CHANGE IN THE STRUCTURAL ELEMENTS OF THE LIGNIN OF THE BROWN ALGA *Cystoseira barbata* AT DIFFERENT AGES

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Natural and isolated lignins of the alga *Cystoseira barbata* of different ages have been cleaved with metallic sodium in liquid ammonia. Nine compounds were identified in the cleavage products by GLC: derivatives of the p-coumaryl and guaiacyl series with free OH groups in the α and γ positions of the side chains of the aromatic nucleus. It has been shown that with the growth of the alga the lignin becomes less condensed, which may be due to an increase in the degree of its methoxylation.

Studying the lignin of the annual brown alga of the Black Sea *Cystoseira barbata* and its change in the process of the growth of the plant we have isolated the dioxane lignin (DLA) and the Björkmann lignin (MWL) of alga of different ages and have studied their compositions [1, 2]. The isolated preparations contained all the functional groups characteristic for lignins, but with the growth of the alga the compositions of the lignins changed [2].

The results obtained permit the assumption that differences in the functional state also involve differences in the structure of the isolated preparations of the *Cystoseira* lignin. To confirm this hypothesis experimentally, and also to establish the aromatic nature of the *Cystoseira* preparations, we have performed the decomposition with metallic sodium and liquid ammonia [3, 4] of the DLA, the MWL, and the native lignins. The total monomeric decomposition products were analyzed by GLC.

The yield of ether-soluble phenols on a single treatment of the *Cystoseira* DLA and MWL with sodium and liquid ammonia amounted for samples of the alga older than three years to 22.01% and 22.04%, respectively, for that aged 2-3 years to 20.03%, and for the youngest samples to 21.01%. The yield of monomeric products of the cleavage of the natural *Cystoseira* lignins with metallic sodium in liquid ammonia for samples more than three years old was 1.31% of the mass of the plant; for those 2-3 years old, 1.28%; and for those up to one year old, 1.20%.

The products of the reductive cleavage of the *Cystoseira* DLA and MWL were as follows (% in the mixture):

	DLA-I (up to one year)	*DLA-II (2-3 years)*	*DLA-III (more than three years)*	*MWL*
Phenol	10,91	—	—	—
1-(4-Hydroxyphenyl)propane	0,42	0,81	1,83	6,99
Guaiacol	6 71	47,21	19,63	4,30
Vanillin	0,12	0,22	0.51	0,97
(4-Hydroxy-3-methoxyphenyl)ethane	—	0,97	4,71	2,58
1-(4-Hydroxy-3-methoxyphenyl)propane	—	31,03	54,97	66,24
1-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	0,62	6,49	14,14	15,05
3-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	—	—	2.62	—

The products of the reductive cleavage of the natural *Cystoseira* lignins of different ages were (% in the mixture):

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	Up to one year	*2-3 years*	*More than 3 years*
Phenol	43,78	0,40	—
1-(4-Hydroxyphenyl)propane	12.02	3,55	3,11
1-(4-Hydroxyphenyl)propan-1-ol	15,45	—	—
Guaiacol	3.43	—	1,24
Vanillin	—	—	1.24
(4-Hydroxy-3-methoxyphenyl)ethane	—	2,12	2,98
1-(4-Hydroxy-3-methoxyphenyl)propane	—	67.02	61,16
1-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	—	11,55	14.36
3-(4-Hydroxy-3-methoxyphenyl)propan-1-ol	—	6,37	15,66

There were a number of differences in the compositions of the alkaline fractions of the cleavage products of the *Cystoseira* lignins. Thus, in the decomposition of the natural lignin of *Cystoseira* aged more than three years, seven compounds were identified which were mainly derivatives of guaiacylpropane. In the alkaline fractions of the *Cystoseira* lignin aged up to one year, it was mainly derivatives of the p-coumaryl series that were detected. In the products of the decomposition of the lignin of *Cystoseira* aged 2-3 years we found six compounds which were derivatives of both the p-coumaryl and the guaiacyl types. The distribution was similar in alkaline fractions of the degradation products of isolated preparations of *Cystoseira* lignin.

