A novel method that permits the quantitative detection and classification of various carbonyl groups in lignins has been developed. The proposed method was optimized with the quantitative trifluoromethylation of a series of carbonyl-containing lignin-like model compounds. This effort was followed by $^{19}$F NMR spectral analyses of the resulting fluorine derivatives allowing for a thorough understanding of their structure/$^{19}$F chemical shift relationships. The various carbonyl groups present in lignins were also investigated by trifluoromethyllating them in the presence of catalytic amounts of tetramethylammonium fluoride (TMAF), followed by hydrolysis with TMAF in tetrahydrofuran. By using a variety of selective reactions, it became possible to assign a number of prominent $^{19}$F NMR signals to a variety of carbonyl groups present in lignins. These studies demonstrated that the proposed method can be applied to the quantitative determination of carbonyl groups that are present in soluble native and technical lignins.

**Keywords:** Nuclear magnetic resonance (NMR); spectroscopy; carbonyl groups; lignins; quantitative analysis; classification; methods

**INTRODUCTION**

Lignin is a complex phenylpropanoid biopolymer formed by an enzyme-initiated radical polymerization of cinnamyl alcohols (Harkin, 1956). Due to the random nature of its formation, lignin does not possess regularity in its repeating units (Ianshekar and Fiechter, 1983; Glasser and Kelley, 1987; Argyropoulos and Menachem, 1997). This peculiarity makes the characterization of its structure a challenging task.

A number of studies have demonstrated the presence of small amounts of carbonyl groups in lignins (Adler and Ellmer, 1948; Adler and Marton, 1959; Marton and Adler, 1961; Gierer and Söderberg, 1959). In particular, milled wood lignins have been reported to contain conjugated cinnamaldehyde structures and α-carbonyl groups (Geiger and Függerer, 1979). Other investigations have shown that technical lignins contain appreciable amounts of α-carbonyl groups in addition to benzaldehyde and quinones (Lin and Dence, 1992; Sarkanen and Ludwig, 1971). The presence of carbonyl groups in lignins, in particular those present as α- and β-quinonoids, quinonemethides, and other extended conjugated enone systems, is responsible for the yellow color of photochemically reverted papers (Argyropoulos et al., 1994; Argyropoulos and Hetnář, 1994; Lin and Kringstad, 1971; Forskåhl et al., 1991; Castellan et al., 1993; Gellerstedt and Patersson, 1977).

Several methods for determining the carbonyl groups in lignins are available (Green, 1963; Lindberg and Misiorny, 1952; Lindberg and Theander, 1954; Heuser, 1953; Miyake, 1970). Among these, the most effective and simple one utilizes the reaction of carbonyl groups with hydroxylamine hydrochloride, forming an oxime and hydrochloric acid. Subsequent titration of the hydrochloric acid provides an estimation of the amount of carbonyl groups in a sample (Gierer and Söderberg, 1959; Miyake, 1970). A modification of this technique, claiming greater reproducibility, has been described by Zakis (1994). The modified procedure calls for the use of triethanolamine to function as the acid acceptor followed by a back-titration. A technique that attempts the distinction of α-carbonyl groups from those of conjugated aldehydes is also available (Lindberg and Misiorny, 1952; Lindberg and Theander, 1954) and is based on sample reduction (sodium borohydride) followed by UV spectroscopic measurements. The latter method requires the use of appropriate lignin model compounds that serve as standards for determining the changes in molar absorptivity of the absorption bands that are caused by the reduction of a particular carbonyl group to the corresponding benzylic alcohol.

