17. A. A. Geronikaki and Kh. A. Abduazimov, Khim. Prirodn. Soedin., 398 (1974).
18. V. M. Reznikov and V. F. Novitskii, Khim. Prirodn. Soedin., 81 (1975).
19. A. Yamaguchi, Mokuzai Gakkaishi, 19, No. 1, 29 (1973).
20. A. Yamaguchi, Mokuzai Gakkaishi, 19, No. 3, 141 (1973).
21. N. F. Éfendieva, Author's Abstract of Candidate's Dissertation, Moscow (1974).
22. H. Nimz, Chem. Ber., 102, 799 (1969).
23. F. Vieböck and A. Schwappach, Chem. Ber., 63, 2812 (1950).
24. G. F. Zakis, L. N. Mozheiko, G. M. Telysheva, in: Methods of Determining the Functional Groups of Lignin [in Russian], Riga (1975), p. 51.
25. J. Gierer and S. Soderberg, Acta Chem. Scand., 13, 127 (1959).
26. H. McNair and E. Bonelli, Basic Gas Chromatography, fourth ed., Varian Aerograph, Walnut Creek, Calif. (1968).

CHANGE IN THE STRUCTURAL ELEMENTS OF COTTON-PLANT LIGNINS IN THE VARIOUS VEGETATION PERIODS

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

UDC 547.621:032.11

In studying the lignin of the cotton plant and its changes during the growth of the plant, we isolated and investigated [1, 2] the dioxane lignin (DLA) from the stems of the cotton plant of variety 108-F in its various vegetation periods.

For the dioxane lignins of the earlier vegetation period (DLA-I and DLA-II) Björkman's method of purification [3] is the best, since it gives a lignin practically free from carbohydrates and having a larger proportion of methoxy groups than that purified by other methods [2]. Having adopted this method of purification and having generalized results obtained previously, we calculated the semiempirical formulas of the lignins per single phenylpropane unit:

Dioxane lignin of the early vegetation period (DLA-I)

$$C_9H_{7,47}O_{1,38}(OCH_3)_{0,47}(OH_{phen})_{1,05}(OH_{alip})_{0,93}(O_{CO})_{0,35}(OOH_{COOH})_{0,17};$$

Dioxane lignin of the flowering period (DLA-II)

$$C_9H_{7,81}O_{1,39}(OCH_3)_{0,73}(OH_{phen})_{0,92}(OH_{alip})_{1,04}(O_{CO})_{0,31}(OOH_{COOH})_{0,065};$$

Dioxane lignin of the ripe stems of the cotton plant (DLA-III)

$$C_9H_{6,85}O_{1,56}(OCH_3)_{1,0}(OH_{phen})_{0,4}(OH_{alip})_{0,87}(O_{CO})_{0,21}(OOH_{COOH})_{0,045}$$

We found catechol groups in all the lignins.

Various methods of determining catechol groups in lignins have been described in the literature, and these groups have been found not only in technical [4, 5] and demethylated [6] lignins but also in natural lignins [7]. To confirm the presence of catechol groups in the dioxane lignins of the cotton plant, we studied the kinetics of the absorption of oxygen by these lignins, and also by some model substances (Fig. 1). For clarity, the amount of oxygen absorbed has been calculated in moles per mole of oxidized substance. In the case of lignins, the calculation was referred to the molecular weight of a phenylpropane structural unit (ppsu).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 100-107, January-February, 1977. Original article submitted July 2, 1976.

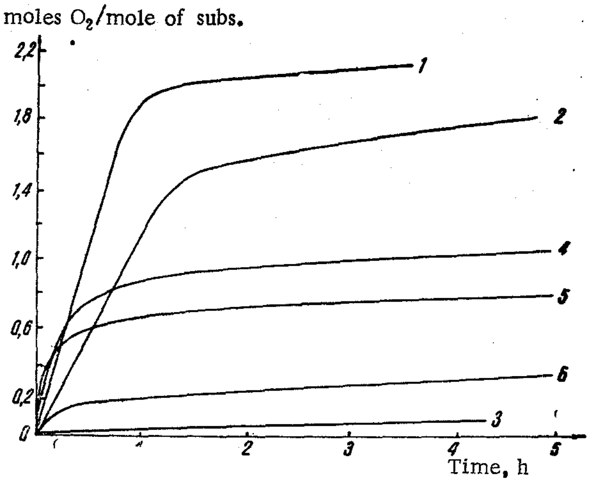

Fig. 1. Kinetics of the absorption of oxygen by alkaline solutions of: catechol (1), 5-hydroxyvanillin (2), ferulic acid (3), DLA-I (4), DLA-II (5), and DLA-III (6).

