the MGL in dioxane. The band at 3435 cm^{-1} is due to vibrations of the OH groups and that at 1282 cm^{-1} probably corresponds to the stretching vibrations of C–O in the C_{ar}–O–C group. The band at 1346 cm^{-1} is assigned to the nitro group ($C-NO_2$). The presence of nitrate $O-NO_2$ groups in the sample is confirmed by the observation of a strong band at 1635 cm^{-1}(Fig. 4).

By comparing the IR spectra of samples Nos. 4 and 5 (VI) and (VII), it can be shown that the intensity of the C=O band in the IR spectrum of sample (VII) is considerably stronger than in the spectrum of (VI) and the amount of C=O groups in sample (VII) is apparently higher than in sample (VI). A similar situation can be found by comparing the intensities of the absorption bands of the nitro groups.

Experimental

Preparation of the MGL. Sieved spruce sawdust (0.25-mm sieve) was extracted in a Soxhlet apparatus with hot water for 6 hr and with 1, 2-dichloroethane for 12 hr. Then the material was dried at room temperature under vacuum over $CaCl_2$ to a moisture content of 0.1-0.15%. The sawdust, with an ash content of 0.25% and a lignin content by Komarov's method of 27.98%, was ground in a vibration ball mill in absolutely dry toluene. Each 80-ml drum was 50% filled with steel balls, 4-5 mm in diameter, and 6.5-7 g of sawdust. The latter was ground for 10 hr in a VNIITISM [All-Union Scientific Research Institute for the Fine Grinding of Building Materials] mill. The lignin was extracted with aqueous dioxane on a shaker for a week. The lignin was isolated and purified by Bjorkmann's method [5]. The MGL was obtained in the form of an almost white powder, its amount being 30.18 g, representing a yield of 21.2% of the content of lignin in the original sawdust. Then the MGL was extracted and simultaneously freed of the bulk of the carbohydrates by Freudenberg's method [6].

Fractionation of the MGL. At room temperature, 30.18 g of the MGL was ground with small portions of butan-1-ol (75 ml) in an agate mortar. Then it was washed with 175 ml of butan-1-ol. The insoluble part of the lignin was separated on a Schott No. 3 funnel. The solution was evaporated to dryness under vacuum. The resulting pale yellow precipitate of lignin was dissolved in a mixture of 1, 2-dichloroethane and ethyl alcohol (1 : 1). The solution was centrifuged off, and the centrifugate was added in drops to absolute diethyl ether; the precipitate which deposited was filtered off on a Schott No. 4 funnel and was washed first with diethyl ether and then with petroleum ether (bp 40-60°C). The yield of fraction soluble in butanol (sample No. 2) was 0.65 g (2.36%). Sample No. 2 was dried at 80°C in a vacuum gun over $CaCl_2$ to constant weight.

The part of the lignin insoluble in butan-1-ol was dissolved in 150 ml of a mixture of dioxane and water (9 : 1) and added to 500 ml of the same mixture. The solution was treated with a small amount (9 g) of Al_2O_3 to decolorize it. The carbohydrates settled together with the alumina. The solution was filtered and the lignin free from the bulk of the sugars was isolated as described above. The yield of the MGL fraction insoluble in butanol (sample No. 1) was 28.35 g (93.94%).

Nitration of the lignin fraction insoluble in butan-1-ol with 4 N nitric acid. In absolute ether. A three-necked flask fitted with a mechanical stirrer, dropping funnel, and thermometer was charged with 1 g of lignin and 20 ml of absolute diethyl ether. The contents of the flask were cooled to 5°C and, with continuous stirring, 30 ml of a 5 N solution of HNO_3 in ether (9.45 g; 6.25 ml, sp. gr. 1.513) was added in drops. The nitration took 1 hr at room temperature with continuous stirring. The precipitate was filtered off and was washed with absolute ether (100 ml) and finally with water until it was free from traces of acid, and then it was brought to absolute dryness under vacuum over P_2O_5. The product (sample No. 3) amounted to 0.88 g (88% yield). In addition, the ethereal solution gave 0.5% of dinitroguaiacol.

In absolute dioxane. The nitration of the MGL (3.09 g) with a solution of 18.75 ml of HNO_3 (d 1.51) in 150 ml of dioxane was carried out under the same conditions. After the end of the reaction, the solution was poured into 200 ml of benzene. The precipitate which formed was filtered off and was washed with benzene (50 ml), ether (50 ml), and finally with water (100 ml), until it was free from traces of acid, and was then dried in vacuum over P_2O_5 to constant weight. The yield of sample No. 4 was 2.42 g (78%). The acidic benzene-ether washing solution was evaporated in vacuum to one quarter of its initial volume. A yellow-red precipitate deposited. The evaporated solution was extracted with 250 ml of ethyl acetate. The solution was washed with water to neutrality and was then evaporated to dryness under vacuum, and the residue was dried over P_2O_5. Yield 1 g (32% of the initial MGL – sample No. 5). The aqueous solution was evaporated to dryness in vacuum and the resulting residue was recrystallized from water. This gave 0.67 g of a substance with mp 186-187°C, corresponding to oxalic acid. In addition, 0.02 of dinitroguaiacol was obtained from the acidic benzene-ether solution.

In carbon tetrachloride. In drops, with continuous stirring, an emulsion of HNO_3 in CCl_4 (18.9 g or 12.5 ml of HNO_3, d 1.513, in 50 ml of CCl_4, which corresponded to 27 moles of HNO_3 per phenylpropane unit) was added to a suspension of 2 g of lignin in 50 ml of CCl_4 cooled to 5°C. The contents of the flask were heated to 20°C and stirring was continued for 1 hr. The lignin gradually passed into solution, and it had all dissolved at the end of the reaction. The dark cherry-red solution was poured into 200 ml of ether, after which a precipitate of nitrolignin deposited, and this was separated off on a Schott No. 4 funnel. Then the precipitate was washed with ether and water and dried over P_2O_5. Yield 1.97 g (98.5%).

The IR spectra of the compounds were determined on a UR-10 double-beam automatic infrared spectrophotometer of the firm Veb Karl Zeiss, German Democratic Republic. The spectral slit widths of the apparatus was 4 cm^{-1} for the 3500 cm^{-1} region, 6.5 cm^{-1} for the 2000 cm^{-1} region, 6.0 cm^{-1} for the 1500 cm^{-1} region, and 4.0 cm^{-1} for the 1000 cm^{-1} region. A LiF prism was used in the 4000-2000 cm^{-1} region and a NaCl prism in the 2000-700 cm^{-1} region. The spectra were obtained with samples moulded with KBr.

Summary

1. The nitration of spruce lignin obtained by mechanical grinding has been carried out with nitric acid in an anhydrous medium under heterogeneous and homogeneous conditions. Fractionation of the nitrolignin has been carried out and the elementary and functional composition of the fractions has been determined; the mean semi-empirical formulas of the C_9 structures have been calculated for both fractions. It has been shown that, depending on the nitration conditions, from one to two nitro groups enter the C_6—C_3 structural element of nitrolignin and, with an excess of HNO_3, nitrate groups as well.

The fall in the content of OCH_3 groups corresponds to the content of phenolic hydroxyl groups in the initial MGL and can be explained by the demethylation of the guaiacyl structures of the lignin on nitration.

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