STRUCTURE OF THE LIGNIN OF COTTON PLANTS OF THE SUBSPECIES *Mexicanium*

E. N. Yanishkevskaya, B. Kh. Pulatov,
Kh. A. Abduazimov

UDC 547.992:542.61+002.61

The nitrobenzene oxidation and cleavage with metallic sodium and liquid ammonia and with thioacetic acid (TAA) of the natural lignin of cotton plants of the subspecies *mexicanium* and of the dioxane lignin (DLA) isolated from it have been performed. A comparison of the yields of the products and their compositions has shown that the most complete and reliable results are given by cleavage with TAA, under which a considerable part of the lignin suffers destruction. The high content of monomeric and dimeric fractions in the products of TAA cleavage shows a low degree of condensation of the lignin of cotton plants of the subspecies *mexicanium*.

Continuing an investigation of the natural and dioxane lignins of cotton plants of the subspecies *mexicanium*, we have subjected the stems, ground and extracted with ethanol—benzene (1:2) and with hot water, and also the dioxane lignin (DLA) isolated by a modification of Pepper's method to alkaline nitrobenzene oxidation (NBO) and to cleavage with metallic sodium in liquid ammonia in order to determine the structural units and relationships between them. The identification and quantitative evaluation of the amounts of degradation products was carried out with the aid of gas-liquid chromatography.

In the treatment of the products of nitrobenzene oxidation, the acidic fraction was extracted first and then the aldehyde fraction, and they were analyzed separately. The yield of products amounted to 18% on the Komarov lignin for DLA and to 13.7% for the natural lignin. Below we give the qualitative and quantitative compositions of the products of nitrobenzene oxidation:

Substance	Cotton plant, % on the Komarov lignin	DLA, %
p-Hydroxybenzoic acid	1.02	0.34
p-Coumaric acid	0.32	—
p-Hydroxybenzaldehyde	0.12	0.21
p-Hydroyacetophenone	0.06	—
Vanillic acid	4.43	4.42
Guaiacol	—	0.73
Vanillin	0.42	1.00
Acetoguaiacone	0.04	0.32
Syringic acid	2.64	4.30
Syringaldehyde	0.12	0.40
Ratio: p-coumaryl	0.31	0.08
guaiacyl	1	1
syringyl	0.56	0.72

Guaiacyl structures predominate in the products of the nitrobenzene oxidation both of the isolated DLA and of the natural lignin of cotton plants of the subspecies *mexicanium*, while in the products of the oxidation of the DLA the proportion of syringyl structures is greater.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 758—761, November-December, 1982. Original article submitted December 17, 1981.

 0009-3130/82/1806-0722$07.50

The method of cleaving the lignin with metallic sodium and liquid ammonia, being milder, permits us to judge the structure of the C_3 side chains of the structural units of the lignin [1]. The cleavage products were extracted with ether at pH 8 and with ethyl acetate at pH 2. The combined yields amounted to 14.2% on the Komarov lignin for the natural lignin and 37% for the DLA. The results of the analysis of the total ether-extracted products of cleavage by sodium and liquid ammonia of the natural lignin of cotton plants of the subspecies *mexicanium* and the DLA, making up 4.2% of the Komarov lignin for the natural lignin and 7% for the DLA were as follows:

Substance	Cotton plant, % on the Komarov lignin	DLA, %
Phenol	—	0.02
p-Hydroxyphenylpropane	0.04	0.05
3-(p-Hydroxyphenyl)propan-1-ol	—	0.03
Guaiacol	—	0.01
Guaiacylethane	—	0.08
Guaiacylpropane	0.61	1.66
Vanillin	0.28	0.66
1-Guaiacylpropan-1-ol	0.09	0.02
Syringylpropane	3.03	3.51
Ratio: p-coumaryl	0.04	0.04
guaiacyl	1	1
syringyl	3.08	1.44

As we can see, the less condensed part of the lignin macromolecule enriched with syringyl structures undergoes cleavage by sodium in liquid ammonia, which is particularly appreciable in the case of the natural lignin.

The qualitative composition of the cleavage products enables us to judge the presence of benzyl alcohol ether groups in the lignin, as is shown by the formation of 1-guaiacyl-propan-1-ol. The presence of vanillin in appreciable amounts may show not only the occurrence of secondary oxidative processes but also the presence in the lignin of a certain amount of formyl groups, as was deduced previously on the basis of features of the PMR spectra [2].

The low yields of cleavage products do not permit us to obtain an idea of the ratio of the structural units of the whole lignin molecule.