The presence of catechol structures should correspond to a considerable increase in the oxidizability of the substance by oxygen in an alkaline medium [5, 6]. The mechanism of this process has been investigated [5]. It is assumed that it is precisely the catechol groups that are responsible for the rapid absorption of oxygen in the first minutes of the process.

As can be seen from Fig. 1, with an increase in the age of the plant the amount of structures containing the catechol grouping in the lignin falls. From the nature of the kinetic curves it may by assumed that the reactivity of the catechol groups in DLA-I and DLA-II is higher than that of catechol itself and of other model substances. This may be a consequence of the presence in the lignin of substitutents in the ortho position to the catechol hydroxyls [6].

It has been proposed to use the kinetic method [5] for calculating the amount of catechol groups in the lignins. But, apparently, it is better to use the actual content of o-dihydroxy groups in lignins for a comparative characterization.

We determined the catechol groups in the DLA by the method of complexometric titration [4]: DLA-I 6.11%; DLA-II 5.0%; DLA-III 1.68%.

All the methods of determining catechol groups described above are indirect. For direct proof we used the method of alkaline nitrobenzene oxidation. Previously, we were unable to detect substances with o-dihydroxy groups in the oxidation products from DLA-I, probably because of their ready oxidizability to quinones under the experimental conditions. When DLA methylated at the phenolic hydroxyls was oxidized, the amount of veratraldehyde in the oxidation products increased sharply in comparison with the amount of vanillin in the oxidation products of the unmethylated lignin [2].

More convincing results were obtained in the oxidation of DLA-I ethylated with diethyl sulfate. In the combined ether-soluble material from the oxidation products we identified by GLC 3,4-diethoxybenzaldehyde (14.8% of the total ether-soluble material), 4-ethoxy-3-methoxybenzaldehyde (10.8%), 4-ethoxy-3,5-dimethoxybenzaldehyde (4.6%), p-hydroxybenzaldehyde + p-ethoxybenzaldehyde (3.5%), and vanillin (40%). The detection of the first-mentioned aldehyde shows the presence of catechol groups in the cotton-plant lignins.

The vanillin and p-hydroxybenzaldehyde are obviously produced from lignin fragments attached in position 4 of the aromatic ring by ether bonds to other structural units.

Taking the catechol groups and aryl—aryl bonds into account, the formulas assume the following forms:

DLA-I, mol. wt. of 1 ppsu = 197.5

$$C_9H_{7.47}\,O_{1.08}\,(OCH_3)_{0.47}\,(OH_{phen})_{0,35}\,(OH_{alip})_{0.93}\,(OH_{cat})_{0.35}\,(O_{CO})_{0.45}\,(O_{ar\text{-}alk})_{0,3}(OOH_{COOH})_{0.17};$$

DLA-II, mol. wt. of 1 ppsu = 201.4

$$C_9H_{7,81}\,O_{1,01}\,(OCH_3)_{0,73}\,(OH_{phen})_{0,32}\,(OH_{alip})_{1.04}\,(OH_{cat})_{0.3}\,(O_{CO})_{0,31}\,(O_{ar\text{-}alk})_{0.38}(OOH_{COOH})_{0,065};$$

DLA-III, mol. wt. of 1 ppsu = 199.6

$$C_9H_{6,85}\,O_{0,96}(OCH_3)_{1,01}\,(OH_{phen})_{0,3}\,(OH_{alip})_{0,87}\,(OH_{cat})_{0,1}\,(O_{CO})_{0,21}\,(O_{ar\text{-}alk})_{0.6}(OOH_{COOH})_{0,045}$$

It can be seen from the formulas that in the DLA of the later vegetation periods the amount of catechol groups has increased from $0.3/C_9$ to $0.1/C_9$ with a simultaneous increase in the amount of methoxy groups, while the number of residual phenolic hydroxyls has scarcely changed. Obviously, during the growth of the plant the methylation of the catechol hydroxyls takes place.