A large part of the decomposition products consisted of p-hydroxyphenylpropane derivatives with different degrees of methoxylation of the benzene ring. Among them were substances with OH groups in the side chain in the α and γ positions. Similar compounds were obtained in the cleavage of a dimeric model of the β-guaiacyl ether of α-guaiacylglycerol and of the lignin of conifer wood [4]. The possibility of the formation of such substances in the reduction of coniferyl aldehyde or coniferyl alcohol units has been reported by Yamaguchi [5].

The absence among the products of the decomposition of the *Cystoseira* lignins of compounds with hydroxy groups in the β position to the aromatic ring is indirect evidence of the fact that in the *Cystoseira* lignins the β-arylalkyl ether bond predominates. The fairly high percentage of guaiacylpropan-1-ol in the products of the cleavage of the algal lignins (particularly in the mature samples) indicates a high content of benzyl alcohol groups in them. The comparatively large yield of dihydroeugenol shows that elimination of the OH groups in the side chain takes place during the reaction. No derivatives of the syringyl series were found in the samples studied.

In addition to phenolic alcohols, vanillin was detected in the phenolic fractions of the decomposition products of the natural *Cystoseira* lignins. According to the literature [6], the formation of vanillin indicates that it is a structural fragment of the *Cystoseira* lignin attached to the macromolecule by means of an alkyl-aryl or an aryl-aryl ether bond. The sum of the decomposition products of guaiacyl nature for the dioxane lignins of *Cystoseira* aged 2-3 years and more than 3 years exceeded 86%. This is probably due to the fact that the guaiacyl products in these lignins are bound predominantly by ether bonds which on decomposition with metallic sodium in liquid ammonia are cleaved most completely [3, 4]. However, it is not excluded that the comparatively high proportion of guaiacyl derivatives and low proportion of p-coumaryl derivatives is a consequence of the incomplete (not more than 22%) degradation of the *Cystoseira* lignins.

The acid fractions of the products of the cleavage of the native *Cystoseira* lignin and of isolated preparations were separated by extraction with ethyl acetate at pH 2. The yield of phenolic products soluble in ethyl acetate amounted, for the DLA-I, DLA-II, and DLA-III, to 26.4, 24.35, and 20.01% of the lignin taken, and for the native lignins of *Cystoseira* aged up to one year, 2-3 years, and more than 3 years it was 2.6, 1.5, and 1.4% of the plant taken, respectively.

To characterize this fraction we used the method of gel filtration on type LH-20 gel in the methanol—water (9 : 1) system, employing the partition coefficients given by Smirnova and Abdazimov [7]. As an example, Fig. 1 gives the gel chromatogram of the cleavage products of DLA-III.

On an eluogram (Fig. 1) of the cleavage products, the regions of oligomers, tetramers, trimers, dimers, and monomers present among the products of the incomplete decomposition of this fraction are distinguished. Since the bulk of the monomers is extracted by ether, the monomeric products are represented in very small amounts. The bulk of the degradation products of the natural and isolated *Cystoseira* lignins is composed of dimers.

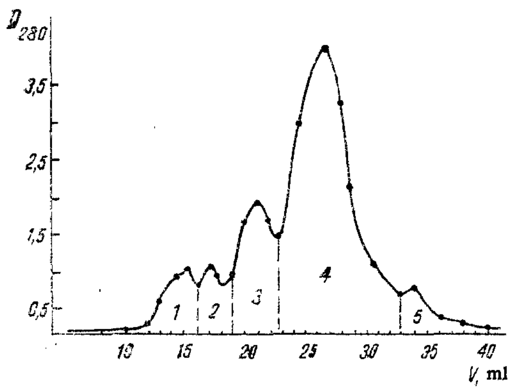

Fig. 1. Gel chromatogram of the products of the cleavage of dioxane lignins from *Cystoseira* aged more than three years (DLA-III) by metallic sodium in liquid ammonia: 1) oligomers; 2) tetramers; 3) trimers; 4) dimers; 5) monomers.

EXPERIMENTAL

The dioxane lignins of *Cystoseira* of different ages (DLA-I, DLA-II, and DLA-III) were isolated by Pepper's method [8] and the mechanical grinding lignin by Bjorkman's method [9]. For the more complete isolation of the dioxane lignins from the young specimens of *Cystoseira* (DLA-I, DLA-II), the plant material was first ground in a rotary mill in an atmosphere of argon for 200 h. All the preparations isolated were purified by Björkman's method [9].

