# THE LIGNIN OF THE SEA ISLAND COTTON PLANT OF VARIETY S-6030 AFFECTED BY FUSARIAL WILT. I.

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A comparative study of the dioxane lignins isolated from healthy and wilt-affected cotton plant stems has shown that Fusarium fungi demethylate lignin and make it more oxidized than the lignin from healthy stems.

We have previously reported the results of a study of the dioxane lignin (DLA) isolated from ripe stems of the sea island cotton plant of variety S-6030 [1]. Cotton plants of the sea island varieties are attacked by fungi of the genus Fusarium, and the medium-fiber varieties by Verticillium. It follows from the literature that the fungi of both types attack not only the cellulose but also the lignin [2]. The changes taking place in the lignin under the action of wilt fungi have been little studied. It is known that fungi of the genus Fusarium are capable of breaking down individual fragments of lignin faster than fungi of the genus Verticillium [2]. However, only partial breakdown of the lignin molecule takes place.

The total amounts of lignin determined by Komarov's method [3] is healthy and wilt-affected stems of a cotton plant of variety S-6030 differed little (24.17% in the stems of the healthy plants and 25.61% in those of the diseased plants).

The yield of DLA obtained by Pepper's method under a current of nitrogen [4] from stems infected by the fungi (7.16% on the weight of the total lignin) was lower than from healthy stems (9.52%). In both cases it amounted to 1/3 of the total lignin. The DLA was purified by two reprecipitations through the addition of its aqueous dioxane solutions to absolute ether.

The developed empirical formula per $C_9$ unit has been calculated for the DLA form a wilt-affected cotton plant on the basis of the results of elementary analysis and functional-group analysis (given without taking the carbohydrates into account, their amount in the DLA being low – 0.85%):

$$C_9H_{8.13}O_{1.77}(OCH_3)_{0.98}(OH_{ph})_{0.29}(OH_{al})_{1.05}(O_{CO})_{0.4}(O_{ar-al})_{0.71}.$$

As compared with the DLA from the stems of a healthy cotton plant of the same variety [1]

$$C_9H_{8.7}O_{0.59}(OCH_3)_{1.18}(OH_{ph})_{0.29}(OH_{al})_{1.19}(O_{CO})_{0.32}(O_{ar-al})_{0.71}$$

it contained a smaller amount of $OCH_3$ groups and of aliphatic hydroxyls. However, its oxygen content was higher ($5.2/C_9$) than in the DLA from healthy stem ($4.28/C_9$), which indicates a higher degree of oxidation of this lignin. As in the DLA from healthy stems, the amount of phenolic OH groups was fairly low ($0.29/C_9$) and the $C_3$ side chain was highly hydroxylated.

The UV spectrum of the DLA from the stems of a wilt-affected cotton plant had the maximum at 280 nm that is characteristic for lignins. The molar extinctions at 280 nm proved to be different for the DLA from the healthy and wilt-affected plants (3200 and 2600, respectively). This indicates different numbers of lignin structural units of different types and, possibly, different types of bonds in the two DLAs.

The IR spectra of the DLAs from the healthy and diseased plants were superficially similar but a calculation of the VOOP* absorption bands [7] showed their nonidentity.

*VOOP – data provided by the All-Russian Society for Conservation of Natural Resources – Editor.

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A study of molecular-weight distribution by column gel chromatography on Sephadex G-75 and molecular weight calculation showed that the two DLAs differed insignificantly. The DLA from the wilt-affected stems had $\bar{M}_w$ = 8200, $\bar{M}_z$ = 16,400, $\bar{M}_n$ = 3800. The polydispersity $\bar{M}_w/\bar{M}_n$ = 2.15.

When the native lignin was subjected to nitrobenzene oxidation, the total yield of aldehydes and acids was higher from the wilt-affected stems (1.94%). The yield of the same products from the healthy stems was 1.65%, which indicates a lower degree of condensation of the native lignin in the diseased plant.