Infrared spectroscopy has been also used for investigating various structures in lignin (Kalboe and Ellefsen, 1962; Faix, 1991; Hergert, 1971) including carbonyls (Marton et al., 1961). Recently, Hortling et al. (1997) reported a semiquantitative technique for the determination of carboxylic and nonconjugated carbonyl groups by IR spectroscopy. However, the application of these techniques was not widespread because their precision...
NMR is rapidly becoming a powerful analytical tool in the hands of wood chemists aimed at providing answers in relation to the structure of lignins. However, the complex structure of these materials has imposed some serious challenges and limitations, even in the application and use of NMR (Argyropoulos, 1995). Efforts to overcome some of the limitations imposed by the lignin or its derivatization with fluorine derivatives were analyzed by 19F NMR. This was followed by the addition of 3 mL of a solution composed of 0.01 N sodium hydroxide and 100 mg of sodium borohydride and stirred at 40 °C for 24 h. The reaction mixture was then acidified to pH 3–4 with dilute (10%) sulfuric acid. The organic solvents were evaporated under reduced pressure, and the lignin was precipitated with the addition of water. The precipitated lignin was then washed and centrifuged three times, dissolved in a mixture of dioxane/water (25:5, v/v), and freeze-dried. Sodium Borohydride Reduction. Lignin (200 mg) was dissolved into 25 mL of a solution composed of (60:40:50, v/v) 2-methoxyethanol, 2-propanol, and water, respectively. This was followed by the addition of 702 μL of 30% hydrogen peroxide. After the solution was added, 200 mg of sodium hydrosulfite (Na2S2O4) dissolved in 5 mL of water. After various reaction times (15, 60, and 240 min), a 1 mL aliquot of the mixture was withdrawn, acidified by the addition of 1 M HCl, and extracted with ethyl acetate. The organic solvent was then evaporated under reduced pressure, and the residue was analyzed by GC/MS.

Sodium Hydrosulfite Reduction of Lignin. Lignin (200 mg) was dissolved in 5 mL of dioxane, and a slow stream of nitrogen was bubbled through the solution for 30 min. To this mixture was added a solution composed of 0.01 N sodium hydroxide in 5 mL of water, and the reaction mixture was kept under stirring at room temperature for 1 h. The mixture was then freeze-dried, and the residue was washed and centrifuged three times with small aliquots of water. Finally, the reduced lignin was dissolved in a mixture of dioxane/water (25:5, v/v) and freeze-dried.

Gel Chromatography/Mass Spectrometry. GC/MS analyses were carried out on a Hewlett-Packard 5972 mass spectrometer interfaced to a Hewlett-Packard 5890A gas chromatograph with a 30 m × 0.25 mm packed silica capillary column DB-5. The injection port temperature was 280 °C, and the oven temperature increase profile was from 100 to 250 °C, with a gradient of 5 °C/min. 19F NMR Spectroscopy. All spectra were recorded on a Varian Unity 300 FT-NMR spectrometer at an operational frequency of 470.3 MHz. The derivatized trifluoromethylated lignin was dissolved in 800 μL of a solvent mixture composed of pyridine and deuterated chloroform (15–20 mg/0.8 mL) at a volume ratio of 1.6:1, v/v. The mixture was stirred with a magnetic bar until the lignin was fully dissolved. To this mixture was added 100 μL of internal standard solution
Characterization. Characterization of Trifluoromethyl-
ated Model Compounds. 1H NMR (CDCl3) (TMS) δ 1.77 (s, 3H); 2.60 (s, 1H); 7.37–7.46 (m, 3H); 7.56–7.61 (m, 2H) ppm. 13C NMR (CDCl3) (TMS) δ 78.690 (d, J = 32.1 Hz); 115.11; 153.83; 129.36 (q, J = 284.9 Hz); 155.83. 19F NMR (CDCl3/CFCl3) (CFCl3) δ –80.57 (s) ppm. MS m/z 246 (M+), 197, 167, 151, 124, 110, 69, 51. Anal. Calcd for C10H11F3O4: C, 46.88; H, 2.15; Found: C, 45.94; H, 2.42. 14H NMR (CDCl3) (TMS) δ 2.92 (s, 3H); 3.841 (d, J = 6.6 Hz); 6.485 (d, J = 8.48 Hz); 7.03–7.11 (m, 4H) ppm. 19F NMR (CDCl3/CD3OD) (CDCl3) (silylated) δ –80.57 (s) ppm. MS m/z 262 (M+), 197, 167, 151, 124, 110, 69, 51. Anal. Calcd for C10H11F3O4: C, 46.88; H, 2.15; Found: C, 45.94; H, 2.42. 15H NMR (CDCl3) (TMS) δ 2.25 (s, 3H); 3.841 (d, J = 6.6 Hz); 6.485 (d, J = 8.48 Hz); 7.03–7.11 (m, 4H) ppm. 19F NMR (CDCl3/CD3OD) (CDCl3) (silylated) δ –80.57 (s) ppm. MS m/z 262 (M+), 197, 167, 151, 124, 110, 69, 51. Anal. Calcd for C10H11F3O4: C, 46.88; H, 2.15; Found: C, 45.94; H, 2.42.
**Figure 1.** Trifluoromethylation of carbonyl-containing (including quinones) lignin-like model compounds.

