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# LIGNINS FROM ANNUAL GRASSY PLANTS

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*Lignins isolated from the annual grassy plants oats* Avena sativa*, rye* Secale *sp., wheat* Triticum *sp., and barley* Hordeum *sp. were characterized chemically. It was found that the grassy lignins belonged to a class of compositionally heterogeneous GSH-type biopolymers and consisted of monomeric units of the guaiacyl, syringyl, and p-coumaryl types.*

**Keywords:** grassy plants, lignin, elemental composition, functional composition, IR spectroscopy, $^{13}$C NMR spectroscopy, ratio of structural units.

Straw from grassy plants is a yearly renewable source of plant biopolymers such as cellulose, lignins, and hemicelluloses that exhibit valuable properties and can be used not only as raw material for production of cellulose and paper products but also in various sectors such as medicine, pharmacology, perfumes, and the chemical and food industries.

Herein we report the characteristics of lignins isolated from stems of plants of the Gramineae family such as wheat (*Triticum* sp.), rye (*Secale* sp.), oats (*Avena sativa*), and barley (*Hordeum* sp.).

The elemental and functional compositions of dioxanelignins from grassy plants (Table 1) showed that the studied samples had characteristically high contents of O-containing functional groups. It is noteworthy that the C contents were low (58.7–60.8%). Data for perennial grasses are given for comparison. Thus, lignin isolated from the perennial grass bamboo (LSBa) typically had a higher C content (62.5%).

The content of $OCH_3$ groups in the studied lignins averaged 15.5 ± 0.5% (Table 1). This was closer to conifer lignins than lignins of the guaiacyl-syringyl type. Calculations of the $C_9$ formulas of the monomeric unit showed that the number of methoxyls was close to 1.0 unit/$C_9$, in particular, the value for LSR and LSBa samples was 1.05 unit/$C_9$; for LSO, 1.09; and for LSW, 0.95. It is possible that this was due to the high relative content in the grassy lignins of *p*-coumaryl or H-type units. The maximum number of phenolic hydroxyls was observed in lignin from rye straw, 4.7% (Table 1). The $OH_{phen}$ value was relatively low (2.6%) in LSW preparation.

IR spectra of the studied preparations contained a series of characteristic bands typical of lignins at 3440–3450, 1710–1730, 1595–1610, 1500–1520, 1460–1470, 1425–1430, 1365, 1330–1340, 1270–1275, 1125–1135, 1035–1070, and 815-850 cm$^{-1}$. The strongest band in the spectra of all grassy lignin preparations was that at 1125–1135 cm$^{-1}$. The order $ROD_{1500} > ROD_{1470} > ROD_{1430}$ was obeyed for all preparations. This differed from studied lignins from other guaiacyl-syringyl (GS) lignins. Furthermore, $ROD_{1270} > ROD_{1230}$ for all samples. This was also a feature that distinguished grassy lignins from GS lignins.

A comparison of all results suggested that H-units (*p*-coumaryl structures) played an important role in the structural organization of the grassy lignin macromolecules. This also was responsible for the low methoxyl content in these lignins. An analysis of all spectral properties showed that the chemical structure of the studied lignins from grassy plants differed from that of GS- and G-type lignins from larch and conifer wood and should be considered GSH-type lignins.

Table 2 presents the number of principal functional groups and $n_x$ fragments calculated per aromatic ring (AR) that was obtained based on an analysis of $^{13}$C NMR spectra of LSO, LSR, and LSW samples.

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TABLE 1. Elemental Composition of Dioxanelignins from Grassy Plants, %

| Lignin of stems | C | H | O | $OCH_3$ | $OH_{phen}$ | COOH | $C_9$-formula |
|---|---|---|---|---|---|---|---|
| Oats (LSO) | 59.2 | 5.30 | 34.9 | 16.0 | 4.3 | 2.7 | $C_9H_{9.72}O_{3.52}(OCH_3)_{1.09}$ |
| Rye (LSR) | 58.7 | 5.53 | 35.5 | 15.0 | 4.7 | 2.2 | $C_9H_{10.29}O_{3.55}(OCH_3)_{1.05}$ |
| Wheat (LSW) | 60.3 | 5.66 | 34.0 | 15.8 | 2.6 | 4.2 | $C_9H_{10.23}O_{3.62}(OCH_3)_{0.95}$ |
| Barley (LSB) | 60.5 | 5.56 | 33.4 | 15.7 | 3.8 | 2.7 | $C_9H_{10.1}O_{3.2}(OCH_3)_{1.01}$ |
| Meadow fescue (LS-MF) | 60.8 | 5.60 | 33.1 | 14.5 | 4.9 | 4.3 | $C_9H_{10.0}O_{3.2}(OCH_3)_{0.91}$ |
| Bamboo (LSBa) | 62.5 | 6.03 | 30.9 | 16.9 | | | $C_9H_{8.45}O_{2.68}(OCH_3)_{1.05}$ |

