SUMMARY

The influence of the main factors on the process of extracting urease from water melon seeds has been studied. The optimum values of the factors, degree of comminution, pH of the extractant, time of extraction, temperature of the process, and ratio of raw material and extractant have been determined.

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AN INVESTIGATION OF THE FRACTIONAL COMPOSITION OF DIOXANE LIGNIN OF THE COTTON PLANT BY THE PMR METHOD

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Analysis of the PMR spectra of six fractions of the dioxane lignins of the cotton plant differing considerably in molecular weight has confirmed their chemical nonidentity. It has been established that the low-molecular-weight fractions are less condensed than the high-molecular-weight fractions. It has been found that aliphatic OH groups are approximately evenly distributed between the α- and γ-C atoms of the lignin side chain.

We have separated the dioxane lignin of the ripe stems of the cotton plant of variety 108-F (DLA) into six fractions differing considerably in molecular weight [1]. The chemical nonidentity of the fractions has been shown by the fact that they contain different amounts of functional groups in the phenylpropane structural units (PPSUs), by a comparison of the relative optical densities of the main bands in their IR spectra, and also by an analysis of the products of alkaline nitrobenzene oxidation [2]. Continuing a study of the fractions obtained, we have investigated their PMR spectra after acetylation. As was shown previously, this method gives extremely valuable information in the comparative study of different dioxane lignins of the cotton plant.

The conditions of recording the PMR spectra and the methods for treating them have been given previously [3]. The results of the present investigation are given in Table 1.

Analysis of the numbers of free aromatic protons (zone I) showed that in the various fractions they ranged from 2.02 to 2.16 per C_9. Knowing that in all fractions the amount of p-coumaryl structures was 10-15 times

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TABLE 1. Distribution of Protons in the Phenylpropane Units of the Fractions of Cotton Plant DLA*

Zone	Limits of the zone, ppm	Fraction I		Fraction II		Fraction III		Fraction IV		Fraction V		Fraction VI	
		A	B	A	B	A	B	A	B	A	B	A	B
I	2,0—3,7	16,25	2.02	15,00	2,12	16,43	2,05	14,94	2,02	15,90	2,06	15,35	2,13
II	3,7—4,3	3,61	0,45	3.66	0,51	3,28	0,41	3,07	0,41	3,35	0,44	2,97	0,41
III	4,3–4,8	3,20	0,39	3,00	0.41	3,28	0,41	2,39	0,31	1,25	0,16	1,65	0,23
IV	4,8–7,5	52,70	6,54	50,66	7,01	51,17	6,40	50,19	6,78	47,28	6,40	48,96	6,80
IVa†	5,9—6,8	31,40	3,90‡	25,50	3,60‡	32,39	4,08‡	29,88	4,02‡	31,38	4,08‡	29,88	4,14‡
	Other H's	21,30	2,64	25,16	3,54	18,78	2,32	20.31	2,76	15,90	2,32	19,80	2,66
V	7,5—7,9	5,00	0,62	7.00	0,98	6,57	0,82	6,13	0,83	7,53	0,98	7,05	0.98
VI	7,9–8,4	18,40	2,28	20,00	2,82	18,30	2,29	22,59	2,94	21.57	2,99	21,35	2,90
VII	8,4—9,5	0,70	0,09	0,66	0 09	0,93	0.11	1,92	0,26	2,09	0,27	2,48	0,34
	Sum	100	12,39	100	14,07	100	12,49	100	13,51	100	13,26	100	13,88

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*A) Percentage content of the signal; B) number of protons per C_9.

† Region of the protons of $-OCH_3$ groups.

‡ The values found from empirical formulas are given.

