Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [*Russian original No. 3, May-June, 2011*]

NITROBENZENE OXIDATION OF LIGNINS FROM SEVERAL PLANTS OF THE FAMILY Gramineae

Z. K. Dzhumanova and G. N. Dalimova*

UDC 547.99.992

It was found based on results from alkaline nitrobenzene oxidation of natural and isolated dioxane lignins from several plants of the family Gramineae such as rice husks, rice straw, and corn and sorghum stems that the studied lignins consisted of three types of structural units with the predominance of guaiacyl structures.

Keywords: rice husk, rice straw, corn stems, sorghum stems, nitrobenzene oxidation, phenolcarboxylic acids, aromatic aldehydes.

Grasses (Gramineae Juss.) are distributed in nature on all continents, represent a significant part of the biomass of many biocommunities, and are considered one of the most widespread and useful plants for man. The main components of the cell wall, e.g., cellulose, carbohydrates, proteins, extractable substances, of many grasses have by now been investigated. One of the main components of grasses is lignin, the content of which in grasses reaches 15–28% and which is not yet fully studied. It is also known that high-molecular-weight compounds of plant xylem, cellulose and lignin, exhibit valuable properties and can be used in various economic sectors.

We reported previously on the isolation of dioxane lignins (DLAs) from rice husk and straw and from corn and sorghum stems. These were characterized by determining the chemical composition and calculating the semi-empirical formula [1, 2].

The goal of our work was to study the structure of natural lignins and DLAs from rice husk and straw and corn and sorghum stems using alkaline nitrobenzene oxidation (NBO).

The yields of NBO products from the studied samples were different for both natural lignins and DLAs isolated from each plant (Fig. 1).

HPLC was used to analyze total oxidation products of natural lignins from the grasses (Table 1). The analysis showed that aromatic aldehydes, ketones, and phenolcarboxylic acids belonging to three types of structural units (*p*-coumaryl, guaiacyl, and syringyl) were formed by NBO of lignins from the samples.

A comparison of the ratio of guaiacyl, syringyl, and *p*-coumaryl structural units in the alkaline NBO products of natural lignins and DLAs from the studied plants found that syringyl structures predominated in the oxidation products of rice-straw DLA. This may have indicated a low degree of condensation in this lignin (Tables 1 and 2). However, guaiacyl structures predominated in the oxidation products of natural lignin and rice-husk DLA. The observation of a rather significant quantity of *p*-hydroxybenzoic acid in the oxidation products of corn stems was a characteristic signature of this plant and reached 5–10%, in agreement with the literature on the content of esterified phenolcarboxylic acids in grasses [3].

The ratio of syringaldehyde to vanillin (S/V) is given in many studies as an indicator of the degree of condensation of lignin preparations. For example, the ratio of syringyl and guaiacyl groups in mechanically milled lignin (MML) of wheat-straw (*Triticum aestivum* L.) internodes showed that the Maule color reaction [treatment of sections of plant material with $KMnO_4$ solution (1%) for 5 min and then HCl and NH_4OH (a red color should appear for a positive reaction)] was negative for lignins with $S/V < 2$ [4].

The S/V ratios for lignins from rice husk and straw were 0.25 and 0.57, respectively, whereas that for natural lignins from corn and sorghum stems differed sharply from them and were 16.28 and 3.65, respectively. A ratio $S/V < 2$ means that the studied lignins from rice husk and straw were similar to conifer lignins whereas those from corn and sorghum stems were similar to larch lignins.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: dalimov@list.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 374–376, May–June, 2011. Original article submitted October 18, 2010.

TABLE 1. HPLC Analysis of Alkaline NBO Products of Natural Lignins from Gramineae Plants, % in Mixture

Compound	Rice husk	Rice straw	Corn stem	Sorghum stem
Phenol	–	–	5.99	0.23
Vanillin	3.2	1.24	2.93	2.41
Acetovanillone	0.2	0.1	–	–
p-Hydroxyacetophenone	–	–	3.21	1.11
p-Hydroxybenzaldehyde	1.1	0.4	4.34	9.34
Syringaldehyde	0.8	0.6	0.18	0.66
Vanillic acid	Tr.	0.1	0.13	14.33
p-Hydroxybenzoic acid	0.6	Tr.	25.64	1.71
Syringic acid	0.2	0.5	0.15	0.12
Ratio				
syringyl:guaiacyl:*para*-coumaryl	0.3:1.0:0.5	0.8:1.0:0.07	0.1:1.0:12.7	0.74:1.0:0.05

TABLE 2. HPLC Analysis of Alkaline NBO Products of Dioxane Lignin from Gramineae Plants