20. $^1$H NMR (CDCl$_3$) δ 4.75 (s, 2H); 7.25–7.34 (m, 2H); 7.41–7.55 (m, 6H); 7.67–7.72 (t, 2H, J = 7.32 Hz) ppm. $^{19}$F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −75.66 (s) ppm. MS m/z 175 (M$^+$ − C$_6$H$_4$FSO$_2$), 152, 105, 77, 51. Anal. Calcld. for C$_6$H$_5$FOSO$_2$: C, 54.87; H, 5.45; F, 32.54. Found: C, 54.91; H, 3.50; F, 32.58. 99% yield.

21. $^1$H NMR (CDCl$_3$) (TMS) δ 3.63 (s, 1H); 3.78 (s, 3H); 3.863 (d, 6H, J = 11 Hz) ppm. $^13$C NMR (CDCl$_3$) δ 55.85; 56.04; 56.17; 61.36; 79.59 (q, J = 27.4 Hz); 82.64; 109.87; 110.48; 112.07; 120.72; 121.66; 124.62; 124.97 (J = 286.59 Hz) ppm. 19F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −76.03 (s) ppm. MS m/z (silylated) 402 (M$^+$), 302, 278, 248, 235, 221, 181, 165. Anal. Calcld. for C$_6$H$_5$FOSO$_2$: C, 56.72; H, 5.26; F, 14.16. Found: C, 56.74; H, 5.29; F, 14.22. 95% yield.

22. $^1$H NMR (CDCl$_3$) (TMS) δ 3.83 (s, 3H); 3.87 (s, 3H); 3.90 (s, 3H), 3.93 (d, 1H, J = 3 Hz) ppm. 4H NMR (d$_2$:1H, 1H) δ 12.46 (d, 1H, J = 12 Hz); 4.640 (d, 1H, J = 12 Hz) ppm. 58.65 (d, 1H, J = 3 Hz) ppm. 6.84–7.12 (m, 6H) ppm. $^{13}$C NMR (CDCl$_3$) (TMS) δ 56.11; 56.19; 56.44; 71.68; 75.65 (q, J = 28.3 Hz) ppm. 108.78; 110.06; 114.41; 113.40; 120.41; 121.80; 122.50 (q, J = 286.59 Hz) ppm. 127.88; 147.37; 148.68; 149.16; 149.95 ppm. $^{19}$F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −76.57 (s) ppm. MS m/z (silylated) 372 (M$^+$), 303, 248, 235, 197, 189, 180. Anal. Calcld. for C$_6$H$_5$FOSO$_2$: C, 50.86; H, 5.14; F, 15.13. Found: C, 50.82; H, 5.20; F, 15.29. 96% yield.

23. $^1$H NMR (CDCl$_3$) (TMS) δ 2.25 (s, 3H); 3.75 (s, 1H); 3.79 (s, 3H), 3.88 (s, 3H); 3.935 (d, 1H, J = 5.99 Hz) ppm. 4.33 (d, 1H, J = 5.99 Hz) ppm. 7.33 (d, 1H, J = 1 Hz) ppm. $^{13}$C NMR (CDCl$_3$) (TMS) δ 21.19; 55.88; 55.93; 61.31; 79.85 (q, J = 28.9 Hz) ppm. 82.64; 109.59; 112.95; 113.93; 118.43; 121.01; 121.19; 125.07 (q, J = 286.59 Hz) ppm. 128.71; 134.83; 143.81; 145.62; 146.35; 148.89; 151.53 ppm. $^{19}$F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −75.93 (s) ppm. MS m/z (silylated) 618 (M$^+$), 480, 451, 411, 365, 343, 323, 271. Anal. Calcld. for C$_6$H$_5$FOSO$_2$: C, 56.72; H, 5.26; F, 14.16. Found: C, 56.78; H, 5.19; F, 14.18. 93% yield.