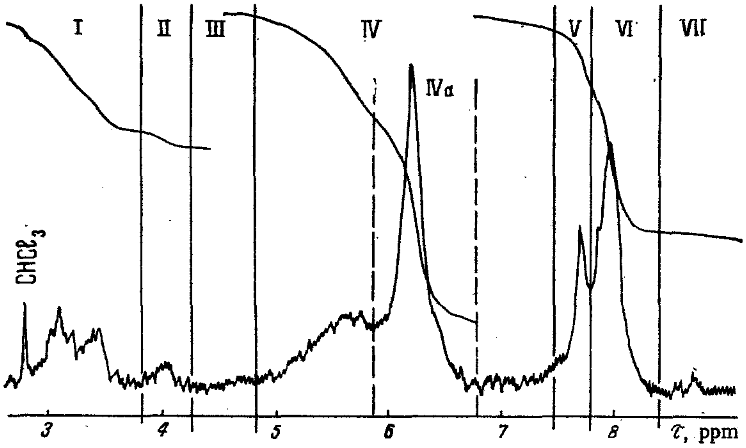


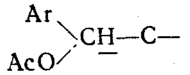
Fig. 1. PMR spectrum of the fifth fraction of cotton-plant dioxane lignin.

smaller than syringyl and guaiacyl structures, their number cannot be taken into account in calculating the degrees of condensation of the fractions. The numbers of condensed nuclei were calculated by a method described previously [3]:

Fraction	OCH_3/C_9	Number of aromatic H's theoretical	Number of aromatic H's from the PMR	Content of nuclei, %
I	1,30	2,70	2,02	68
II	1,20	2,80	2,16	64
III	1,36	2,64	2,05	59
IV	1,34	2,66	2,02	64
V	1,36	2,64	2,06	58
VI	1,38	2,62	2,13	49

The low-molecular-weight fractions (V and VI) are less condensed than the high-molecular-weight fractions (I and II). The fact that fraction VI was the least condensed can also be judged on the basis that it contained the largest number of methoxy groups per PPSU (1.38) but at the same time more than two aromatic protons were detected in the PMR spectrum, which indicates bonds of the PPSUs of this fraction with one another through the protons of the C_3 side chain.

A feature of the calculation of the degree of condensation of nuclei from the characteristics of PMR spectra is that a decrease in the number of aromatic protons by 0.1 leads to a substantial change (by 10%) in the degree of condensation of the nuclei. Because of this high sensitivity, one should probably not rely on the absolute percentage of condensed nuclei in the fractions but operate with these magnitudes for a comparative estimate.

The PMR spectra of all the fractions showed clear signals (τ = 4.05 ppm) corresponding to the protons of benzyl acetate groups  $\overset{Ar}{\underset{AcO}{>}}CH{-}\underset{|}{C}{-}$ (zone II). The number of these protons varies within fairly wide limits of 0.41–0.51 per PPSU. Consequently, in each second PPSU of the lignin molecule there is a hydroxy group in the α position to the aromatic ring. We have not detected these hydroxyls by other methods.

The calculation of the protons relating to phenylcoumaran structures (zone III) showed that in the low-molecular-weight fractions (V and VI) their number was considerably smaller than in the high-molecular-weight fractions (I and II). Phenylcoumaran structures are involved in the condensed parts of the lignin molecule and a decrease in their number in the low-molecular-weight fractions is an additional argument indicating the lower degree of condensation of the latter.

In zone IV of the PMR spectra, apart from the protons of methoxy groups, there are the H_α, H_β, and H_γ protons of the C_3 side chain. If to their number we add the hydrogens of zones II, III, and VII we obtain the total numbers of protons in the fractions with the exception of the H's of the functional groups: I, 6.40; II, 7.18; III, 5.71; IV, 6.23; V, 5.70; VI, 6.18. According to the biogenetic theory of the structure of lignin and also from the schemes of the structure of lignin given in the literature, the number of such protons ranges about six. As can be seen, in all the fractions apart from the second the number of protons ranges from 5.70 to 6.40, which agrees well with the literature. A comparison of these figures with the results of elementary analysis shows that the latter method gives high values for hydrogen (1–2 atoms per C_9). A similar situation is found in the literature [4, 5]. This can be explained by different degrees of accuracy of the methods used.

