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LIGNINS OF *Rumex acetosa* SEEDS

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Spinach seeds were analyzed quantitatively. Wilstetter lignin was isolated and characterized by alkaline hydrolysis of spinach seed—lignocarbohydrate complex. Its elemental composition and molecular weight were determined. Its IR spectrum was analyzed.

Key words: *Rumex acetosa*, spinach seeds, lignin hydrochloride, lignocarbohydrate complex.

Common spinach or sour spinach (*Rumex acetosa*) is a perennial herbaceous plant of the smartweed (Polygonaceae) family that is used as a leafy vegetable.

The chemical composition of spinach seeds must be studied in detail in order to find pathways to utilize them.

The chemical composition of spinach seeds is as follows (%): C, 40.42; H, 6.38; N, 2.05; Komarov lignin, 11.55; cellulose, 13.91; easily hydrolyzed polysaccharides (EHPS), 31.91; difficultly hydrolyzed polysaccharides (DHPS), 0.77; Wilstetter lignin, 10.49; and lignocarbohydrate mix, 57.28.

The content of Komarov lignin in spinach seeds is characteristic of herbaceous plants. Its seeds have a high EHPS content and a very low DHPS content. The spinach-seed carbohydrate complex probably is the EHPS type, i.e., hemicellulose. There is much less cellulose in spinach seeds than in herbaceous plants.

It was necessary to free the Komarov lignin, cellulose, and polysaccharides from tannin-like substances in order to determine them or else the results for these components were elevated.

Lignin hydrochloride or so-called Wilstetter lignin was isolated in order to study it more completely. The yield of lignin hydrochloride from spinach seeds was 10.49% of the plant mass or 90.85% of the Komarov lignin. This indicates that it is labile since almost all lignin was isolated. It is a dark pink powder that is soluble in dioxane—water (9:1), DMSO, and weakly basic solutions. The elemental and functional composition (%) of the isolated Wilstetter lignin from spinach seeds is: C, 49.95; H, 5.96; N, 2.07; OH, 11.89; CO, 3.78; COOH, 1.02.

Spinach seeds are not strongly lignified. Therefore, the comparatively low C content and relatively high H content is reasonable. Lignin in lignified plants usually does not contain N. However, the part of the N that is still involved in formation N-containing components in the immature plant may be found in amino acids bonded to lignin. It is known that methionine acts as a methylating agent in lignin biosynthesis [2] and hordenine is converted to lignin as young barley runners grow and develop [3].

It can be seen that spinach-seed lignin has all principal lignin functional groups. The presence of carboxylates in spinach-seed lignin, which is typical of tomato- and pomegranate-seed lignins, distinguishes them from lignins of certain herbaceous plants, for example, those of cotton and althaea [4, 5].

IR spectra of spinach-seed Wilstetter lignin contains the following absorption bands (cm^{-1}): 3378, stretching vibrations of phenol and alcohol OH involved in H-bonds; 2940, asymmetric stretching of C–H bonds in methyl and methylene groups; 1656, carbonyl stretching vibrations; 1523, skeletal vibrations of aromatic rings; 1374, deformations of C–H bonds in methyl and methoxy groups; 1243, 1155, 1082, 1021, asymmetric stretching vibrations of C–O–C, C–C, and C–O bonds; 862, 766,

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out-of-plane deformations of C–H bonds in aromatic rings. It should be noted that frequencies in the range 1500-1610 cm^{-1} are characteristic only for lignins.

The molecular weight of spinach-seed Wilstetter lignin as determined by viscosimetry was 16,230.

In order to study the lignin more completely, we hydrolyzed natural spinach-seed lignin with NaOH (8%) as before [1]. The suspended particles that formed upon acidification of the basic spinach-seed hydrolysate with conc. H_2SO_4 gave a dark brown amorphous powder that was insoluble in water and organic solvents and poorly soluble in bases. It can be assumed that the suspended particles were formed by hydrolysis of the lingocarbohydrate components of the studied specimens and are probably their small fragments.

The chemical nature of the suspended particles was studied by hydrolyzing them with H_2SO_4 (2 N) for 48 h at 100°C. The presence of carbohydrates was established using phenolsulfonic acid and paper chromatography. The hydrolysate contained qualitatively glucose, xylose, arabinose, and galactose. Thus, the hydrolyzed part of the suspended particles is carbohydrate in nature.