Characterization of Trifluoromethylated Lignin. Dioxane lignin: $^{19}$F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −67.71 to −76.79 ppm. $^1$H NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ 72.70 to −72.99 ppm. 74.23 to −74.54 ppm. −75.20 to −75.83 ppm. −76.87 to −77.11 ppm. −77.78 (d, J = 6.6 Hz) ppm. −78.21 to −78.26 ppm. −78.78 (d, J = 7.0 Hz) ppm. −82.66 ppm. −84.25 ppm. −85.22 ppm.

Kraft lignin: $^{19}$F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −73.78 ppm. −74.29 ppm. −74.48 ppm. −74.92 ppm. −76.06 ppm. −76.40 ppm. −77.61 ppm. −77.78 (d, J = 6.6 Hz) ppm. −79.14 ppm. −79.24 ppm. −79.24 (d, J = 7.1 Hz) ppm.

Sulfinic acid hydrolysis: $^{19}$F NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ −74.28 ppm. −75.77 ppm. −75.81 ppm. −76.53 ppm. −76.64 ppm. −77.68 ppm. −78.77 (d, J = 7.5 Hz) ppm. −79.19 ppm. −79.24 (d, J = 7.1 Hz) ppm.

Kraft lignin: $^{13}$C NMR (CDCl$_3$/pyridine) (CFCl$_3$) δ 72.70 (d, J = 6.6 Hz) ppm. 74.29 ppm. 75.77 ppm. 76.53 ppm. 76.64 ppm. 77.68 ppm. 78.77 ppm. 79.19 ppm. 79.24 ppm. 79.24 ppm.

RESULTS AND DISCUSSION

The detailed chemical reactions used to quantitatively trifluoromethylate the carbonyl groups (including quinones) that are present in lignin are shown in Figure 1. The precise trifluoromethylation conditions used for lignins were developed from an understanding of the reaction details for various model compounds (Avhazi and Argyropoulos, 1996b).

The acquisitions of the $^{19}$F NMR spectra for all trifluoromethylated lignins were carried out in a mixture of CDCl$_3$ and pyridine (1:1.6, v/v), due to the relatively low solubility of lignins in common organic solvents. The particular choice of CDCl$_3$/pyridine (1:1.6, v/v) was made on the basis of our previous work on $^{31}$P NMR spectra of phosphitylated lignins (Argyropoulos et al., 1993; Argyropoulos, 1994a,b; Fillipov et al., 1991). For this reason all $^{19}$F chemical shifts for trifluoromethylated carbonyl-containing lignin model compounds were recorded in CDCl$_3$/Py (DMF and DMSO were not suitable) (Tables 1–4).

The $^{19}$F NMR signals of trifluoromethylated ketones for lignin end-groups range between −80.28 and −80.42 ppm (upfield from CFC$_3$), whereas those of dimeric units are confined between −73.39 and −76.57 ppm. Trifluoromethylated derivatives of cinnamic-like aldehydes appeared between −78.23 and −78.24 ppm, whereas benzaldehyde analogues occupied the range from −77.63 to −77.90 ppm. The latter signals appeared as doublets due to the coupling of fluorine to the adjacent proton present on the trifluoromethylated carbon, with coupling constants ranging between 6.1 and 8.0 Hz (Avhazi and Argyropoulos, 1996b).

The $^{19}$F NMR chemical shifts of trifluoromethylated ortho- and p-quinones were spread over a wide range, and their position was found to be sensitive to steric effects. The $^{19}$F NMR spectra of a trifluoromethylated o-quinone model compound showed two signals at −68.8 and −74.9 ppm, whereas the signals of trifluoromethyl-
ated p-quinone model compounds ranged from −76.0 to −80.2 ppm (upfield from CFCl$_3$).

Figures 2–4 show the $^{19}$F NMR spectra for a variety of trifluoromethylated lignin samples, black spruce milled wood lignin, residual kraft lignin, Sucrolin, Alcell organosolv, steam explosion lignin from yellow poplar (Andersons and Faix, 1995; Milne et al., 1992), and milled straw lignin. These spectra contain a number of $^{19}$F NMR signals that spread over 20 ppm, with a number of common signals for all of the lignins.

To ensure that the trifluoromethylation reaction was selectively carried out on the carbonyl groups in lignin, $^{19}$F NMR spectra of lignin samples before and after reduction with sodium borohydride were acquired. As anticipated, the $^{19}$F NMR spectra of the reduced and trifluoromethylated lignins showed no signals (Figure 5).

