LITERATURE CITED

1. W. O. Godtfredsen, W. von Daehne, L. Tybring, and S. Vangedal, J. Med. Chem., 9, 15 (1966).
2. L. Fieser and M. Fieser, Reagents for Organic Synthesis, Vol. 4, Wiley, New York (1974).

STUDY OF THE FRACTIONAL COMPOSITION OF THE DIOXANE LIGNIN FROM THE COTTON PLANT

N. A. Veksler, M. S. Rakhmatullaev, L. S. Smirnova, and Kh. A. Abduazimov

UDC 547.458.84 + 549.927.2

It has been shown previously [1] that the dioxane lignin (DLA) from ripe cotton-plant stems is polydisperse. For a detailed study we have fractionated it in terms of molecular weights by successive precipitation from dioxane solution with ether by the triangle method [2]. This method gives a small number of fractions, but each of them has a comparatively narrow molecular-weight distribution. The separation yielded six fractions (I-VI) with successively decreasing molecular weights, since with an increase in the volume fraction of precipitant lignin fractions with ever-smaller molecular weights precipitate. Fractions (I) and (III) were the largest.

To check the efficiency of separation and to calculate molecular weights, the fractions obtained were subjected to gel chromatography in an analytical column containing Sephadex G-75 with dimethyl sulfoxide (DMSO) as solvent and eluent. Fig. 1a shows eluograms of the fractions and, for comparison, an eluogram of the unfractionated DLA is given. From these eluograms we plotted integral curves of molecular weight distribution (MWD), using the coefficients found previously [3]. The weight-average and number-average molecular weights ($\overline{M}_w$ and $\overline{M}_n$) of the fractions were calculated from the integral MWD curves. These values and their ratios ($\overline{M}_w/\overline{M}_n$), which characterize the degree of polydispersity of the fractions, are given below:

Fraction No.	Yield, %	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
Initial DLA	100	12000	4200	2,9
I	19,8	21800	15000	1,45
II	5,8	19000	11200	1,7
III	22,5	12000	7500	1,6
IV	12,8	5700	3700	1,5
V	5,7	4100	3200	1,3
VI	12,6	3000	2200	1,4

The polydispersities of the fractions were different, varying from 1.7 to 1.3. The fractions of lowest molecular weight, (V) and (VI), were the most homogeneous. By comparing the $\overline{M}_w$ and $\overline{M}_n$ values of the fractions we can see that the selected method of fractionation is fairly effective for DLA, since the molecular weights of the fractions differ considerably.

It is known [4] that spruce DLA is inhomogeneous in relation both to molecular weight and to chemical composition. Consequently, for each fraction we performed an elementary analysis and a quantitative analysis of functional groups. The results* of the analytical investigation of the DLA fractions are given below (%):

Fraction No.	C	H	OCH_3	OH_{tot}	CO	OH_{COOH}	OH_{ph}	COOH	Carbohydrates
Initial DLA	59,94	6,40	19,52	11,02	2,92	0,48	3,38	1,27	2,80
I	58,12	6,24	18,49	11,45	2,62	0,48	2,88	1,27	3,28
II	58,22	6,40	18,40	11,58	2,58	0,49	3,40	1,30	3,40
III	58,24	6,46	19,30	11,54	3,00	0,48	3,42	1,27	3,30
IV	59,74	6,30	19,47	11,90	2,96	0,53	3,41	1,40	3,80
V	59,02	6,22	19,34	11,78	2,87	0,55	3,44	1,46	4,30
VI	57,56	6,25	18,39	12,88	4,02	0,72	3,46	1,94	8,90

* The results are given without being recalculated to carbohydrates.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 69-74, January-February, 1979. Original article submitted September 22, 1978.

