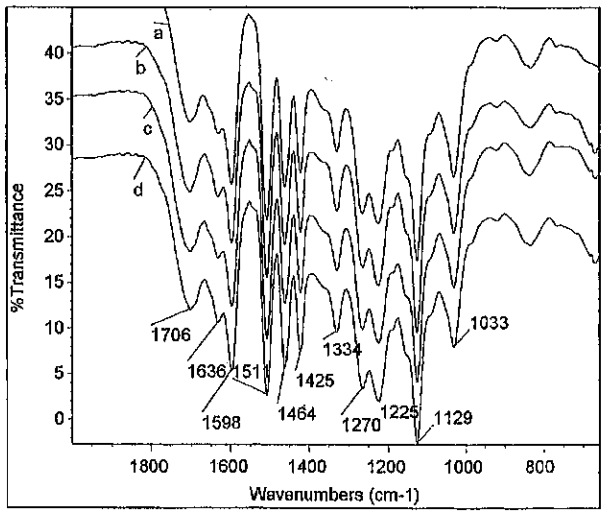

Fig. 4. The FT-IR spectra of pure lignin (PL) fractions isolated with 2% H_2O_2 at pH 11.5 for 12 h at 20°C (spectrum a); 40°C (spectrum b); 50°C (spectrum c); and 70°C (spectrum d) from the dewaxed and water-treated rye straw.

spectral profiles and the relative intensities of the bands were rather similar in four spectra, which confirmed that the 'core' of lignin structure did not change significantly during the alkaline peroxide treatment at the different temperatures. The band at 1706 cm^{-1} has been assigned to the unconjugated ketone and unconjugated carbonyl stretching, while the band at 1636 cm^{-1} has been attributed to carbonyl stretching conjugated with aromatic rings (Vazquez et al., 1997). Aromatic skeleton vibrations in the PL preparations are assigned at 1598, 1511, and 1425 cm^{-1}. Absorption at 1464 cm^{-1} indicates C–H deformations and aromatic ring vibrations. The bands at 1334, 1270, and 1225 cm^{-1} have been assigned to ring breathing with C–O stretching. The 1334 cm^{-1} band has been associated with syringyl units, and 1270 cm^{-1} band with guaiacyl units. The bands at 1129 and 1033 cm^{-1} indicate the aromatic CH in-plain deformation for syringyl type and guaiacyl type, respectively. Aromatic C–H out of bending appears at 840 cm^{-1}. Not surprisingly, it can be observed that a great similarity existed among the spectra of alkaline peroxide-soluble lignins and the lignin preparation, solubilized under analogous conditions (pH 11.5, 50°C, 12 h) but in the absence of peroxide, supporting the previous finding that alkaline peroxide treatment did not affect the overall structure of lignin from rye straw.

3.7. ^{13}C-NMR spectrum

The PL fraction, obtained by treatment of the straw sample with 2% H_2O_2 at 70°C for 12 h at pH 11.5, was also studied by ^{13}C-NMR spectroscopy (Fig. 5). Most of the observed signals have been previously assigned in straw and wood lignin spectra (Nimz et al., 1981; Scalbert et al., 1986; Imamura et al., 1994; Kondo et al., 1995). As can be seen from Fig. 5, the most striking characteristic of the ^{13}C-NMR spectrum is the almost absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show a signal at 63.2 ppm (C-5, Xyl internal unit) for the associated hemicelluloses, however, the peak intensity is rather weak. The carbonyl resonances from uronic acids and esters may contribute to signal at 174.8 ppm, which indicates C-6 in methyl uronates (Himmelsbach and Barton, 1980).

The signals for aromatic part of the lignin appear in the region between 104.4 and 170.0 ppm. The syringyl (S) residues were indicated by signals at 152.3 (C-3/C-5, S), 138.2 (C-4, S etherified), 134.2 (C-1, S etherified), 133.4 (C-1, S nonetherified), 106.8 (C-2/C-6, S with α-CO), and 104.4 ppm (C-2/ C-6, S). Guaiacyl (G) residues gave signals at 149.8 and 149.3 ppm (C-3, G etherified), 148.0 and 147.1 (C-4, G etherified), 145.7 (C-4, G nonetherified), 134.2 (C-1, G etherified), 133.4 (C-1, G nonetherified), 119.3 (C-6, G), and 114.9 ppm (C-5, G). The *p*-hydroxyphenyl (H) residues appeared as two signals at 128.7 and 128.0 ppm (C-2/C-6, H). These signals confirmed that the lignin preparation could be justified as SGH-lignin. The signals at 168.2 (C-γ, PC ester), 159.9 (C-4, PC ester), 144.7 (C-α, PC ester), 130.2 (C-2/C-6, PC ester), 125.9 and 125.3 (C-1, PC ester), and 115.9, 115.7, and 115.4 ppm (C-3/C-5, PC ester) represented the esterified *p*-coumaric acid. Etherified ferulic acid was observed with signals at 167.4 (C-γ, FE ether, data not shown in the spectrum), 144.4 (C-α, FE ether), and 122.4 ppm (C-6, FE ether). Esterified

ferulic acid was identified with a signal at 122.9 ppm (C-6, FE ester). It seems clear that the *p*-coumaric acid is linked to lignin by ester bonds, while the ferulic acid is linked to lignin by ether and ester bonds. On the basis of study on the hydroxycinnamic acids, particularly ferulic and *p*-coumaric acids in the cell walls of wheat straw, we (Sun and Lawther, 1998) previously reported that *p*-coumaric acid was mostly esterified to lignin or polysaccharides, while ferulic acid appeared almost equally in etherified linkages with lignin and in esterified bonds to arabinose in hemicelluloses. Similarly, in the cell walls of rye straw, in addition to the etherified linkages between ferulic acid and lignin, ferulic acid at least in part, also esterified to hemicellulose.