TABLE 2. Number of Principal Functional Groups and Fragments in Grassy Lignin Preparations, $n_x$/AR

| Fragment | LSB | LSO | LSR | LSW | $\delta_C$ (range of CS, ppm) |
|---|---|---|---|---|---|
| C(O)O- | 0.31 | 1.18 | 0.84 | 0.45 | 185–164 C(O)O – in ester bonds |
| $C_{ar}O$ | 0.32 | 0.66 | 0.42 | 0.35 | 164–156 C-4 H, H′ |
| $C_{ar}O$ | 0.71 | 0.61 | 0.62 | 0.66 | 156–150 C-3/C-5 S |
| $C_{ar}O$ | 1.07 | 1.00 | 0.95 | 0.90 | 150–140 C-3/C-4 G, G′; C-3/C-5 S |
| $C_{ar}O$ | 0.42 | 0.37 | 0.39 | 0.41 | 134–138 C-4 S, S′ |
| $C_{ar}C$ | 1.71 | 1.48 | 1.39 | 1.25 | 140–123 C-1 S, S′; C-1 G, G′ |
| $C_{ar}C$ | 0.50 | 0.28 | 0.45 | 0.44 | 123–119 C-1 H, H′ |
| $C_{ar}C$ | 0.87 | 0.54 | 0.85 | 0.72 | 119–114 |
| $C_{ar}C$ | 0.10 | 1.37 | 0.11 | 1.25 | 114–95 |
| $CH_{ar}$ | 0.87 | 0.56 | 0.84 | 0.73 | 132–125 C-2/C-6 H, H′ |
| $CH_{ar}$ | 0.36 | 0.35 | 0.37 | 0.36 | 125–117 C-6 G, G′ |
| $CH_{ar}$ | 0.62 | 0.48 | 0.51 | 0.52 | 117–114 C-5 G, G′, C-3/C-5 H, H′ |
| $CH_{ar}$ | 0.63 | 0.55 | 0.57 | 0.61 | 108–114 C-2 G, G′ |
| $CH_{ar}$ | 0.35 | 0.24 | 0.34 | 0.29 | 108–105 C-2/C-6 S, S′ ($\alpha$-CO) |
| $CH_{ar}$ | 0.53 | 0.35 | 0.51 | 0.48 | 105–102 C-2/C-6 S, S′ |
| $CHO_{alk}$ | 1.79 | 1.70 | 1.74 | 1.57 | 90–64 $C_{\alpha\beta}$ in $\beta$-O-4, $\alpha$-O-4 |
| $CH_2O_{alk}$ | 0.85 | 1.04 | 0.90 | 1.02 | 74–64 $C_\gamma$ in $CH_2$-OR, $CH_2$-OH |
| $CH_3O$ | 1.08 | 1.08 | 1.07 | 1.05 | 56–54 Ar-$OCH_3$ |
| $C_\beta$ | 0.06 | 0.07 | 0.07 | 0.08 | 54–50 |
| $C_{alk.}$ | 1.90 | 1.66 | 2.05 | 1.99 | 5–45 CH, $CH_2$ aliph. |

Significant numbers of resonances were observed in the range 5-45 ppm (resonances of aliphatic C atoms in CH, $CH_2$, and $CH_3$ groups not bonded to O atoms). This indicated that the chemical structure of the side aliphatic chains was highly varied. However, their number and position coincided in spectra of all preparations.

Resonances with chemical shifts (CSs) 53.5 and 53.8 ppm indicated that coumarane and pinoresinol structures were present. Grassy lignins characteristically show two resonances for $OCH_3$ groups at 55.7 (guaiacyl structure) and 55.9 ppm (syringyl structure).

Resonances in the range 100–160 ppm were due to aromatic structural units. This range can be subdivided into four intervals. The first is 100–117 ppm for resonances of tertiary aromatic C atoms that contain O functionalization in the *ortho*-position (C-2 and C-5 in uncondensed G-units or C-2 and C-6 in S-units). The second is 117–125 for resonances of tertiary aromatic C atoms (C-2/C-6 in H-units and C-6 in G-units). The third includes 125–142 for resonances of quaternary aromatic C atoms, mainly C-1 and C-5. Finally, the interval 142–160 ppm corresponds to resonances due to esterified aromatic C atoms.

Characteristic resonances with CS 152.1 and 152.4 ppm were observed in the $^{13}C$ NMR spectra. These were due to C-3 and C-5 atoms bonded to $OCH_3$ groups. Unsubstituted C-2 and C-6 C atoms in syringyl units of the preparations caused the appearance of resonances with CS 102-104 ppm. Resonances at 119.0 and 119.1 ppm indicated that guaiacyl units (C-6) were present. Resonances characteristic of *p*-coumaryl units were related to peaks with CS 131.4–131.5 ppm (C-2 and C-6 atoms in H-units). Also, resonances with CS 160.1 and 166.7 ppm were related to C-4 and $C_\beta$-atoms in *p*-coumaryl esters, respectively.