0009-3130/79/1501-0056$07.50

© 1979 Plenum Publishing Corporation

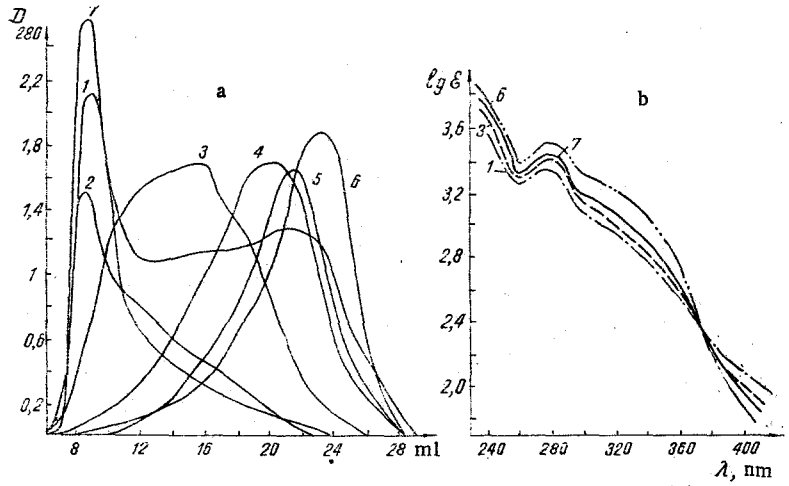


Fig. 1. Gel eluograms (a) and UV spectra (b) of the DLA fractions: 1) fraction I; 2) (II); 3) (III); 4) (IV); 5) (V); 6) (VI); 7) initial DLA.

In view of the fact that the initial DLA contained about 3% of carbohydrates bound to the lignin, we studied the distribution of the carbohydrates in the lignin fractions [5]. As can be seen from the figures given above, in the low-molecular weight fractions (V) and (VI) the amount of carbohydrates increased. To determine the nature of the carbohydrate component we subjected the sixth fraction to acid hydrolysis, and in the hydrolyzate we found glucose and xylose in a ratio of 1 : 15 by GLC [6]. Consequently, it is mainly pentosans that are bound to the lignin.

On the basis of the results of elementary and functional analysis taking the carbohydrate content into account we calculated the semiempirical formulas of each fraction per C_9 phenylpropane structural unit (PPSU):

Initial DLA, mol. wt. 205

$$C_9H_{8.02}O_{0.74}(OCH_3)_{1.33}(OH_{ph})_{0.42}(OH_{al})_{0.85}(O_{CO})_{0.22}(OOH_{COOH})_{0.06}(O_{ar\text{-}al})_{0.58}$$

Fraction (I), mol. wt. 211.2

$$C_9H_{7.76}O_{0.88}(OCH_3)_{1.3}(OH_{ph})_{0.37}(OH_{al})_{0.98}(O_{CO})_{0.2}(OOH_{COOH})_{0.059}(O_{ar\text{-}al})_{0.63}$$

Fraction (II), mol. wt. 210.7

$$C_9H_{8.1}O_{1.05}(OCH_3)_{1.20}(OH_{ph})_{0.44}(OH_{al})_{0.92}(O_{CO})_{0.2}(OOH_{COOH})_{0.063}(O_{ar\text{-}al})_{0.56}$$

Fraction (III), mol. wt. 212.0

$$C_9H_{8.11}O_{0.98}(OCH_3)_{1.36}(OH_{ph})_{0.44}(OH_{al})_{0.92}(O_{CO})_{0.23}(OOH_{COOH})_{0.061}(O_{ar\text{-}al})_{0.56}$$

Fraction (IV), mol. wt. 205.9

$$C_9H_{7.44}O_{0.66}(OCH_3)_{1.34}(OH_{ph})_{0.43}(OH_{al})_{0.93}(O_{CO})_{0.22}(OOH_{COOH})_{0.067}(O_{ar\text{-}al})_{0.57}$$

Fraction (V), mol. wt. 208.5

$$C_9H_{7.38}O_{0.82}(OCH_3)_{1.36}(OH_{ph})_{0.44}(OH_{al})_{0.91}(O_{CO})_{0.22}(OOH_{COOH})_{0.071}(O_{ar\text{-}al})_{0.56}$$

Fraction (VI), mol. wt. 212

$$C_9H_{7.65}O_{0.91}(OCH_3)_{1.38}(OH_{ph})_{0.47}(OH_{al})_{0.81}(C_{CO})_{0.32}(OOH_{COOH})_{0.093}(O_{ar\text{-}al})_{0.53}$$