The results of a study of the molecular weight distribution (MWD) of the dioxane lignins by gel chromatography in a column with Sephadex G-75 using DMSO as eluent and solvent showed that a change in the method of purification had little effect on the MWD. Below we give the number-average molecular weight ($\overline{M}_n$), the weight-average molecular weight $\overline{M}_w$, and the mean molecular weight $\overline{M}_z$ found by the statistical treatment of the MWD curves [8] (for plotting the MWD curves we used the coefficients given by Alekseev et al. [9]:

Preparation	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_n:\overline{M}_w:\overline{M}_z$
DLA-I	12440	16960	21900	1:1.36:1,76
DLA-II	8820	13710	17870	1:1.55:2.03
DLA-III	5760	15190	20280	1:2.64:3.51

The facts given above and the eluograms [2] show that all the DLAs are polydisperse. Only on the eluogram of DLA-III can distinct separation into high- and low-molecular weight fractions be seen. The first fraction amounts to 67% and has a mean molecular weight of 22,600, i.e., it contains 113 structural units in the molecule, and 20% consists of the low-molecular-weight fraction with a molecular weight of 2400 (12 structural units).

The UV spectra of all the DLAs, taken in aqueous dioxane, show the maximum at 280 nm corresponding to the absorption of aromatic rings with substituents, which is characteristic for all lignins.

The IR spectra of the DLAs have the bands characteristic for the natural lignins. A broad band at 3400 cm^{-1} corresponds to OH groups and hydrogen bonds. Absorption in the 2940-2950 cm^{-1} region is due to the stretching vibrations of C–H bonds, including OCH_3 groups. A band at 1720-1730 cm^{-1} is that of β-carbonyl groups in COOH's. Absorption at 1420, 1460, 2860, 2870, and 1330 cm^{-1} is due to C–H bonds in OCH_3 groups. Bands at 1600-1620 cm^{-1} and 1520-1530 cm^{-1} relate to the skeletal vibrations of double bonds in aromatic rings. Bands at 1225-1230 cm^{-1} correspond to the absorption of phenolic groups. Bands at 1125, 1130, and 1030 cm^{-1} relate to the absorption of various ether bonds.

The IR spectrum of DLA-III differs from the spectrum of the other lignins by the fact that all the bands in it are sharper. The intensity of the 3400 cm^{-1} band is smaller than in the other spectra, which shows a decrease in the total number of hydroxyls in this lignin. In the DLA-III spectrum, the 1520 cm^{-1} band is stronger than the 1600 cm^{-1} band, in contrast to the spectra of the other lignins. A decrease in the intensity of the 1520 cm^{-1} band in comparison with the 1600 cm^{-1} band has been observed [6] in the IR spectrum of demethylated lignin, containing a large number of catechol groups, as compared with the spectrum of the initial material.

In order to study the structure of the cotton-plant lignins we subjected the DLAs and the native lignins to decomposition with sodium in liquid ammonia [11, 12]. The total monomeric decomposition products were analyzed by GLC. The products of the reductive decomposition of the DLAs of the cotton plant were as follows (% on the DLAs):

Substance	DLA-I	DLA-II	DLA-III
Phenol	0,204	—	0.014
4-Hydroxyphenylethane	—	0,023	0.022
Guaiacol	0.05	0,023	0.041
Vanillin	0.25	1,38	2.35
Vanillyl alcohol	0.04	0.44	0.21
1-(4-Hydroxy-3-methoxyphenyl)propane	1.69	3.58	6.45
(4-Hydroxy-3-methoxyphenyl)ethane	0.034	—	—
1-(4-Hydroxy-3-methoxyphenyl)ethanol	—	0.21	0.53
1-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	0.21	0.33	0.84
3-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	—	—	0.57
1-(4-Hydroxy-3,5-dimethoxyphenyl)propane	1.16	3.95	1.87
1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-1-ol	0,12	0.07	0.37

Below we give the products of the reductive decomposition of natural lignins of the cotton plant and, for comparison, information on the decomposition of ripe stems of the cotton plant [13] (% on the Komarov lignin):