More complete degradation of the lignin is given by cleavage with thioacetic acid (TAA) [3]. We performed the TAA cleavage of stems of cotton plants of the subspecies *mexicanium* that had been comminuted and extracted with ethanol—benzene (1:2) and with hot water and also of the isolated DLA. A comparison of the amounts of Komarov lignin in the initial raw material and in the residue after cleavage showed that about 84% of the initial lignin had undergone degradation. In the case of the DLA the degree of cleavage can be judged only from the yield of products (which is less accurate because of possible losses during the working up of the reaction mixture), and this amounted to 48%. Gel chromatography on Sephadex LH-20 showed the presence in the total cleavage products of oligomeric, tetrameric, trimeric, dimeric, and monomeric fractions, amounting, according to Nimz's method [3] to 2, 11, 14, 40, and 33% for the natural lignin and 5, 11, 19, 39, and 26% for the DLA, respectively (Fig. 1). The large amount of monomeric and dimeric fractions show the low degree of condensation of the lignin of the cotton plant of the subspecies *mexicanium*, which was also confirmed by the low molecular mass and the high hydrogen content in the semi-empirical formula of the DLA and from the characteristics of the PMR spectrum [4].

In order facilitate the separation of the products obtained, the reaction mixture was first extracted at pH 8 with ether, giving the monomeric fraction, and then, after acidification to pH 2, the higher-molecular-weight fractions were extracted with ethyl acetate. Gel chromatography of the total materials obtained on a column of Sephadex LH-20 showed that with such treatment the fractions of monomers were separated almost completely (Fig. 2).

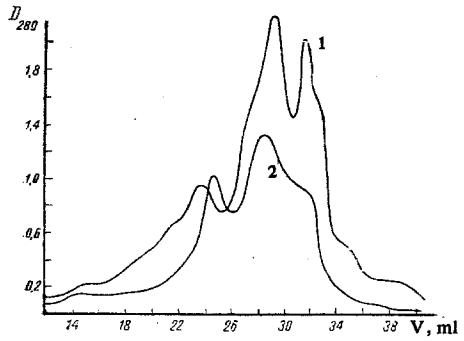

Fig. 1

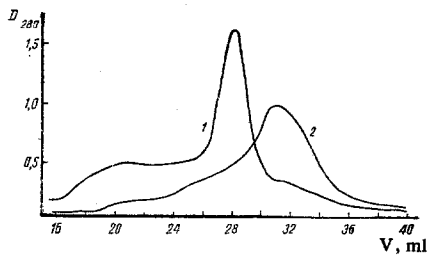

Fig. 2

Fig. 1. Eluograms of the total products of TAA cleavage: 1) natural lignin; 2) dioxane lignin.

Fig. 2. Eluograms of the products of the TAA cleavage of the natural lignin of cotton plants of the subspecies *mexicanium* extracted at pH 2 (1) and pH 8 (2).

The compositions of the monomeric fractions were studied by the GLC method (% on the total):

Substance	Cotton plant	DLA
Phenol	2.35	—
p-Hydroxyphenylpropane	0.26	0.18
Guaiacol	0.53	0.31
Guaiacylethane	8.88	8.55
Guaiacylpropane	23.11	23.92
Vanillin	0.56	—
1-Guaiacylpropan-1-ol	1.60	3.04
3-Guaiacylpropan-1-ol	17.0	14.64
Syringylpropane	26.50	26.52
1-Syringylpropan-1-ol	—	1.76
Ratio: p-coumaryl	0.01	0.004
guaiacyl	1	1
syringyl	0.54	0.56

In the products of the cleavage of the DLA the p-coumaryl structures were present in far smaller amount than in the products of the degradation of the lignin of the stems, while the ratio between the quaiacyl and syringyl structures was almost the same in these products. As also in the products of alkaline nitrobenzene oxidation, the predominating products were the guaiacyl structures, and the proportion of p-coumaryl structures was very small.

EXPERIMENTAL

The nitrobenzene oxidation of the natural lignin was carried out by Leopold's method [5], and that of the DLA by Geronikaki and Abduazimov's method [6]. From an ethereal solution, 5% sodium bicarbonate solution extracted the acids, and then 6% sodium bisulfite solution extracted the aldehydes and ketones. The acids were analyzed in the form of their methyl esters under the conditions described previously [7], and the aldehydes by Leopold's method [5]. The products were identified from their retention times and by the introduction of markers.