**Signal Assignment.** Structural elucidation of several trifluoromethylated carbonyl-containing moieties in lignins was first carried out by comparing their $^{19}$F NMR chemical shifts to the various trifluoromethylated lignin model compounds. The $^{19}$F NMR spectral analyses of different fluorinated lignins displayed numerous well-resolved sharp signals ranging from −64 to −87 ppm, corresponding exactly to the region of various trifluoromethylated lignin model compounds, allowing for some tentative signal assignments. These assignments were tentative because the $^{19}$F NMR chemical shifts of trifluoromethylated quinones occupied a wide range, overlapping with those of ketones (Ahvazi and Argyropoulos, 1996b). As such, complete signal identification could not be carried out solely on the basis of model compound chemical shift information.

The presence of different aldehydes in trifluoromethylated lignins was detected on the basis of their $^{19}$F NMR chemical shifts and coupling constants ($J_{F-H}$). The $^{19}$F NMR signals of trifluoromethylated aldehydes were spread over two regions: from −77.6 to −77.9 ppm and from −78.8 to −79.1 ppm. These regions were assigned to benzoic and cinnamic aldehyde type structures, respectively. The absence of the characteristic aldehydic doublets signals from the $^{19}$F NMR spectra of some lignins could be due to signal overlap.

We clarified these signal assignments by examining $^{13}$C NMR signal splitting by fluorine nuclei in two-dimensional $^{19}$F–$^{13}$C heteronuclear NMR experiments. This is because the $^{13}$C NMR spectra for a number of trifluoromethylated model compounds showed distinct signals with appreciably different $J$-coupling constants.

More specifically, the $^{13}$C NMR chemical shifts for CF$_3$ groups (quartet) appeared between 123 and 126 ppm, with a $^1J_{C-F}$ coupling constant of 285 Hz. Furthermore, a long-range $^2J_{C-F}$ coupling constant was found to be approximately 30 Hz, confined (quartet) between 68 and 80 ppm, allowing for the differentiation of the ketonic from the quinonic signals (Table 5).

In an effort to select a suitable set of parameters that would cover all possible $^{19}$F–$^{13}$C long-range coupling constants that may be encountered in lignin, several HMOC experiments were conducted on different carbonyl-containing lignin model compounds. These studies revealed that, during an HMOC experiment, minor variations in the selected $J$-coupling constants could have serious implications on cross-peak intensity. For example, Figure 7 shows HMOC spectra of trifluoromethylated 3,4-dimethoxybenzaldehyde (13) with $J$ values 28, 30, 32, and 282 Hz. The cross-peak at −72.25 and 73.70 ppm (Figure 7, signal I) is our target signal. However, when the $J$ value was varied, another cross-peak at −72.37 and 130.9 ppm (Figure 7B–D, signal

### Table 1. Fluoride Ion Induced Trifluoromethylation of Carbonyl Compounds of Ketones with Trifluoromethyltrimethylsilane

<table>
<thead>
<tr>
<th>Entry</th>
<th>Precursor</th>
<th>Product</th>
<th>Overall % Yield</th>
<th>$^{19}$F NMR(ppm) CDCl$_3$</th>
<th>CDCl$_3$/Pyridine</th>
<th>GC-MS m/z</th>
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<td>1)</td>
<td></td>
<td></td>
<td>96</td>
<td>−81.35</td>
<td>−80.30</td>
<td>190 (M$^+$), 151, 127, 121, 105, 91</td>
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<td></td>
<td></td>
<td>98</td>
<td>−81.76</td>
<td>−80.57</td>
<td>206 (M$^+$), 188, 167, 149, 137, 119</td>
</tr>
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<td>3)</td>
<td></td>
<td></td>
<td>96</td>
<td>−81.60</td>
<td>−80.38</td>
<td>236 (M$^+$), 197, 167, 151, 124, 110</td>
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<td>4)</td>
<td></td>
<td></td>
<td>95</td>
<td>−81.47</td>
<td>−80.28</td>
<td>266 (M$^+$), 227, 197, 181, 155, 123</td>
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<tr>
<td>5)</td>
<td></td>
<td></td>
<td>95</td>
<td>−81.56</td>
<td>−80.42</td>
<td>250 (M$^+$), 211, 181, 139, 124, 107</td>
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<td>6)</td>
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<td></td>
<td>94</td>
<td>−74.79</td>
<td>−73.39</td>
<td>252 (M$^+$), 233, 213, 183, 165, 127</td>
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<td>7)</td>
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<td></td>
<td>96</td>
<td>−75.11</td>
<td>−73.76</td>
<td>312 (M$^+$), 273, 243, 212, 135, 108</td>
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Table 2. Fluoride Ion Induced Trifluoromethylation of Carbonyl Compounds of Aldehydes with Trifluoromethyltrimethylsilane