Substance	Vegetation Period		
	Early	Flowering	Late
Phenol	0.12	0.055	0,017
(4-Hydroxyphenyl)ethane	0,018	—	0,011
1-(4-Hydroxyphenyl)propane	0.027	0,018	0.18
1-(4-Hydroxyphenyl)propan-1-ol	0,14	—	0.069
3-(4-Hydroxyphenyl)propan-1-ol	2,52	0.1	0.2
Guaiacol	—	0.024	—
Vanillin	2,03	1.26	0.62
Vanillyl alcohol	—	0.014	0.05
(4-Hydroxy-3-methoxyphenyl)ethane	—	0.062	0.047
1-(4-Hydroxy-3-methoxyphenyl)ethanol	0,021	0,076	—
1-(4-Hydroxy-3-methoxyphenyl)propane	2.7	3,02	3,9
1-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	0.85	0.71	2,92
3-(1-4-Hydroxy-3-methoxyphenyl)propan-1-ol	0.26	0.14	0.45
1-(4-Hydroxy-3,5-dimethoxyphenyl)propane	0.3	4.74	11.23
1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-1-ol	0.47	1.56	2.23

Figure 2 gives the chromatograms of the decomposition products from DLA-III.

In the reduction by sodium in liquid ammonia, ether bonds in the lignins are cleaved, and aryl—alkyl ether bonds most readily [11, 12]. The total yield of monomeric phenols rises with an increase in the age of the plant on the reduction both of the DLA and of the natural lignin. This obviously indicates an increase in the number of ether bonds and a decrease in the degree of condensation of the lignin. It is known that the capacity for condensation decreases for structural units in the sequence p-coumaryl, guaiacyl, syringyl, and therefore, obviously, the lignin of the ripe stems also contains a larger amount of more highly methoxylated units.

The reduction products were found to contain substances relating to three types of structures. Their quantitative ratios change according to the vegetation period. A tendency is observed to an increase in the amount of syringyl and a decrease in the amount of p-hydroxyphenyl structures relative to guaiacyl structure. This can be well seen in the reduction of the native lignins of the cotton-plant stems.

In the reduction of the DLAs, the pattern is more complex: a large part of the decomposition products is represented by p-hydroxyphenylpropane derivatives with different degrees of methylation of the benzene ring. Among them are substances with OH groups in the side chain in the α and γ positions. Analogous substances of the guaiacyl series were obtained in the cleavage of the dimeric model represented by the β-guaiacyl ether of α-guaiacylglycerol and of the lignins of coniferous trees [12]. The possibility of the formation of such substances in the reduction of coniferyl aldehyde or coniferyl alcohol units has been reported by Yamaguchi et al. [14] and has been confirmed on model substances [15].

We have not yet detected substances with a β-OH group in the propane side chain. It is likely that in the lignins of the cotton plant the majority of structural units are bound by β-aryl—alkyl bonds.

It must be mentioned that among the decomposition products substances have been found with one or two carbon atoms in the side chain: vanillin, vanillyl alcohol, p-hydroxyphenylethane, guaiacylethane, and 1-guaiacylethanol. It has not yet been accurately determined whether these substances are formed in the cleavage of the C—C bonds during the decomposition reaction or are lignin structures [16].