In each of the six fractions, the number of aliphatic protons is 4.45–3.20. It is difficult to judge from this the degree of substitution of the C_3 side chain, since the differences in the numbers of protons are small and the number of CO groups, aryl–alkyl bonds, and aliphatic hydroxyls in the fractions differ.

From the numbers of protons in zones V and VI it is possible to calculate the numbers of phenolic and aliphatic hydroxyls in the PPSUs of the fractions, since the protons of the aromatic (zone V) and aliphatic (zone VI) acetoxy groups appear there. In none of the spectra that we obtained was a clear separation of the signals of the acetoxy protons (aromatic and aliphatic) observed because of their partial overlapping. However, the total signal of acetoxy protons was expressed very clearly. A calculation of hydroxyls is given below (1, from the formula; 2, from the PMR spectrum):

Fraction	Phenolic OH's		Aliphatic OH's		Total OH's	
	1	*2*	*1*	*2*	*1*	*2*
I	0,37	0,21	0,98	0,76	1,35	0,97
II	0,44	0,33	0,92	0,94	1,36	1,27
III	0,44	0,27	0,92	0,76	1,36	1,03
IV	0,43	0,27	0,93	0,97	1,36	1,24
V	0,44	0,33	0,91	0,98	1,36	1,31
VI	0,47	0,33	0,81	0,99	1,35	1,32

In all the fractions, the numbers of aromatic hydroxyls calculated with the aid of the PMR spectra are lower than those obtained by chemical methods while, as a rule, the number of aliphatic protons is higher. This is explained by the fact that part of the aromatic acetate groups present in the ortho position to the diphenyl bond appeared in zone VI.

It can be seen from the facts given above and previously [3] that the PMR-spectroscopic method of calculating the total number of hydroxyls in all the preparations studied (F-I, F-II, F-III, DLA-I, DLA-II, DLA-K) [6, 7] gives lower values than chemical methods of determination. Better agreement of the results is achieved only for the low-molecular-weight fraction. It has been observed that the intensity of the coloration of the DLA falls sharply after acetylation. The pigments strongly bound to the lignin probably become soluble in diethyl ether and leave the lignin after acetylation. These substances contain OH groups which are possibly the reason for the high values in analyses.

Knowing the number of aliphatic and benzyl hydroxyls, and also taking into account the fact that other PPSUs of lignin are usually attached to the β-C atom of the phenylpropane chain through a –O–bond, it may be considered that about half the OH groups are attached to the α-atoms and the others to the γ-atoms of the C_3 side chain.

In region VII the signals of protons appear that belong to methyl and methylene aliphatic groups. It can be seen from Table 1 that with an increase in the molecular weight of the fractions the amounts of such protons in them rises from 0.09 to 0.34. This is probably connected with the degree of condensation of the fractions.

Thus, the use of the PMR method has confirmed that the fractions of cotton-plant dioxane lignin differ from one another not only by their molecular weights but also chemically. This method has enabled us to compare the degree of condensation of the lignin fractions and to differentiate and calculate the amounts of α- and γ-aliphatic hydroxy groups.

EXPERIMENTAL

The dioxane lignin fractions were acetylated for 24 h with a tenfold amount of an equimolar mixture of acetic anhydride and pyridine at room temperature. The acetylated fractions were precipitated in water and, after drying, were purified by reprecipitation of their dioxane solutions in absolute ether. The preparations were carefully dried in a vacuum desiccator over P_2O_5.

PMR spectra were taken on a JNM-4H-100/100 MHz spectrometer at 22–24°C (c 10–12% by weight, 0–HMDS, τ scale, solvent deuterochloroform).

SUMMARY

On the basis of an analysis of the PMR spectra of six fractions of dioxane lignin from the ripe stems of the cotton plant, their chemical nonidentity has been confirmed. It has been established that the low-molecular-

weight fractions (V and VI) are less condensed than the high-molecular-weight fractions (I and II). It has been found that the aliphatic OH groups are approximately evenly distributed between the α- and γ-C atoms of the lignin side chain.

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