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COMPARISON OF LEAF AND STEM CELL-WALL COMPONENTS IN BARLEY STRAW BY SOLID-STATE ^{13}C NMR

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Key Word Index—*Hordeum vulgare*; Poaceae; lignin; cellulose; xylan; cutin; cell walls; solid-state NMR.

Abstract—Barley straw was separated mechanically into leaf and stem fractions. Their ^{13}C NMR spectra were recorded at low field (25 MHz) using magic-angle spinning and cross-polarisation (CP-MAS) under conditions optimised for quantitation of lignin. The stem fraction contained more lignin with a higher proportion of ether-linked syringyl residues. The leaf fraction contained more phenolic acids and cutin. The molecular rigidity of the components of the stem cell walls was estimated from the ^{13}C spin-lattice relaxation time T_1, which decreases with thermal motion. Crystal-interior cellulose and syringyl lignin were the most rigid polymeric components. These data have implications for the capacity of the straw to withstand mechanical stress and to resist degradation during digestion by ruminants.

INTRODUCTION

Cereal straw consists primarily of cell walls, but these are derived from a wide variety of cell types [1, 2]. The leaf fraction, which is itself anatomically complex, is more readily digestible by ruminants than is the stem. The stem fraction, on the other hand, has longer, stronger fibre cells and therefore is more suitable than the leaves for pulping and the manufacture of composite materials. The properties of digestibility and mechanical strength are strongly influenced by the interaction of lignin and carbohydrate within the straw cell walls. The most effective of the few methods that can be used to study lignin-carbohydrate complexes directly, without solubilisation, is solid state ^{13}C NMR [3–7]. This technique also has the advantage that the contribution of individual polymers to the rigidity of the intact cell wall can be deduced from NMR relaxation parameters, which are dependent on thermal motion [6–9].

In this paper we describe the application of ^{13}C NMR spectrometry to lignified cell walls from the leaf and stem fractions of barley straw. The ^{13}C spin-lattice relaxation time T_1, was also determined for the stem fraction to provide an insight into the origin of its mechanical properties. The ^{13}C T_1 is sensitive to thermal motions in the same frequency range as the NMR experiment itself, i.e. of the order of 10^7–10^8 Hz [6, 9].

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RESULTS

Quantification of lignin and related polymers in the cell walls

The CP-MAS spectra of the leaf and stem fractions of barley straw, with and without dipolar dephasing, are shown in Fig. 1 and the spectral assignments [5–12] are listed in Table 1. Dipolar dephasing selectively reduced the intensity of signals from carbon nuclei with either one or two bonded protons, e.g. the carbohydrate signals, and thus allows the lignin region of the spectrum to be examined with less interference.

The spectra of both leaf and stem fractions were very similar in the carbohydrate region (60–110 ppm) although the presence of small quantities of pectic rhamnogalacturonan in the leaf fraction may be inferred from spectral intensity at 100 ppm and 18 ppm. In the aromatic region the spectra were very different. The leaf fraction was clearly less lignified: it contained only about half the quantity of aromatic components present in the stem, when both were estimated as lignin (Table 2). The aromatic region of the spectrum from the stem fraction was typical of lignin with a moderately high proportion of β-aryl ether linked syringyl residues (153 ppm). The corresponding part of the spectrum from the leaf fraction had less β-aryl ether linked syringyl residues and much more spectral intensity assignable to phenolic acids (168 and 125 ppm) [11, 12]. However, the leaf fraction also showed a strong hydrocarbon CH_2 signal at 30–32