Cleavage with sodium in liquid ammonia and the chromatography of the products obtained were carried out as described previously [8, 9].

The thioacetic acid (TAA) cleavage of the natural lignin was performed by Nimz's method [3] with some modifications. An absolutely dry powder (25 g) with a particle size of 0.25 mm was dried in vacuum for 25 min and was then treated with a mixture of 200 ml of TAA and 5 ml of the BF_3—ether complex. The reaction mixture, protected with a calcium chloride tube, was shaken at room temperature for 7 days. The unchanged TAA was distilled off in vacuum using a liquid-nitrogen trap. The dry residue was treated five times with a 5% solution of

sodium acetate. The extracts were re-extracted with ethyl acetate and the ethyl acetate solution was washed with water and evaporated as completely as possible in a rotary evaporator. To the residue was added the sodium-acetate-washed powder and 250 ml of a 2 N solution of caustic soda in ethanol–water (3:1), and the mixture was heated in a current of nitrogen at 60°C for 24 h. Then the solid matter was separated off and it was heated once more with 200 ml of 2 N caustic soda solution in ethanol–water (3:1) for 20 h.

The solid matter was separated off and was washed with ethanol–water (3:1) (3 × 100 ml) and with methanol. The combined solutions were reduced with Raney nickel (68 g) at the boil in a current of nitrogen with mechanical stirring for 8 h. The cooled solution was decanted off, and the catalyst was washed by being boiled first with 1 N NaOH in ethanol–water (1:1) (2 × 125 ml), and then with 125 ml of ethanol–water (1:1). The ethanol was evaporated off from the combined solutions at a temperature not exceeding 30°C in a rotary evaporator. The residue was acified to pH 8 with dilute (1:1) hydrochloric acid and was extracted with ether (yield 1.5 g) and it was then acidified to pH 2 and extracted with ethyl acetate (yield 1.1 g).

The cleavage of the DLA with TAA was performed under the same conditions at a ratio of 25 ml of TAA to 1 g of DLA, omitting the stage of treating the residue. In place of this, after the elimination of the TAA residues the reaction mixture was dissolved in ethyl acetate and the solution was washed with 5% sodium acetate solution and with water, the ethyl acetate was evaporated off, and hydrolysis and reduction were performed. The yield of the total ether-extracted material was 0.27 g and of the total ethyl-acetate-extracted material 0.21 g.

The gel chromatography of the products obtained was performed on Sephadex LH-20 using methanol–water (9:1) as eluent solvent.

The GLC analysis of the total ether-extracted material was performed under the same conditions as for the products of cleavage with sodium in liquid ammonia.

SUMMARY

A comparison of the results of oxidation with nitrobenzene and of cleavage with sodium in liquid ammonia and with thioacetic acid of the natural lignin and the dioxane lignin of cotton plants of the subspecies *mexicanium* has shown that the most complete and reliable results are given by cleavage with thioacetic acid. In this case, a considerable part of the lignin undergoes degradation. Analysis of the products obtained permits the conclusion that the amount of p-coumaryl structural units in the lignin under investigation is very low, and the guaiacyl:syringyl ratio is 1:0.54 for the natural lignin and 1:0.56 for the isolated DLA. The high amount of monomeric and dimeric fractions in the products of TAA cleavage, and also the low molecular mass and high hydrogen content in the semiempirical formula, together with features of its PMR sectrum, show a low degree of condensation of the lignin of the cotton plant of the subspecies *mexicanium*.

LITERATURE CITED

1. O. P. Grushnikov and V. V. Elkin, Advances and Problems in Lignin Chemistry [in Russian], Moscow (1973), p. 258.
2. K. Lundquist and T. Olsson, Acta Chem. Scand., B31, 788 (1977).
3. H. Nimz, Chem. Ber., 102, 799 (1969).
4. E. N. Yanishevskaya, B. Kh. Pulatov, and Kh. A. Abduazimov, Khim. Prir. Soedin., 755 (1982). [In this issue].
5. B. Leopold, Acta Chem. Scand., 4, 1523 (1950).
6. A. A. Geronikaki and Kh. A. Abduazimov, Khim. Prir. Soedin., 242 (1976).
7. G. N. Dalimova, A. A. Geronikaki, E. N. Yanishevskaya, and Kh. A. Abduazimov, Khim. Prir. Soedin., 74 (1979).
8. A. A. Geronikaki and Kh. A. Abduazimov, Khim. Prir. Soedin., 93 (1977).
9. N. A. Veksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 100 (1977).