As can be seen from the figures given below, in the combined aldehydes and acids substances were identified that belonged to three types of structural units: p-coumaryl, guaiacyl, and syringyl. In the products of the oxidation of the healthy stems their ratio was 0.13:1:0.66, and in the diseased stems 1:1:1.11. In the lignin of the diseased stems, for each guaiacyl unit there were 7.7 times more p-coumaryl structures and 1.7 times more syringyl structures (I – healthy stems; II – diseased stems):

| Oxidation product | From the native lignin, % of the Komarov lignin | | From the DLA, % on the DLA | |
|---|---|---|---|---|
| | I | II | I | II |
| p-Hydroxybenzaldehyde | 0.015 | 0.011 | 0.28 | — |
| p-Hydroxybenzoic acid | -- | 0.42 | 0.12 | 10.74 |
| p-Coumaric acid | 0.034 | 0.39 | 1.28 | 6.35 |
| Guaiacol | — | — | 0.12 | 0.025 |
| Vanillin | 0.27 | 0.33 | 3.60 | 4.88 |
| Vanillic acid | 0.05 | 0.47 | 13.96 | 1.24 |
| Acetoguaiacone | 0.04 | 0.024 | — | — |
| Ferulic acid | 0.013 | — | — | — |
| Syringaldehyde | 0.24 | 0.212 | 1.05 | 0.84 |
| Syringic acid | 0.002 | 0.70 | 7.58 | 3.10 |

On the nitrobenzene oxidation of DLA, the total yield of aldehydes and acids was somewhat higher in the case of the DLA from the wilt-affected cotton plant (37% as compared with 35% from the DLA of the healthy stems). Consequently, as in the native lignin, the DLA from the stems affected by fusarial wilt was less condensed than the DLA from the healthy stems. The ratio of p-coumaryl-, guaiacyl, and syringyl structures in the DLA from the healthy stems was 0.08:1:0.38, and in the diseased stems the proportion of p-coumaryl components and, to a smaller extent, the proportion of units with syringyl structures had increased: 2.8:1:0.64.

In the calculatin of the $OCH_3$ groups present on an average in one phenylpropane structural unit from the ratio of the structural units according to the results of nitrobenzene oxidation, it was found that the DLA from the healthy stems contained 1.20 $OCH_3/C_9$, and the DLA from the diseased stems 0.51 $OCH_3/C_9$. Consequently, from the amount of $OCH_3$ groups in the products of nitrobenzene oxidation, as well, after attack by wilt the lignin was impoverished in methoxy groups.

## EXPERIMENTAL

The dioxane lignin was obtained by a method described previously [1]. The elementary composition of the DLA was: C – 59.34%; H – 5.18%.

Functional group analysis [5] (%): methoxy groups – 15.15; carbonyl groups – 5.52; total OH groups – 10.79; phenolic hydroxyls – 2.31; carbohydrates bound to the lignin – 0.85 [6].

UV spectra were taken on a SF-26 spectrophotometer in aqueous dioxane (1:9) and IR spectra on a UR-20 instrument in tablets with potassium bromide. Gel chromatography was performed on a 1.2 × 45 cm column filled with G-75 gel in DMSO. $V_e$ = 28.7 ml, $V_0$ = 10 ml for dextran blue with a molecular weight of 2,000,000. To calculate the molecular weights we used coefficients found previously [8].

The nitrobenzene oxidation of the native lignin and the DLA was performed as described in [9], and the GLC of the total acids and aldehydes as in [10].

SUMMARY

A comparative study of the dioxane lignins isolated from healthy and wilt-affected stems of the cotton plant of variety S-6030 has shown that Fusarium fungi demethylate the lignin of cotton-plant stems and make it more oxidized as compared with the lignin from healthy stems.

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# THE REACTION OF NITROLIGNIN WITH AMMONIA

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The reaction of nitrolignin with ammonia has been studied. It has been established that it takes place mainly through the carbonyl and carboxy groups of the lignin. A dark brown product with a high nitrogen content readily soluble in water and exhibiting biological activity was obtained. On the basis of the results of elementary and functional analyses, a semiempirical formula has been calculated for the substance obtained, and its viscosity and electrical conductivity have been determined. The molecular weight, determined by the sedimentation method in an ultracentrifuge, was more than 60,000. It was established by gel chromatography that the product of the interaction of nitrolignin with ammonia was polydisperse.

Nitrolignin (NL), obtained by nitrating hydrolysis lignin from cottonseed husks at the Andizhan hydrolysis factory is used in the drilling of deep wells as a viscosity-lowering agent [1], and it is biologically active [2] and possesses a limited solubility in water.

In the nitration process, the functional composition of the macromolecule of lignin undergoes considerable changes: the amount of $OCH_3$ and OH groups falls and the amount of carboxy and carbonyl groups increases greatly. New functional groups ($NO_2$, etc.) are introduced into the lignin molecule [3, 4]. The introduction of nitrogen into the lignin macromolecule leads to an increase in its solubility.

The aim of our work was to increase the solubility of nitrolignin in water, to raise its biological activity, and to study the changes taking place in the lignin macromolecule on its reaction with ammonia.

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