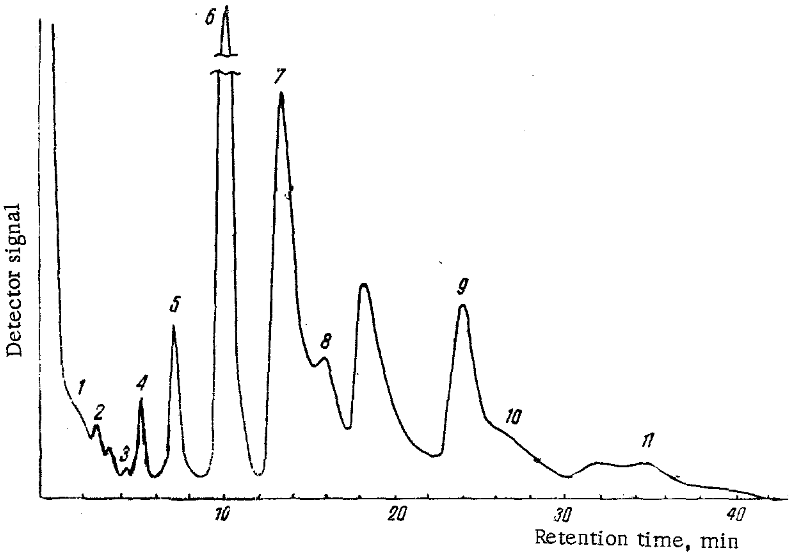

Fig. 2. Gas—liquid chromatogram of the products of the reduction of DLA-III with sodium in liquid ammonia: 1) phenol; 2) guaiacol; 3) (4-hydroxyphenyl)-ethane; 4) vanillyl alcohol; 5) 1-(4-hydroxy-3-methoxyphenyl)ethanol; 6) 1-(4-hydroxy-3-methoxyphenyl)-propane; 7) vanillin; 8) 1-(4-hydroxy-3-methoxyphenyl)propan-1-ol; 9) 1-(4-hydroxy-3,5-dimethoxyphenyl)propane; 10) 3-(4-hydroxy-3-methoxyphenyl)-propan-1-ol; 11) 1-(4-hydroxy-3,5-dimethoxyphenyl)-propan-1-ol.

The results of investigations on models [15] has shown that vanillyl alcohol is obtained in good yields by the decomposition with sodium in liquid ammonia of guaiacylglycerol and also in low yields from guaiacylpropane-1,2-diol. We assume that vanillin is split off from the structural fragments of the lignin macromolecules with which it is bound by an ether bond. It has been established [17] that vanillin is not completely reduced even on prolonged contact with sodium and liquid ammonia. The comparatively high yield of the reduction products, particularly for the lignin of the ripe stems, shows that the structural units in it are bound mainly by aryl—alkyl ether bonds.

EXPERIMENTAL

The dioxane lignins from the ripe stems (DLA-III) were isolated as described previously [1] and those from the stems of earlier periods (DLA-I and DLA-II) by a modification of a method given previously [2].

The methoxy groups were determined by the method of Vieböck and Schwappach, the total hydroxy groups, the carbonyl groups, and the phenolic hydroxyls by handbook methods [18], and catechol groups by Prokshin's method [5].

The kinetics of the absorption of oxygen in an alkaline medium by the lignins and model compounds were studied by literature methods [6, 7].

Purification of the Dioxane Lignins. The DLA-III was purified as described previously [1]. The DLA-I and DLA-II were precipitated from concentrated aqueous dioxane extracts in absolute ether. After separation and drying, the precipitated lignins were dissolved in 90% acetic acid and were reprecipitated in a tenfold volume of water. After centrifuging and washing, the isolated lignins were dried over P_2O_5, dissolved in aqueous dioxane (9:1), and reprecipitated in absolute ether.

Decomposition with Sodium with Liquid Ammonia. A 10-g sample of the comminuted (0.25 mm) plant which had been extracted with ethanol—benzene (1:1) and dried over P_2O_5 was treated with a solution of metallic sodium in liquid ammonia at —33°C. The sodium (10 g) was added gradually over 7-8 days as the solution became decolorized. After the end of the reaction and the elimination of the ammonia, first moist ether and then water were added;

the solution was neutralized with a current of CO_2 to pH 8. Then the decomposition products were extracted with ether from the aqueous solution. The ethereal extract was dried with anhydrous sodium sulfate, concentrated to small volume, and subjected to GLC analysis. In the case of the decomposition of the DLAs, 1 g of lignin and 0.75 g of sodium were used.

The gas–liquid chromatography of the concentrated ethereal extracts after decomposition was performed on a "Khrom-4" chromatograph with a flame-ionization detector and a stainless-steel column (370 × 0.3 cm) filled with 15% of Apiezon L on Chromaton NAW DMCS (0.16-0.2 mm). The column temperature was 205°C and the evaporator temperature 255°C. The rate of flow of the carrier gas (helium) was 40 ml/min. On the chromatograms, the decomposition products were identified from their retention times and by the addition of markers. Quantitative estimation was performed by the area normalization method [19].