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<th>Entry</th>
<th>Precursor</th>
<th>Product</th>
<th>Overall % Yield</th>
<th>$^{19}$F NMR (ppm) CDCl$_3$</th>
<th>$^{19}$F NMR (ppm) CDCl$_3$/Pyridine</th>
<th>GC-MS m/z</th>
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<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td>97</td>
<td>-78.848 (d, $J_{CF} = 6.1$ Hz)</td>
<td>-77.680 (d, $J_{CF} = 6.1$ Hz)</td>
<td>MS m/z 178 (M$^+$), 159, 127, 107, 69, 79</td>
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<td>9)</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td>98</td>
<td>-77.745 (Acetone-D$<em>6$) (d, $J</em>{CF} = 6.1$ Hz)</td>
<td>-77.901 (d, $J_{CF} = 6.1$ Hz)</td>
<td>MS m/z (silylated) 336 (M$^+$), 267, 249, 225, 197, 151</td>
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<td>10)</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td>99</td>
<td>-77.587 (Acetone-D$<em>6$) (d, $J</em>{CF} = 7.5$ Hz)</td>
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<td>MS m/z (silylated) 424 (M$^+$), 469, 383, 356, 283, 247</td>
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<td>11)</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td>95</td>
<td>-78.981 (d, $J_{CF} = 6.1$ Hz)</td>
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<td>MS m/z 252 (M$^+$), 205, 183, 167, 155, 140</td>
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<td>-78.916 (d, $J_{CF} = 6.1$ Hz)</td>
<td>-77.756 (d, $J_{CF} = 7.5$ Hz)</td>
<td>MS m/z 236 (M$^+$), 219, 187, 167, 139, 124</td>
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<td>14)</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td>99</td>
<td>-79.458 (d, $J_{CF} = 6.1$ Hz)</td>
<td>-78.229 (d, $J_{CF} = 7.5$ Hz)</td>
<td>MS m/z 202 (M$^+$), 184, 165, 133, 115, 91</td>
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<td>15)</td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
<td>99</td>
<td>-79.607 (d, $J_{CF} = 6.1$ Hz)</td>
<td>-78.241 (d, $J_{CF} = 6.1$ Hz)</td>
<td>MS m/z 248 (M$^+$), 219, 195, 179, 161, 147</td>
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Table 3. Fluoride Ion Induced Trifluoromethylation of Quinones with Trifluoromethyltrimethylsilane

<table>
<thead>
<tr>
<th>Entry</th>
<th>Precursor</th>
<th>Product</th>
<th>Overall % Yield</th>
<th>$^{19}$F NMR (ppm) CDCl$_3$</th>
<th>$^{19}$F NMR (ppm) CDCl$_3$/Pyridine</th>
<th>GC-MS m/z</th>
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<td>-80.75 (-80.81)</td>
<td>-79.72 (-79.80) (minor/major)</td>
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<td><img src="image19.png" alt="Image" /></td>
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<td>MS m/z 193 (M$^+$-CF$_3$), 173, 145, 124, 69, 51</td>
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<td><img src="image22.png" alt="Image" /></td>
<td>94</td>
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<td>-77.69 (-78.85)</td>
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<td>19)</td>
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<td>89</td>
<td>-69.59 (-75.87)</td>
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<td>MS m/z 584 (silylated) (M$^+$-CF$_3$), 489, 399, 378, 327, 285</td>
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<td><img src="image26.png" alt="Image" /></td>
<td>98</td>
<td>-73.55 (-75.66)</td>
<td>MS m/z 175 (M$^+$-C$_8$H$_4$F$_3$O), 152, 105, 77, 51</td>
<td></td>
</tr>
</tbody>
</table>