The presence of lignin in the suspended particles was proven using a color reaction with fluoroglucinol [6]. Thus, suspended particles isolated during alkaline hydrolysis of spinach seeds are fragments of the lignocarbohydrate complex. The colloidal state of these fragments is stabilized by the protective action of the polysaccharides [7]. Upon acidification, as in our instance, or upon cryogenic treatment, the colloidal state of the suspended particles changes [8] and they settle.

The following absorption bands typical of lignin and carbohydrate preparations were found in the IR spectrum of the spinach-seed suspended particles (lingocarbohydrate mixture) (cm^{-1}): 3421, stretching vibrations of phenol and aliphatic OH groups involved in H-bonds; 2941, 2887, stretching vibrations of C–H bonds in methyls; 1716, 1654, stretching vibrations of C=O carbonyls and carboxylates; 1542, 1509, skeletal vibrations of aromatic rings; 1396, deformations of O–H bond in phenol hydroxyls; 1156, 1081, 1022, C–O and C–O–C stretching vibrations; and 1081, 1044, skeletal vibrations of pyranose ring. The IR spectrum of the suspended particles is not as well resolved as that of Wilstetter lignin owing to the overlap of lignin and carbohydrate bands in the studied specimens. This can be seen in the broadening of the band for OH absorption from 3200-3600 to 3000-3700 cm^{-1}.

The elemental composition and molecular weight of the spinach-seed lignocarbohydrate mix (%) are: C, 40.96; H, 6.70; N, 0.51; MW, 78,300. The molecular weight was determined by viscosimetry using an Ostwald viscosimeter.

The relatively low C and H contents indicate that the suspended particles are not only lignin. Pure lignin preparations typically have a higher C content (58-62%).

Thus, spinach-seed lignin has properties and gives results for quantitative analysis that are similar to lignins of herbaceous plants.

EXPERIMENTAL

Paper chromatography was performed using butanol:pyridine:water (6:4:3) with anilinium acid phthalate developer and FN 11 paper.

Raw Material Preparation. The alcohol:benzene extract of spinach seeds that were defatted with hexane and $CHCl_3$ was obtained by the TAPPI-6m-59 method [6]. Extracted seeds were ground to a powder in a Tsiklon laboratory grinder. Plant material prepared this way was used for further analyses.

Elemental Analysis. The contents of C, H, and N in lignin hydrochloride and in the lignocarbohydrate complex were determined on an EA-1108 CHNS analyzer (Italy).

Functional Composition. The contents of OH, carbonyl, and carboxylates in the lignin were established by the literature methods [9].

IR spectra of lignin hydrochloride and the lignocarbohydrate mix were recorded on a Model 2000 Fourier IR spectrometer (Perkin—Elmer, Sweden) in KBr disks.

Lignin content was determined using a modified Komarov method with 72% H_2SO_4 [6]; **cellulose content**, using the Kuerschner—Hoffer method based on alcoholic HNO_3 [6]; **EHPS content**, by an ebulliostatic method [6] based on direct titration. Hot basic Cu solution was titrated with sugar solution on a special apparatus, an ebulliostat, that enabled the analysis to be performed in a stream of water vapor without allowing air to reach the surface of the reacting liquid.

Content of DHPS was also established using an ebulliostatic method [6].

Lignin hydrochloride (Wilstetter lignin) was isolated as before [2].

Acid hydrolysis of suspended particles was carried out analogously to the literature [8]. Suspended particles (0.1 g) in H_2SO_4 (4.0 mL, 2 N) in sealed ampuls were heated on a water bath for 48 h at 100°C. The ampuls were opened. The hydrolysate was centrifuged. The centrifugate, the hydrolyzed part of the suspended particles, was evaporated to 1 mL.

Phenol-sulfate Specimen. Diluted hydrolysate (0.5 mL) of suspended particles was treated with phenol (0.5 mL, 0.5%) and conc. H_2SO_4 (2.5 mL). This gave a brown color typical of carbohydrates.

Color reaction for lignin in suspended particles was performed with fluoroglucinol [6].

Molecular weights of lignin hydrochloride and lignocarbohydrate mix of spinach seeds were determined by viscosimetry using an Ostwald viscosimeter by the literature method [10].

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