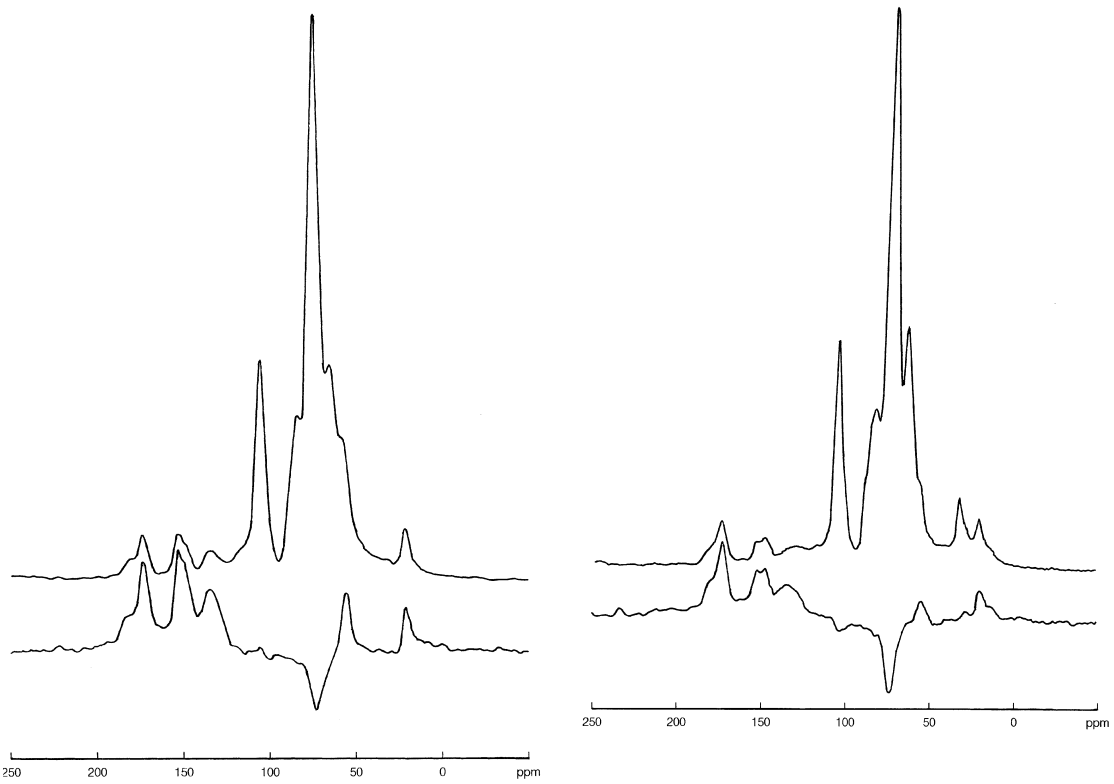

Fig. 1. ^{13}C NMR spectra of stem (A) and leaf (B) fractions from barley straw. Upper spectra: standard CP-MAS conditions. Lower spectra with dipolar dephasing to eliminate signals from non-protonated (except methyl) carbon nuclei and thus emphasise the spectral region associated with the aromatic rings of lignin.

Table 1. Resonance assignments for ^{13}C spectra [14–16]

Chemical shift, ppm	Assignment
179	COOH of non-esterified phenolic acids
173	COOH of acetyl
168	COO- of esterified phenolic acids
153	C-3/C-5 of ether-linked syringyl units
149	C-3 of ether-linked guaiacyl units
147	C-3/C-5 of guaiacyl and syringyl units, not ether-linked
136	C-1 of syringyl units
133	C-1 of guaiacyl and C-4 of syringyl units
116	C-5 of guaiacyl and C3/C-5 of 4-hydroxyphenyl units
109	C-1 of arabinofuranosyl units
105	C-1 of cellulose
101	C-1 of xylan
89	C-4 of crystal-interior cellulose
84	C-4 of crystal-surface cellulose
82	C-4 of xylan
72, 75	C-2/C-3/C-5 of 4-linked polysaccharides
69	C-2/C-3 of pectic galacturonan
65	C-6 of crystal-interior cellulose
62	C-6 of crystal-surface cellulose, C-5 of xylan
57	lignin OCH_3
31	cutin CH_2
21	acetyl OCH_3

ppm which, in the absence of major quantities of lipid or protein, may be assigned to cutin [13, 14]. It may therefore be assumed that a part of the aromatic material in this fraction corresponded to the phenolic components of cutin, as in wheat bran [15] and ryegrass cell walls [5].

^{13}C *Relaxation in stem cell walls*

The ^{13}C T_1 values (Table 3) were in general long in comparison with those of wood, making it impracticable to generate single-pulse (SP-MAS) ^{13}C spectra which would have given more accurate quantitation of lignin [6]. As might be anticipated, the C-4 resonance of crystalline cellulose (89 ppm) gave the longest ^{13}C T_1 within the carbohydrate part of the spectrum, indicating the greatest rigidity. Reasonable agreement between a number of resonances assigned to crystal-surface cellulose and arabinoxylans showed

Table 2. Lignin content of barley leaf and stem cell walls

Fraction	Lignin (mol %)	Lignin (mass %)
Leaf	10.8	9.0
Stem	22.3	18.9

Table 3. ^{13}C spin-lattice relaxation time T_1 in barley stem cell walls

ppm	Polymer	^{13}C T_1, s
153	lignin (syringyl C-3, C-5)	32
148	lignin (guaiacyl C-3, C-4)	17
133	lignin (S + G C-2, C-5, C-6)	32
105	cellulose, C-1	18
88	crystal-interior cellulose, C-4	33
83	crystal-surface cellulose, C-4	16
72	general carbohydrate	17
62–64	cellulose (C-6), xylan (C-5)	14
57	lignin (methoxyl)	16
21	hemicellulose (acetyl)	10

that these were undergoing chain motions (rather than local motions of individual functional groups) on a considerably larger scale than the interior chains of the cellulose microfibrils.

Within the lignin component, the ^{13}C T_1 value for non-protonated carbons of the guaiacyl residues (147 ppm). These T_1 values for ring carbon nuclei must reflect overall polymer motions, whereas for the lignin methoxyl groups (56 ppm) for the very short ^{13}C T_1 may be assumed to result from rapid rotation of the methyl group and is therefore not relevant to the rigidity of the cell-wall structure as a whole. It may therefore be concluded that syringyl-rich areas of the lignin network were more rigid than guaiacyl-rich areas.