On analyzing the amount of methoxy groups per C_9 unit, we see that it rises in the low-molecular weight fractions. On the basis of the OCH_3/C_9 ratio we calculated the amounts of guaiacyl and syringyl units and their ratio in each fraction. In this calculation we took into account the fact that the DLA of the cotton plant contains only two types of elementary structural units with methoxy groups – guaiacyl with one OCH_3 group and syringyl with two – and also the fact that the amount of p-coumaryl structures in the DLA is an order of magnitude smaller (according to the results of alkaline nitrobenzene oxidation [7]) than the amount of guaiacyl units:

TABLE 1

Frequency, cm^{-1}	Relative optical densities of the fractions						
	initial DLA	I	II	III	IV	V	VI
3440—3450	0,77	0,91	0,90	0,88	0,95	0,92	1,01
2940—2945	0,53	0,54	0,56	0,49	0,50	0,51	0,53
2880—2890	0,31	0,32	0,35	0,27	0,31	0,30	0,32
2850—2855	0,31	0,30	0,32	0,26	0,29	0,29	0,30
1720—1740	0,28	0,22	0,24	0,20	0,20	0,20	0,25
1595	0,75	0,79	0,79	0,76	0,71	0,76	0,68
1515	1,00	1,00	1,00	1,00	1,00	1,00	1,00
1465	0,93	1,00	1,00	0,97	0,96	0,92	0,89
1425	0,77	0,83	0,85	0,78	0,76	0,79	0,71
1270—1275	1,00	0,95	0,91	0,93	0,94	1,00	1,00
1225—1230	1,12	1,13	1,01	1,09	1,03	1,08	1,01
1125—1130	1,45	1,50	1,40	1,55	1,48	1,47	1,42
1085—1080	0,82	0,90	0,89	0,83	0,81	0,88	0,85
1035—1040	0,96	1,03	1,02	1,03	1,01	1,00	1,05
875	0,29	0,13	0,15	0,11	0,12	0,15	0,14

Fraction No.	Guaiacyl, %	Syringyl, %	Guaiacyl/ syringyl
Initial DLA	67	33	1:0.5
I	70	30	1:0.43
II	71	29	1:0,41
III	61	39	1:0,64
IV	66	34	1:0,51
V	64	36	1:0,56
VI	62	38	1:0,61

As can be seen from these figures, guaiacyl units predominated in all the fractions. With an increase in the molecular weight of the fractions, the proportion of syringyl units in them increased. Consequently, the fractions differed in the chemical structure of the aromatic nuclei.

The amounts of phenolic hydroxyls in fractions (II), (III), (IV), and (V) were practically the same (0.44). This means that in 44 out of each one hundred structural units position 4 did not participate in the formation of ether bonds. The amount of such structures in the high-molecular-weight (I) was smaller (37 out of 100), and in the low-molecular-weight fraction (VI) it was greater (47 out of 100).

The amount of acidic groups in all the lignin fractions was small, but it was somewhat greater in the low-molecular-weight fractions than in the others.

The amounts of CO groups in fractions (I-V) were practically the same: For each 100 PPSUs there were 23-20 fragments with these groups. In fraction (VI) the number of such unit was somewhat greater: 32 out of 100.

The amounts of aliphatic hydroxyls in fractions (I-V) differed little: Out of 100 structural units, 91-98 contained a hydroxy group. In fraction (VI) there were 81 such structures out of 100, but it contained a larger amount of carbonyl groups. It follows from the facts given that the low-molecular-weight fraction (VI) differed from the others also by the structure of the nonaromatic part of the molecule. It was more oxidized since it contained more CO and COOH groups. However, it is impossible to speak of a similar structure of the nonaromatic parts of the lignin in other fractions on the basis of these facts alone, since the nature of the CO and OH groups has not been elucidated.

It can be seen from the formulas that the lignin must contain fragments in which the C_3 side chain contains OH and CO groups simultaneously. The frequency of repetition of such a combination in fractions (I-V) was 1-2 structures out of 10, and in fraction (VI) 1-3 out of 10.

The dissimilar structures of the fractions are also shown by the different amounts of unidentified oxygen (from 0.66 to 1.05) and hydrogen (from 7.38 to 8.11) in them.

Thus, the fractions differed not only in molecular weight but also in chemical structure.