Decomposition with Metallic Sodium in Liquid Ammonia. A 5-g sample of the comminuted (0.25 mm) plant that 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 (5 g) was added gradually as the solution became decolorized. In the case of the decomposition of the MWL and the DLA, 1 g of lignin preparation and 0.75 g of sodium was taken. After the completion 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. The low-molecular-weight phenols were extracted with ether and subjected to GLC analysis. The residue was acidified with sulfuric acid to pH 2 and was extracted four times with ethyl acetate. Separation of the ethyl acetate layer in the treatment of the *Cystoseira* was rendered difficult by the formation of a stable emulsion consisting of slimy polysaccharides of alginate nature. The polysaccharides were eliminated from the preparation by centrifugation. The fractions extracted by ethyl acetate were studied by gel chromatography.

The gas-liquid chromatography of the concentrated ethereal extracts was performed on a Chrom-4 chromatograph with a flame-ionization detector using a stainless steel column (370 × 0.4 cm) filled with 15% of Apiezon L on Chromaton NAW DMCS (0.16-0.20 mm). The temperature of the column was 205°C and that of the evaporator 250°C. The rate of flow of carrier gas (helium) was 35 ml/min. On the chromatograms, the decomposition products were identified from their retention times and by the addition of standard substances. Quantitative evaluation was carried out by the method of area normalization [10].

The gel chromatography of the concentrated ethyl acetate extracts was performed in an analytical column (1 × 32 cm) containing Sephadex LH-20, the solvent and eluent being methanol—water (9 : 1). The column was calibrated as described by Shtreis and Sevost'yanov [11].

SUMMARY

By decomposing *Cystoseira* lignins with metallic sodium in liquid ammonia the presence of p-coumaryl and guaiacyl structural units in them has been established and it has been shown that with the growth of the alga the lignin becomes less condensed, which is apparently connected with an increase in the degree of its methoxylation.

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CLEAVAGE OF THE LIGNINS OF THE ALGA *Cystoseira barbata* BY THIOACETIC ACID

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The most complete cleavage of the aryl-alkyl ether bonds in *Cystoseira* lignins takes place under the action of thioacetic acid. It has been shown that the lignins of *Cystoseira* of different ages differ in the composition of the monomeric structural units. In the lignins of younger specimens of *Cystoseira* (age up to one year), p-coumaryl structures predominate and as the alga gets older a tendency is observed to an increase in the amount of guaiacyl structures and a decrease in the amount of p-coumaryl derivatives. It has been established that the β-arylalkyl ether bond is one of the main bonds between the structural units of *Cystoseira* lignin.

The structural units of the macromolecule of the lignin of specimens of the brown alga *Cystoseira barbata* of different ages are derivatives of the p-coumaryl and the guaiacyl series [1].

Continuing a study of the chemical structures of *Cystoseira* lignins, we have performed the thioacetic acid cleavage of lignins isolated from *Cystoseira* of different ages by Pepper's method (DLA) and by Björkman's method (MWL), and also of the natural lignins of the alga. This reaction leads to the more complete cleavage of the lignin macromolecule than the action of metallic sodium in liquid ammonia. According to Nimz [2, 3], under the action of thioacetic acid in the presence of BF_3 a selective cleavage of the arylglycerol β-aryl ether bonds of the lignin takes place without the formation of secondary C—C bonds as the result of condensation and polymerization. In this process, up to 92% of the initial lignin is cleaved [4].

The thioacetic acid cleavage of the MWL and DLA preparations and also of the natural *Cystoseira* lignins of different ages was performed as described in the literature [5, 6]. The combined monomeric degradation products were extracted with ether at pH 8 and were studied by the GLC method. After the extraction of the monomeric fraction, the solution was acidified to pH 2 and the remaining phenolic compounds were extracted. This fraction was studied by gel chromatography on Sephadex LH-20 in the methanol—water (9 : 1) system. Calibration of the analytical column was carried out on the basis of results obtained by Smirnova and Abduazimov [4].

As a result of the reaction with thioacetic acid, the *Cystoseira* lignin preparation was 86-96% cleaved. On cleavage with metallic sodium and liquid ammonia, the yield of phenolic products did not exceed 22% [1]. Below we give the yield of degradation products of the natural lignins and the dioxane lignins of *Cystoseira* of different ages (% on the initial amount):

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