TABLE 3. Ratio of Guaiacyl, Syringyl, and *p*-Coumaryl Structural Units in Lignins of Grassy and Several Woody Plants

| Lignin preparation | Structural unit type* | | Lignin preparation | Structural unit type* | |
|---|---|---|---|---|---|
| | H | S | | H | S |
| LSB | 0.79 | 0.98 | Birch lignin | 0.28 | 1.22 |
| LSO | 0.48 | 0.65 | Acacia lignin | 0.03 | 0.63 |
| LSR | 0.53 | 0.69 | Larch lignin | – | – |
| LSW | 0.71 | 0.85 | | | |

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*For all samples G = 1.0.

TABLE 4. Distribution of C Atoms Among Structural Fragments in Lignin Samples, $n_x$/AR

| Lignin preparation | $\Sigma\ C_{\beta\text{-}\beta,\ \beta\text{-}5}$ (50–54 ppm) | $\Sigma C_{\text{aliph}}$ (5–45 ppm) | ΣC(O)O (164–185 ppm) |
|---|---|---|---|
| LSB | 0.06 | 1.90 | 0.31 |
| LSO | 0.07 | 1.66 | 1.18 |
| LSR | 0.07 | 2.05 | 0.84 |
| LSW | 0.08 | 1.99 | 0.45 |

Thus, the results from a comparative quantitative analysis of CSs of resonances in NMR spectra (Table 3) indicated that the grassy lignin macromolecules were constructed from three principal types of structural units, i.e., guaiacyl, syringyl, and *p*-coumaryl.

Table 4 shows that larch lignin was a compositionally homogeneous polymer because its macromolecules were constructed exclusively of guaiacyl units. Birch and acacia lignins were dominated by guaiacyl and syringyl units. In contrast with this, grassy lignins were high in *p*-coumaryl units. The ratio of G:S:H units could be estimated as 100:80:60.

Resonances occurring at weak field (CS > 170 ppm) confirmed that carboxylic acids were present in the grassy lignin preparations.

The LSO preparation was unique for the number of ester bonds (Table 4) (1.18 C at./AR) (LSO > LSR > LSW > LSB). The C-4 atoms in *p*-coumaryl units appeared in the range 156–164 ppm. Their number was greatest in the LSO sample (LSO > LSR > LSW > LSB). The lengths of the side chains in the different lignin preparations were different, in particular, for LSR, $n_{\Sigma 10\text{-}45}$ = 2.05 C at./AR; for LSO, 1.7 C at./AR.

Thus, a comparison using IR and $^{13}$C NMR spectroscopy and calculations of semi-empirical $C_9$ formulas of the monomeric unit showed that the chemical structures of lignins from annual grassy plants differed considerably from those of woody lignins. It was found that the grassy lignins belong to a class of compositionally heterogeneous GSH-type biopolymers and consisted of guaiacyl, syringyl, and *p*-coumaryl monomeric units. The average ratio of G:S:H units was 100:80:60.

## EXPERIMENTAL

IR spectra were recorded in KBr pellets using a Specord-M 80 instrument and an MIR8000 IR-Fourier spectrometer (DTGS detector). IR spectra were processed quantitatively by a known method [1] using a baseline and normalization to the strongest absorption band.

$^{13}$C NMR spectra were recorded in pulse mode (Bruker AM-300 spectrometer) at operating frequency 75.5 MHz. The spectral width was 18,000 Hz. The pulse length was 2 μs. The time between pulses was 5–10 s. The solvents were DMSO-$d_6$ and DMF-$d_6$ containing chromium *tris*-acetylacetonate (0.02 M) as relaxant. The solution concentration was 30%. The number of scans was 20,000–100,000. Quantitative calculations involving $^{13}$C NMR spectra were performed according to methods reported before [2–4] using the NUTS program.

Starting plant materials were straw of oats (*A. sativa*), rye (*Secale* sp.), wheat (*Triticum* sp.), and barley (*Hordeum* sp.) (Komi Republic). The component compositions of the plant tissues for oats, rye, wheat, and barley (%) were Kuerschner cellulose 44.7, 49.0, 48.7, and 41.3; Komarov lignin 22.5, 20.0, 21.4, and 22.2; readily hydrolyzed polysaccharides 21.0, 17.4, 20.5, and 19.6; difficultly hydrolyzed polysaccharides 39.2, 43.3, 33.9, and 34.2; water-soluble substances 14.6, 9.4, 14.3, and 12.1; extractable substances 4.2, 3.1, 5.6, and 4.1; ash 5.0, 2.6, 3.1, and 3.4, respectively. The component analysis was carried out using standard methods [5].

Preparations of lignins from straw of oats (LSO), rye (LSR), wheat (LSW), and barley (LSB) were isolated by the literature method [6] using treatment for 2 h with aqueous dioxane in the presence of HCl (0.7%) at reflux. Yield of LSO, 19.7%; LSR, 37.1; LSW, 37.9; LSB, 14.0 of Komarov lignin in the starting raw material.

Elemental analysis of lignins was performed on a Hewlett–Packard (USA) analyzer. Functional groups were determined by standard methods typical of lignin chemistry [7].

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