II) was apparent as a result of an isotope shift effect due to the $^{13}$C$-^{19}$F interaction. This is not surprising because isotopic substitution causes changes in shielding effects: for instance, the $^{19}$F NMR chemical shift of...
CF₃I is shielded by 0.149 ppm more for the ¹³CF₃I isotopomer than in ¹²CF₃I (Harris, 1983). This signal, however, was easily distinguished from the primary correlation because it was confined in the -CF₃ ¹³C chemical shift region, and, in addition, it was slightly shifted (0.1 ppm) from the parent ¹⁹F peak. Nevertheless, isotope shift effects in HMQC spectra of trifluoromethylated lignins could increase the complexity of signal assignment. Because such potential problems were known, a number of trifluoromethylated lignins were subjected to HMQC experiments. The accumulated spectral data, however, despite the long acquisition times (24 h), were inconclusive due to the low carbonyl contents of lignins that gave low signal-to-noise ratios.

Because 2D NMR was of limited utility in aiding the ¹⁹F NMR signal assignments for trifluoromethylated lignins, our attention was focused to the application of selective chemical derivatization techniques. Two different reactions were considered, namely, the Dakin oxidation (Bailey and Dence, 1969; Reeves and Pearl, 1965) and sodium hydrosulfite reduction (Rabjohn, 1963; Fieser and Fieser, 1967; Grundmann, 1977).

The Dakin reaction causes the selective oxidation of various carbonyl groups present in lignins (Reeves and...
Carbonyl groups are oxidized to \( p \)-quinones when a free hydroxyl group is present para to the side chain. In contrast, when the phenolic group is etherified, the system is totally unreactive. Furthermore, \( R \)-unsaturated aldehydes react with alkaline hydrogen peroxide with the formation of the corresponding benzaldehydes and benzoic acids, whereas nonphenolic benzaldehydes are converted directly to the corresponding benzoic acids.

Therefore, a lignin sample subjected to the Dakin reaction should be enriched in \( p \)-quinones and depleted of aldehydes and \( \alpha \)-carbonyls that bear free phenolic hydroxyl groups. The total concentration of etherified \( \alpha \)-carbonyl structures, however, should remain the same before and after the Dakin reaction.

Sodium hydrosulfite is a mild reducing agent that has been reported (Rabjohn, 1963; Rieser and Fieser, 1967; Grundmann, 1977) to selectively reduce quinones in the presence of aldehydes or ketones. To select the best reaction conditions for selective reductions of lignins, a series of exploratory experiments were carried out. These experiments were also aimed at confirming that cinnamyl and benzyl aldehydes as well as model \( \alpha \)-ketones would not be reduced by sodium hydrosulfite. More specifically, di-\( \text{tert} \)-butyl-\( \text{o} \)-quinone, \( \text{p} \)-quinone, acetovanillone, and syringaldehyde were reduced by sodium hydrosulfite. Both \( \text{o} \) - and \( \text{p} \)-quinones were reduced quantitatively to their corresponding alcohol in 15 min, whereas acetovanillone and syringaldehyde were not affected, even after a 4 h reaction. The reduction of lignin with sodium hydrosulfite was complete within 1 h.

Figure 8 shows the \( ^{19} \text{F} \) NMR spectra of trifluoromethylated samples of residual dioxane lignin before (A) and after Dakin oxidation (B) and after sodium hydrosulfite reduction (C). On the basis of the above accounts, and the chemical shift data of Tables 1–4, a number of major carbonyl signals are tentatively assigned.

The comparison of \( ^{19} \text{F} \) NMR spectral analyses of trifluoromethylated dioxane lignin showed a number of prominent signals that were significantly affected by the Dakin and sodium hydrosulfite reactions. For example, the intensities of signals located at \( -67.7 \), \( -73.0 \), and \( -78.2 \) ppm in the original spectrum of the dioxane lignin (Figure 8A) were reduced almost completely (Figure 8C) after their reaction with sodium hydrosulfite. Therefore, these signals were assigned to \( \text{o} \) - and \( \text{p} \)-quinones on the basis in chemistry known to occur between sodium hydrosulfite and quinones.

