2. The maximum yield of rice husk lignin cleavage products amounted to 91% on the Komarov lignin, on the addition of 0.1% of anthraquinone referred to the initial raw material, which increases the yield of low-molecular-weight products by a factor of 1.8.

3. In the process of hydrogenolysis, anthraquinone promotes the demethoxylation of the structural units of lignin with syringyl nuclei.

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CLEAVAGE BY SODIUM IN LIQUID AMMONIA OF KENAF LIGNINS AND THE BIOLOGICAL ACTIVITY OF THE CLEAVAGE PRODUCTS

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The natural lignin of kenaf stems and fractions of the dioxane lignin of kenaf stems isolated previously were cleaved by sodium in liquid ammonia in order to study their structure. It was established that the kenaf lignins consist of three types of structural units: p-coumaryl, guaiacyl, and syringyl, with a predominance of the guaiacyl types. An antioxidation activity of the monomeric products of the degradation of the kenaf lignins has been found.

The fractionation of kenaf dioxane lignin (KDL) has been reported previously [1]. The fractions obtained were fairly homogeneous and differed considerably in molecular weight. In the present paper we give the results of the cleavage by sodium in liquid ammonia of fractions of kenaf lignin. For comparison, the natural lignin (a flour of kenaf stems) was also cleaved. The yields of cleavage products are given below (the values marked with asterisks are given as percengages of the Komarov lignin, and the others as percentages of the weight of the lignin):

Sample	Sum of the monomeric phenols, pH 8	Sum of the phenolic substances, pH 2
Natural lignin	14,6*	51,68*
KDL, initial	15,92	18,95
KDL-1F	11,39	19,83
KDL-2F	13,01	20,85
KDL-3F	13,94	14,22
KDL-4F	19,94	21,69
KDL-5F	23,39	29.90
KDL-6F	24,56	28,90

The yields of monomeric cleavage products increased on passing from KDL-1F to KDL-6F, which permits us to conclude that the degree of condensation decreases on passing from the first fraction to the sixth.

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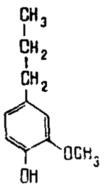
The total amounts of the phenolic substances extracted at pH 2 by ether rose on passing from the initial KDL to KDL-2F and from KDL-3F to KDL-5F, increasing, in general, from the initial KDL to the KDL-6F, which also indicates a decrease in the degree of condensation of the low-molecular-weight fractions. In actual fact in the fractions with a lower molecular weight there was a larger amount of uncondensed groups, as has also been reported in [2], which probably affected the yields of the cleavage products of these lignins. Natural kenaf lignin was cleaved to a greater extent than the isolated lignins, since they were less condensed.

The sum of the monomeric cleavage products was analyzed by the GLC method (Table 1). The main cleavage products were guaiacylpropane and syringylpropane (I, II). Guaiacylpropane was present in the largest amount in the products of the cleavage of the KDL-6F. The presence among the cleavage products of guaiacylpropan-1-ol, guaiacylethan-1-ol, and p-hydroxyphenylpropan-1-ol indicated the presence of benzyl alcohol groups (III) in the kenaf lignin macromolecules.

Similarly, the detection of guaiacylpropan-3-ol in the products of the cleavage of the kenaf lignins must be regarded as an indication of the presence of primary OH groups (IV).



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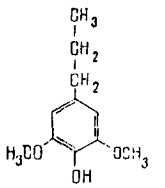

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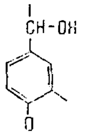

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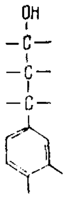

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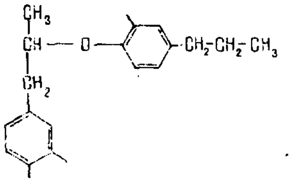

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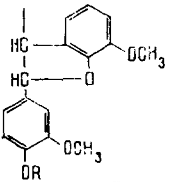

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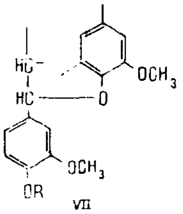