To characterize the fractions we recorded their IR spectra. Since visual evaluation was difficult because of the similarity of the spectra, for the quantitative evaluation of the intensities of the absorption bands we used the relative optical densities (RODs), which were determined by the internal-standard method. As the internal standard we used the 1515 cm^{-1} band, which corresponds to the vibrations of the benzene rings in lignin. The basis lines were drawn through the minima of the absorptions at 1800 and 700 cm^{-1} and through those at 3800 and 2750 cm^{-1}. The ROD values were calculated by the method of Karklin' and Érin'sh [8].

Table 1 gives the RODs of the main absorption bands in the lignin fractions. All the main bands are present in the IR spectra of all the fractions and of the initial lignin, but their ROD values differ, which shows the different amounts in the fractions of the groups to which these absorption bands correspond. This relationship appears particularly clear, for example, in the case of the 3440-3450 cm^{-1} band, which characterizes the absorption of hydroxy groups. The greatest intensity of this band and, consequently, the highest value of the ROD is found in fraction (VI) where the OH-group content is a maximum (Table 1). A similar correlation can be found for the RODs of the β-carbonyl and carboxy bands (1720-1740 cm^{-1}), for the aryl–alkyl ether, including methoxy, bands (1270-1275 cm^{-1}), and for the aromatic bands (1595, 1425 cm^{-1}).

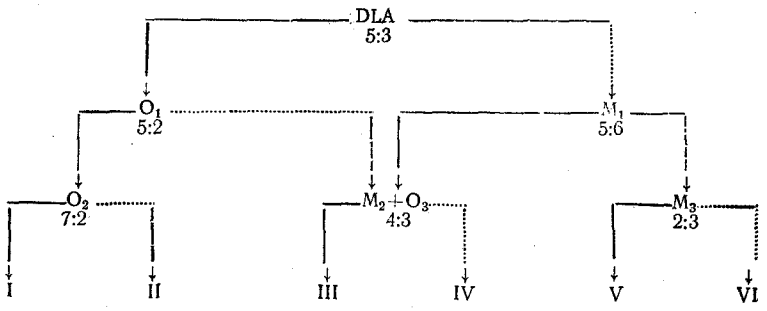
The UV spectra of the lignin fractions and of the initial DLA taken in aqueous dioxane are characteristic for the natural lignins and have maxima at 280 nm and shoulders at 300-360 nm. Fig. 1b, shows the spectra of fractions (I), (III), and (VI) and of the unfractionated DLA. The spectra of fractions (II), (IV), and (V) are close to the spectra of the unfractionated lignin and are not given in Fig. 1. Fraction (VI) has the highest absorption at 280 nm in spite of its higher content of syringyl structures, the molar extinction of which is smaller than that of guaiacyl and coumaryl structures [9]. It is obvious that in fraction (VI) the absorption at 280 nm is due to a high content of structures with conjugated bonds, including CO groups. The higher absorption of this fraction in the 300-360 nm region is also connected with an increased content of CO groups in it.

The high-molecular-weight fractions absorb more strongly in the long-wave region of the spectrum (at about 400 nm), which may be a consequence of the presence of condensed structures in them.

EXPERIMENTAL

The dioxane lignin was obtained from ripe stems of cotton plants of variety 108-F by a method described previously [10], but with preliminary washing of the plant with hot water.

Fractionation was carried out in a three-necked round-bottomed flask placed in a thermostat at a temperature of 25°C and fitted with a mechanical stirrer, a bubbler for the passage of nitrogen, a reflux condenser, and a dropping funnel. With vigorous stirring and the passage of nitrogen, 600 ml of absolute diethyl ether was added to a solution of 10 g of DLA in 1000 ml of dioxane. The first ratio of solvent (dioxane) to precipitant (ether) was 5 : 3. The subsequent ratios are shown in Scheme 1. The precipitate O_1 was dissolved in aqueous dioxane (1 : 100) and after precipitation with ether precipitate O_2 and mother liquor M_2 were obtained, the latter being concentrated in vacuum and subjected to further separation.