The spectrum also indicated that β–*O*–4 linkages (C-α in β–*O*–4, 72.2; C-β in β–*O*–4, 86.1 ppm; C-γ in β–*O*–4, 60.1 ppm) were the major linkages between lignin structural units. The common carbon–carbon linkages such as β–β (C-γ in β–β units, 71.7 ppm, data not shown in the spectrum) and β-5 (C-4 in β-5 units, 144.7 ppm, overlapped with C-α, PC ester) were also present. These signals indicated that the linkages in this rye straw lignin is mainly composed of β–*O*–4 ether bonds together with small amounts of β–β and β-5 carbon–carbon linkages. These results suggested that alkaline peroxide under the conditions used here might not attack the β-aryl ether structure to a significant extent. The signals representing the γ-methyl, α and β-methylene groups in n-propyl side chains appeared in the spectrum between 14.1 and 33.8 ppm. A very strong signal at 56.0 ppm corresponded to the OCH_3 in syringyl and guaiacyl units.

On the basis of the forgoing data, it can be concluded that treatment by alkaline peroxide under the conditions given did not affect the overall structure of lignin from rye straw. Similar results have been reported by Dence (1996),

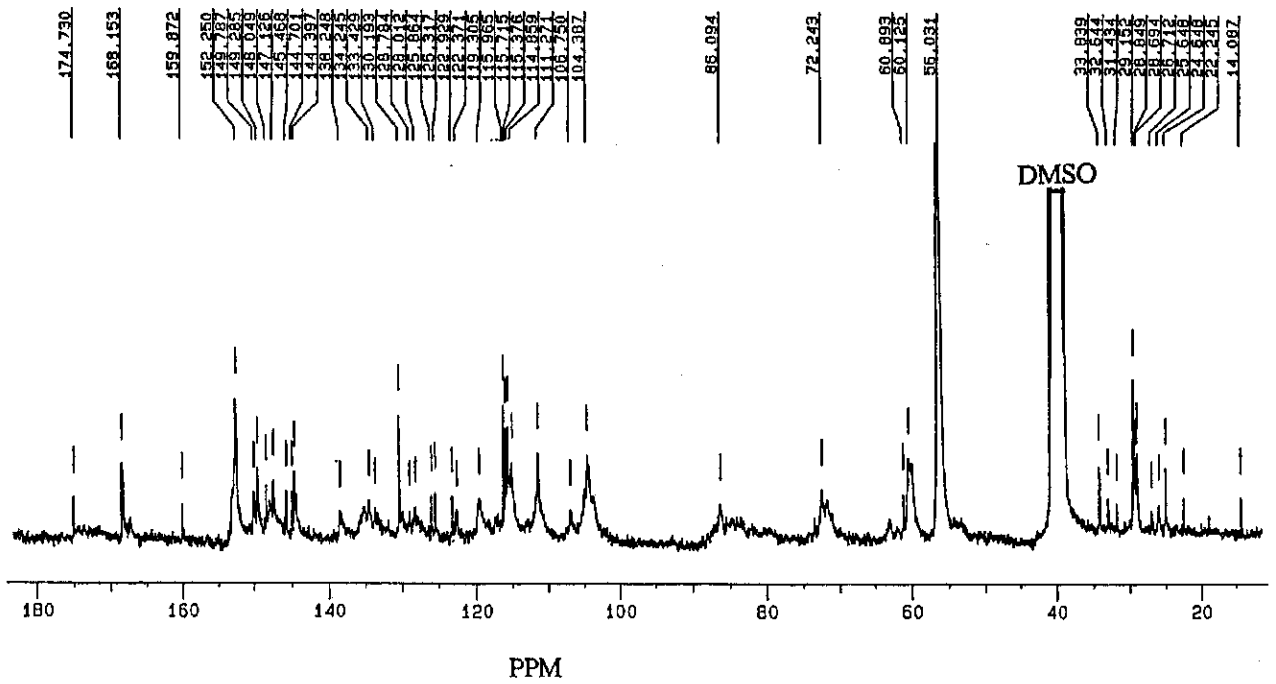

Fig. 5. ^{13}CNMR spectrum of pure lignin (PL) fraction extracted with 2% H_2O_2 at 70°C for 12 h at pH 11.5 from the dewaxed and water-extracted rye straw.

Lachenal et al. (1992) in the studies on the behaviour of lignin in kraft pulp during hydrogen peroxide delignification. The authors stated that hydrogen peroxide was unable to attack phenols of the type present in lignin under alkaline conditions. That is, no degradation of the phenolic ring was observed during the alkaline peroxide treatment. However, at a relatively higher temperature such as 90°C, some depolymerization of lignin may occur and carboxyl groups are created (Dence, 1996).

In conclusion, the six lignin preparations, obtained by treatment of the dewaxed and water-extracted rye straw with 2% H_2O_2 at pH 11.5 for 12 h at 20–70°C, and one alkali lignin fraction, isolated under similar conditions but in the absence of peroxide, showed similar chemical composition and physico-chemical properties. They were relatively free of polysaccharides and contained almost equal amounts of noncondensed guaiacyl and syringyl units with fewer *p*-hydroxyphenyl units. They seem more condensed than wood lignins, but corresponded to the condensation degree of wheat straw lignins. Meanwhile, the lignin in rye straw cell walls appeared to be very closely associated to glucuronic acid or 4-*O*-methylglucuronic acid by ester bonds. *p*-Coumaric acid was found to be linked to lignin by ester bonds, while ferulic acid was linked by their phenolic groups via ether bonds to lignin and also principally linked by their carboxyl groups via ester bonds to hemicelluloses.

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