VII

No guaiacylpropan-2-ol was found among the monomeric cleavage products of any of the samples studied, which shows indirectly, as has been stated by Grushnikov and Elkin [3], the presence of β-alkyl aryl ether bonds (β-O-4) (V). Monomeric phenols with hydroxy groups in the α-position to the aromatic rings (guaiacylpropan-1-ol, p-hydroxyphenylpropan-1-ol) are probably formed in the cleavage of α-alkyl aryl ether bonds. The latter are represented in phenylcoumaran structures (VI), where they are part of a heterocyclic system, and also in open noncyclic structures (VII) [3, p. 282]. The formation of guaiacylpropan-3-ol on the cleavage of kenaf lignins can be explained by a breakdown of alkyl aryl ether bonds in which the γ-C atom of the propane side chain takes part. For example, the detection of a dimeric compound with a γ-O-4 bond in the products of the hydrogenolysis of the wood of coniferous species is known [4]. Furthermore, phenol can also be formed on the cleavage of other fragments of lignin containing guaiacylpropane structures.

Monomeric phenols with an ethyl side chain (p-hydroxyphenylethane, guaiacylethane, guaiacylethan-1-ol), which were detected among the cleavage products of all the samples studied, are probably the products of secondary transformations taking place during the treatment with sodium in liquid ammonia and are not present in the lignin molecule. A similar phenomenon has been reported in [5, 6].

Guaiacyl units predominated in all the samples. The total phenolic cleavage products extracted at pH 2 by ether were studied by the gel-chromatographic method. The gel eluograms of these total extracts indicated that they contained oligomeric, tetrameric, trimeric, dimeric, and monomeric cleavage products.

Thus, the cleavage by sodium in liquid ammonia of natural and dioxane kenaf lignins has shown that they consist of three types of structural units: p-coumaryl, guaiacyl, and syringyl. The main cleavage products were guaiacylpropane (I) and syringylpropane (II).

TABLE 1. Monomeric Products in the Cleavage of Kenaf Lignins

Substance	Natural lignin, % on the Komarov lignin	KDL init	KDL-1F	KDL-2F	KDL-3F	KDL-4F	KDL-5F	KDL-6F
		% on the weight of the lignin						
Phenol	0,027	—	—	1,79	—	—	0,14	—
Guaiacol	0,59	0,096	0,41	0,57	0,070	0,053	0,25	—
p- Hydroxyphenylethane	0,036	0,35	0,16	0,59	0,38	—	0,90	6,31
p-Hydroxyphenylpropane	—	—	—	—	—	0,106	—	—
p-Hydroxyphenylpropan-1-ol	0,56	0,99	0,93	1,19	1,12	2,23	—	—
Guaiacylethane	0,33	0,43	0,18	0,19	0,28	0,67	—	0,24
Guaiacylpropane	2,84	4,50	2,53	5,37	2,59	6,15	6,26	8,13
Guaiacylpropan-1-ol	2,64	2,90	—	5,36	—	4,13	5,47	—
Guaiacylethan-1-ol	—	—	2,62	—	3,09	—	—	—
Guaiacylpropan-3-ol	0,99	—	—	5,25	0,15	—	0,55	—
Vanillin	—	—	—	—	—	—	—	4,0
Syringylpropane	4,39	4,04	3,01	6,96	4,04	6,52	9,73	5,8
Unidentified	2,97	2,58	1,50	5,17	2,16	—	—	—
Ratio								
p-coumaryl	0,085	0,17	0,19	0,13	0,25	0,20	0,08	0,50
guaiacyl	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
syringyl	0,60	0,51	0,52	0,39	0,68	0,60	0,80	0,47

Cleavage with sodium in liquid ammonia, as a milder method of oxidation, has given information on the structure of the side chains of the monomeric structural units of the kenaf lignin.

It is known that, with respect to its antioxidant properties and capacities for binding peroxide radicals, lignin is not merely not inferior but is actually superior to some natural antioxidants [7]. Catignani and Carter [8] consider that with respect to its level of antioxidation action lignin is close to vitamin E.

In the light of what has been said above and in order to establish the antioxidant action of the monomeric products of the cleavage of kenal lignin — the total phenolic substances, consisting of p-coumaryl, guaiacyl, and syringyl units — we have carried out biological trials of these sums by the procedure of [9].