Scheme of the fractionation of DLA

Scheme 1

The course of fractionation is shown in Scheme 1, where the O's represent precipitates and the M's mother liquors. For precipitation, O_2 was dissolved in aqueous dioxane (1 : 100), while the other precipitates were dissolved in dioxane. The dotted arrows in Scheme 1 denote the production of mother liquors concentrated for the subsequent precipitations. The six fractions obtained were purified by precipitation from dioxane solutions in absolute diethyl ether. The yields of the purified fractions are given above. The fractions were analyzed by methods described previously [9]. Gel chromatography was performed in an analytical column (1 × 45 cm) containing Sephadex C-75 with DMSO as solvent and eluent [10]. The values of $\bar{M}_w$ and $\bar{M}_n$ were calculated by a standard method [2].

The IR spectra were taken on a UR-20 in tablets with potassium bromide, and the UV spectra on a SF-4 spectrophotometer in dioxane–water (9 : 1) as solvent. The values of log ε were calculated per phenylpropane structural unit, and the molecular weights are given in the formulas of the fractions.

SUMMARY

1. The dioxane lignin of ripe cotton-plant stems has been separated into six fractions of different molecular weights which were fairly homogeneous and differed considerably in their molecular weights.

2. It follows from the semiempirical formulas that in all the fractions guaiacyl structural units predominated. The chemical nonidentity of the fractions is shown by the different amounts of functional groups in the phenylpropane structural units and by the relative optical densities of the main bands in the IR spectra of the fractions.

3. The low-molecular-weight fraction differed markedly from the others by a higher content of carbohydrates bound to the lignin and by a greater degree of oxidation.

LITERATURE CITED

1. N. A. Veksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 122 (1978).
2. S. R. Rafikov, S. A. Pavlova, and I. I. Tverdokhlebova, Methods for Determining the Molecular Weights and Polydispersities of High-Molecular-Weight Compounds [in Russian], Moscow (1963), p. 33.
3. A. D. Alekseev, V. M. Reznikov, B. D. Bogomolov, and O. M. Sokolov, Khim. Drev., No. 4, 49 (1969).
4. É. I. Chupka, G. L. Burkov, A. V. Obolenskaya, and V. M. Nikitin, Khim. Drev., No. 3, 69 (1969).
5. A. N. Belozerskii and N. I. Proskuryakov, A Practical Handbook on Plant Biochemistry [in Russian], Moscow (1951), p. 12.
6. T. T. Gorovits, Khim. Prir. Soedin., 49 (1969).
7. A. A. Geronikaki and Kh. A. Abduazimov, Khim. Prir. Soedin., 398 (1974).
8. V. B. Karklin' and P. P. Érin'sh, Khim. Drev., No. 7, 83 (1971).
9. D. P'yu, V. Konnore, and A. Kunishi, in: The Chemistry and Biochemistry of Lignin, Cellulose, and Hemicelluloses [in Russian], Moscow (1969), p. 107.
10. N. A. Beksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 645 (1974).

DIOXANE LIGNINS OF KENAF BAST AND TOW

G. N. Dalimova, A. A. Geronikaki, E. N. Yanishevskaya, and Kh. A. Abduazimov

UDC 547.621:032.11

The present paper gives the results of a comparative study of the dioxane lignins isolated from the outer (bast) and inner (tow) parts of the stems of kenaf of the cultivated variety Uzbekskii 15-74 gathered on the territory of the Sverdlov kolkhoz [collective farm], Tashkent Oblast'. Kenaf, like the cotton plant, belongs to the family Malvaceae.

The dioxane lignins from the bast (DLALK) and from the tow (DLAKK) were isolated by a method described previously [1]. The isolated dioxane lignins consisted of brown amorphous powders soluble in the same solvents as the DLAs of Althea [2] and the cotton plant [3]. After purification by Bjorkman's method [4], they contained 3.24% (DLAKK) and 3.88% (DLALK) of bound carbohydrates [5].

Below we give the elementary and functional analyses of the dioxane lignins obtained (%):

Elementary composition and amounts of functional groups	DLAKK	DLALK
C	60.18	59.60
H	6.14	6.02
O	33.68	34.38
OCH_3	20.31	19.78
OH_{tot}	10.44	10.78
OH_{alip}	8.17	7.99
OH_{ph}	2.27	2.79
CO	2.44	2.35
COOH	0.48	0.56

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 74-82, January-February, 1979. Original article submitted October 26, 1978.

0009-3130/79/1501-0060$07.50 © 1979 Plenum Publishing Corporation