Nitrobenzene Oxidation of Ethylated Lignin. A mixture of 1 g of the lignin, 1 ml of nitrobenzene, and 10 ml of 8% NaOH was heated in an autoclave at 180°C for 2 h. The excess of nitrobenzene and the products of its reduction were distilled off with steam. After acidification of the reaction mixture to pH 1, it was extracted with ether. The aldehydes were extracted from the concentrated ethereal solution with an 8% solution of sodium bisulfite. After the decomposition of the bisulfite compounds of the aldehydes (the acidified bisulfite solution was heated) and extraction with ether, 0.1 g of combined ether-soluble material was obtained. The gas-chromatographic analysis of this material was performed under the same conditions as the analysis of the decomposition products.

SUMMARY

1. The dioxane lignins of the cottonplant of various vegetation periods have been studied, and it has been shown that the lignin changes in the course of the development of the plant: the amount of methoxy groups increases and the amount of phenolic hydroxy groups decreases.

2. Gel chromatography of the lignins has shown that they are all polydisperse with a predominance of the high-molecular-weight fraction.

3. The 3,4-diethoxybenzaldehyde detected in the products of the alkaline nitrobenzene oxidation of dioxane lignin of the early vegetation period after its ethylation with diethyl sulfate is a direct proof of the presence of catechol structural units in cotton-plant lignin.

4. A quantitative determination of the catechol structures in the lignins of the cottonplant has shown that their amount decreases as the plant develops.

5. By decomposing the lignins with sodium and liquid ammonia, the presence in them of guaiacyl, syringyl, and p-coumaryl structural units has been confirmed and it has been shown that the lignin becomes less condensed as the plant grows, which is apparently connected with an increase in the degree of its methoxylation.

LITERATURE CITED

1. N. A. Veksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prirodn. Soedin., 645 (1974).
2. N. A. Veksler, L. S. Smirnova and Kh. A. Abduazimov, Khim. Prirodn. Soedin., 80 (1976).
3. A. Björkman, Svensk, Papperstidn., 59, 477 (1956), 60, (158) (1967).
4. G. F. Prokshin, Izv. Vyssh. Uchebn. Zaved., Lesnoi Zh., No. 5, 141 (1956).
5. G. F. Prokshin, Izv. Vyssh. Uchebn. Zaved., Lesnoi Zh., No. 6, 113 (1969).
6. G. F. Zakis, V. Ya. Neiberte, and A. Ya. Mel'ke, Khim. Drev., 14, 98 (1973).
7. B. D. Bogomolov and S. D. Pal'mova, Nauch. Tr. Arkangel'skogo Lesotekhn. Inst., No. 38, 51 (1973).
8. Ch'ien Jen-yüan, Determination of Molecular Weights of Polymers [in Russian], Moscow (1962).
9. A. D. Alekseev, V. M. Reznikov, B. D. Bogomolov, and O. M. Sokolov, Khim. Drev., No. 4, 49 (1969).
10. M. N. Yakobson, P. P. Ernin'sh, B. A. Tsinite, and G. P. Veveris, Khim. Drev., No. 3, 44, (1976).
11. N. N. Shorygina and A. F. Semechkina, Izv. Akad. Nauk SSSR, Ser. Khim., No. 4, 715 (1963); No. 5, 884 (1964); Khim. Drev., No. 1, 57 (1968); Zh. Obshch. Khim., 23, 1593 (1953); 28, 3265 (1958).

12. N. N. Shorygina and A. F. Semechkina, Tr. ILKhP i KhD Akad. Nauk LatvSSR, 19, 65 (1960).
13. S. A. Saidalimov, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prirodn. Soedin., 643 (1976).
14. A. Yamaguchi, M. Miyazaki, and A. Sakakibara, Mokuzai Gakkaishi, 19, No. 1, 23 (1973).
15. A. Yamaguchi, Mokuzai Gakkaishi, 19, No. 3, 141 (1973).
16. N. P. Mikhailov, N. N. Shorygina, and N. K. Vorob'ev, in: The Chemistry and Uses of Lignin [in Russian], Riga (1974), p. 321.
17. V. M. Reznikov and V. F. Novitskii, Khim. Prirodn. Soedin., 77, 283 (1975).
18. G. F. Zakis, L. N. Mozheiko, and G. M. Telysheva, Methods of Determining the Functional Groups of Lignin [in Russian], Riga (1975), pp. 16, 124, 118, 41 (1975).
19. H. McNair and E. Bohelli, Basic Gas Chromatography, fourth ed., Varian Aerograph, Walnut Creek, Calif. (1968).