Compound	DLARH	DLARS	DLACS	DLASS
Vanillin	13.5	5.7	2.8	3.6
Acetovanillone	0.9	0.3	–	–
p-Hydroxybenzaldehyde	4.0	2.6	2.5	1.5
Syringaldehyde	1.2	5.0	0.3	4.1
Vanillic acid	Tr.	0.4	4.8	3.5
p-Hydroxybenzoic acid	2.9	Tr.	2.2	3.2
Syringic acid	0.7	2.5	0.1	2.8
Ratio				
syringyl:guaiacyl:*para*-coumaryl	0.13:1.0:0.26	1.17:1.0:0.05	0.06:1.0:0.05	0.6:1.0:0.9

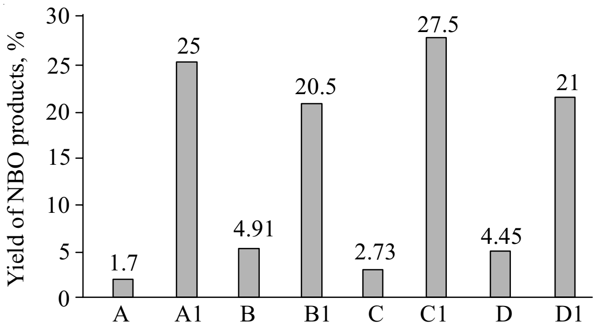

Fig. 1. Yields of nitrobenzene oxidation products: rice husk (A), DLARH (A1), rice straw (B), DLARS (B1), corn stem (C), DLACS (C1), sorghum stem (D), DLASS (D1).

The high yield of vanillin for NBO of natural lignin and that isolated from rice husk (3.2 and 13.5%, respectively) may indicate a high content in these lignins of structures with a C_α–C_β double bond in the propane side chain of the phenylpropane structural unit. The yield of vanillic acid in such instances is greatly reduced. This can be seen in Tables 1 and 2. The presence of a hydroxyl on C_6 of the propane side chain gives an analogous result [5]. The yield of vanillin decreased sharply and the ratio of vanillic acid to vanillin changed simultaneously upon NBO with a CO group on C_α.

Thus, alkaline NBO of natural lignins and those isolated from the grasses rice, corn, and sorghum showed that, with the exception of natural lignin from corn stems, guaiacyl groups predominated in all samples of natural lignins. The same trend was observed in general upon alkaline NBO of DLA from the studied plants. The predominance of *p*-coumaryl groups in natural lignin from corn stems may indicate that this plant contained a large amount of phenolcarboxylic acids, in particular, *p*-hydroxybenzoic acid.

EXPERIMENTAL

Alkaline NBO of Natural Lignins. Meal of non-woody plants (1.0 g) that was extracted by an alcohol:benzene (1:2) mixture, nitrobenzene (1.5 mL), and NaOH solution (15 mL, 2 N) was placed in a stainless-steel autoclave. The lignin was oxidized at 160°C for 3 h with constant stirring (rocker).

The autoclave was cooled after the oxidation was finished. The contents were rinsed with NaOH solution (2 N, 3 × 10 mL). The reaction mixture was filtered through a No. 1 glass filter and washed with H_2O (10 mL) and $CHCl_3$ (20 mL). The filtrate was extracted with $CHCl_3$ (5 × 50 mL) to remove the excess of nitrobenzene and its reduction products. The $CHCl_3$ layer was decanted. The aqueous layer was acidified by conc. HCl to pH 2.5 to convert phenolates to phenols. The acidified solution was treated with NaCl until saturated. Phenolic substances were extracted by $CHCl_3$ (5 × 50 mL).

The combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The solid was dissolved in acetone. The amount of solution was adjusted to 1 g. Then the prepared sample was studied by HPLC.

Alkaline NBO of DLAs. Lignin (0.2 g), nitrobenzene (1.0 mL), and NaOH solution (2 N, 10 mL) were placed in a stainless-steel autoclave. The lignin was oxidized at 160°C for 3 h with constant stirring.

The reaction products were worked up as above and studied by HPLC.

HPLC was carried out on an AT 1100 instrument (Agilent Technology, USA) with a diode-matrix detector, autosampler, and thermostatted column by the Polyphenol method using a C8 Hypersil column (150 × 4 mm, 5 μm). The mobile phase was a gradient of solution A (80%) (0.1 M NaH_2PO_4, pH 3 buffer) and solution B (20%) (MeOH). The flow rate was 1.25 mL/min. The column was thermostatted at 20°C. Peaks were detected at 210 nm.

REFERENCES

1. Z. K. Dzhumanova and G. N. Dalimova, *Uzb. Khim. Zh.*, No. 2, 5 (2009).
2. Z. K. Dzhumanova, G. Berdimbetova, and G. N. Dalimova, *Khim. Prir. Soedin.*, 338 (2009).
3. G. N. Dalimova and Kh. A. Abduazimov, *Khim. Prir. Soedin.*, 160 (1994).
4. T. B. T. Lam, K. Iiyama, and B. A. Stone, *J. Sci. Food Agric.*, **51**, 493 (2006).
5. K. V. Sarkanen and C. H. Ludwig, eds., *Lignins: Occurrence and Formation, Structure, Chemical and Macromolecular Properties, and Utilization*, Interscience, New York, 1971.