The biological trials of the four total sets of products from the cleavage of kenaf lignins (natural kenaf stem lignin, the initial KDL and KDL-1F and KDL-3F) showed that they exhibited an appreciable antioxidant action. Thus, on the addition of alcoholic solutions of the combined monomeric products of cleavage to homogenates of rat liver (incubation at 37°C for 60-240 min), processes involved in the spontaneous peroxide oxidation of the lipids were suppressed, which was shown by a fall in the amount of malondialdehyde (determined by the thiobarbiturate method) in the samples [10].

Table 2 shows that the sums of the monomeric phenol products of the cleavage of kenaf lignins taken for analysis in the final concentration of 10^{-5} g/ml suppressed the peroxide oxidation of the lipids in experiments with a high degree of reliability. The greatest effect was observed when the samples were incubated for 180 and 240 min. After this time, the amount of malondialdehyde in the samples was smaller than in the controls by 36-54%.

The antioxidant activity of the total extracts studied that has been found is apparently due to the presence of phenolic compounds with p-coumaryl, guaiacyl, and syringyl structures. This conclusion is confirmed by results published by Catignani and Carter [8], who considered structures of the type of ferulic, vanillic, syringic, and p-hydroxybenzoic acids to be responsible for the antixoidant properties of lignin.

EXPERIMENTAL

Cleavage with sodium in liquid ammonia was performed as in [11].

Chromatographic analysis was carried out on a Chrom-4 instrument with a flame-ionization detector using a stainless steel column (0.3 × 370 cm) filled with 15% of Apezon L Chromaton NAW DMCS (0.16-0.20 mm). The temperature of the column was 205°C and that of the evaporator 255°C and the rate of flow of carrier gas (helium) was 40 ml/min. The cleavage products were identified from their retention times and by the addition of standard substances. Quantitative estimation was performed by the area-normalizing method [12].

TABLE 2. Influence of the Total Sets of the Monomeric Phenolic Cleavage Products in a Final Concentration of 10^{-5} g/ml on the Amount of Malondialdehyde in Rat Liver Tissue (M ± m), nm per 1 mg of Protein

Preparation	Time of incubation, min			
	60	120	180	240
Control	0,462±0,019	0,808±0,045	1,432±0,098	1,813±0,057
Sum of the monomeric products of cleavage by sodium in liquid ammonia of				
kenaf stems	0,325±0,011 p<0,001	0,602±0,010 p<0,01	1,117±0,027 p<0,02	1,155±0,034 p<0,001
the dioxane lignin of kenaf stems, KDL init	0,305±0,06 p<0,001	0,620±0,033 p=0,01	0,735±0,033 p<0,001	0,980±0,061 p<0,001
1st fraction of kenaf dioxane lignin, KDL-1F	0,283±0,012 p<0,001	0,597±0,009 p<0,002	0,689±0,032 p<0,001	0,825±0,012 p<0,001
3rd fraction of kenaf dioxane lignin, KDL-3F	0,275±0,029 p<0,001	0,502±0,033 p<0,001	0,670±0,008 p<0,001	0,834±0,018 p<0,001

Gel-chromatographic analysis was performed on an analytical column (1.0 ± 45.0 cm) containing Sephadex LH-20 with ethanol—water (9:1) as eluent and solvent.

The amounts of malondialdehyde in the rat liver homogenates after the addition of alcoholic solutions of the total extracts of the monomeric products of cleavage by sodium and liquid ammonia were determined by the thiobarbiturate method described in [10].

CONCLUSIONS

1. The cleavage by sodium in liquid ammonia of the natural lignin and of fractions of kenaf dioxane lignin has shown the presence in their macromolecules of three types of structural units — p-coumaryl, guaiacyl, and syringyl, with a predominance of the guaiacyl type — and has given information on the structure of the side chains of the monomeric structural units of the lignin.

2. The results of cleavage by sodium in liquid ammonia have shown a decrease in the degree of condensation of the kenaf lignin fractions with a decrease in the molecular weights of the fractions, i.e., on passing from KDL-1F to KDL-6F.

3. An antioxidant activity of the total set of monomeric phenols from the cleavage of kenaf lignins has been found.

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