DISCUSSION

Structural features of the cell walls

Separation of straw into leaf and stem fractions is possible on a commercial scale [15]. The prospects for utilisation of both fractions depend on the degree to which their polysaccharides are protected by lignin and other non-carbohydrate components. The spectral data for both leaf and stem cell walls were consistent with the presence of cellulose and arabinoxylans, but there were considerable differences in the associated non-carbohydrate polymers. Cutin appeared to be a substantial component of the leaf cell walls. Cutin, although not the associated waxes, appears in the Klason or acid-detergent lignin fraction from the classical analysis of plant materials and its aromatic constituents contribute to acetyl bromide lignin. It is therefore readily confused with lignin and may be assumed to have broadly similar nutritional properties, remaining relatively indigestible and protecting associated polysaccharides from digestion. However there are likely to be differences of detail because cutin is more hydrophobic and is probably differently linked to polysaccharide [16, 17].

Molecular rigidity in stem cell walls

The decrease in molecular rigidity of the polysaccharides, from the centre of the microfibrils outwards into the xylan matrix, was expected and confirmed that useful data can be obtained from this type of experiment even in the absence of H_2O and at the high motional frequencies probed by the ^{13}C T_1. The finding that the β-aryl ether linked syringyl residues within the lignin were considerably more rigid than the guaiacyl residues, has implications for the performance of the cell walls as composite structural members. The rigidity of the syringyl residues was unexpected, since syringyl lignin, with C-5 substituted, offers less opportunity than guaiacyl lignin for cross-linking into a three-dimensional network. Instead it seems more likely that the syringyl lignin is tightly embedded within the ordered, close-packed structure of the secondary walls of sclerenchyma cells, whereas the guaiacyl lignin is preferentially located in the less ordered middle lamella and primary walls [18, 19].

The structural tissues of the straw stem may be considered as composite materials in two senses. At the nm scale, cellulose microfibrils provide the fibre phase of the composite and syringyl-rich lignin forms the matrix, with xylans bonding the two together. At the μm scale, the fibres are the elongated sclerenchyma cells themselves and the middle lamella, composed principally of guaiacyl-rich lignin, is the matrix that holds them together [1, 2]. Straw has to withstand compressive as well as tensile stresses, respectively on the inner and outer sides of a stem bent by wind or rain. The rigidity of the lignin matrix is in strong contrast to the weakness and flexibility of the non-cellulose polysaccharide matrix in tensioned tissues such as flax fibres, where the cellulose carries essentially all the tensile load and the function of the matrix is to protect the cellulose by diverting incipient cracks away from a path across the microfibrils (or fibre cells, on the larger scale) [20]. The compressive strength of composite materials, on the other hand, depends on the rigidity of the matrix and its ability to withstand interlaminar shear. The very rigid syringyl lignin is well adapted for this function and suitably placed to attach the cellulosic layers of the secondary cell wall to one another, although the xylan connecting lignin and cellulose together appears to provide some flexibility. On the cellular scale, the guaiacyl-rich lignin of the middle lamella, which is not reinforced by cellulose, is probably the weakest point in the whole structure of the tissue and can be shattered mechanically [21] to separate the cells during pulping.

EXPERIMENTAL

Plant material

Barley straw (*Hordeum vulgare* cv. Golden Promise) was air-dried and separated by hand into leaf and stem fractions. Each fraction was milled to pass through a 2 mm sieve, then vacuum-dried immediately before NMR.

NMR

The NMR experiments were carried out on the Bruker MSL100 spectrometer at 25 MHz for ^{13}C. About 250 mg of sample was packed into zirconia rotors for MAS at approximately 3.5 kHz. The CP contact time was 2 ms. The measurement of the ^{13}C T_1 was carried out by the saturation-recovery method [22].

The total quantity of lignin present in each cell preparation was calculated by integration of the CP-MAS spectra between 143 and 160 ppm. It was assumed that this spectral region contained the signals from C-3 and C-5 of syringyl units and C-3 and C-4 of guaiacyl units, i.e. 20% of the carbon atoms in the lignin. It was also assumed that the lignin contained 54% C by mass, compared with 44% for the rest of the cell wall. For nonlignified cell walls the carbohydrate regions of the CP-MAS spectra are approximately quantitative if the cell walls are dry [6, 8], but although the contact time was optimised for quantitation of lignin, the lignin and cutin values are liable to be slightly underestimated. This is because the optimum contact time is a compromise between ensuring as complete as possible cross-polarisation efficiency and losing signal intensity by ^{1}H $T_{1\rho}$ decay. The cross-polarisation process is slow for lignin due to the lack of protons [6] and still slower in cutin hydrocarbons due to their great mobility [13].

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