Another important signal, centered at \( -74.5 \) ppm, which appeared consistently in all of the different trifluoromethylated lignin samples (Figures 2–4), was also identified. This signal, which was assumed to represent \( \alpha \)-carbonyl-containing \( \text{O} \)-4 structures or quinones (Table 4), was found to be drastically reduced after Dakin oxidation, whereas it remained unaffected upon treatment with sodium hydrosulfite (Figure 8B). As such, this signal was assigned to be due exclusively to \( \alpha \)-carbonyl groups of \( \text{O} \)-4 units bearing a free phenolic hydroxyl group para to the side chain.
The fine structural elucidation for a number of signals located at -75 to -79 ppm (Figure 8) was restricted because various trifluoromethylated carbonyl signals in lignin partially overlap in this region. For instance, the 19F NMR signals of aldehydes, quinones, and also α-carbonyls could be all found in this region.

The last set of 19F NMR signals in trifluoromethylated lignin spectra located between -82 and -85 ppm (Figure 8) were assigned to different unhindered ketones. Comparison with model compound data (Table 1) allowed the assignments of two different classes of ketones in this region. The signal that was not affected by the Dakin oxidation appeared at -84.2 ppm and was assigned as being due to the α-carbonyl of etherified lignin end-groups. However, the signal at -82.7 ppm was found to be seriously reduced by Dakin oxidation. This signal was assigned to the ketonic structures bearing a free phenolic hydroxyl group in the para position of the aromatic ring such as 5-5′-biphenyl or 4-O-5′ units. Traces of acetone used to wash and dry the glassware were found to give rise to the signal at -82.15 ppm. Therefore, the sensitivity of this technique dictates that when acetone is used for cleaning purposes, it should be thoroughly removed.

Quantitative Evaluation of the Carbonyl Groups in Lignins. The quantification of the total amount of carbonyl groups in lignin was carried out by using 3,3′-bis(trifluoromethyl)benzophenone as an internal standard. This compound had all of the characteristics of a reliable internal standard required for accurate measurements: it is a pure crystalline solid possessing two equivalent CF3 groups giving a sharp signal at -62.511 ppm. Its position is in the proximity of the lignin signals, so as to allow the use of a narrow sweep width during spectral acquisition, and at the same time does not overlap with any of the lignin signals, allowing for precise integrations. The use of this internal standard permitted the quantitative determination of all carbonyl groups present in all examined lignins. This was made possible because adequate delay time between pulses was used (10 s). This selection was based on detailed measurements of the 19F spin-lattice relaxation times for trifluoromethylated lignins and the internal standard. As anticipated, the longest T1 was that of the internal standard.

To examine the reproducibility and quantitative reliability of our measurements, several native and technical lignins were selected and their carbonyl contents were determined after trifluoromethylation. The total carbonyl content for each lignin sample was determined four times, and the calculated mean values and standard deviations are shown in Table 6. Notably, the total amount of carbonyls determined by 19F NMR was found to be different from sample to sample with high precision. A further investigation aimed at substantiating the present technique as an analytical tool for the quantification of the various carbonyl groups in different soluble lignins was conducted. In particular, the quantitative derivatization of carbonyls by trifluoromethylation was examined by selecting lignin samples for which the carbonyl contents were determined according to two different techniques, oximation and UV spectroscopy, during the 1991 International Round Robin effort (Andersons and Faix, 1995; Milne et al., 1992). It was thus possible to compare the results furnished by quantitative 19F NMR with those produced by independent methods in other laboratories for the same samples as presented in Table 7.

The proximity of the two sets of data in Table 7 qualifies the 19F NMR technique as a novel and promising analytical tool for detecting and determining the
most prominent carbonyl-containing groups present in different soluble lignins.

**Conclusions.** The quantitative trifluoromethylation of carbonyl groups can be applied for the detection and quantitative determination of the various carbonyl groups present in lignins. By applying selective reactions such as borohydride and hydrosulfite reductions and Dakin oxidation, it became possible to assign a number of prominent 19F NMR signals in trifluoromethylated lignins. The quantification of the total amount of carbonyls can be carried out using 3,3′-bis(trifluoromethyl)benzophenone as an internal standard. The total amounts of carbonyls determined according to the proposed technique in a variety of samples were found to be different from one another and yet close to reported values using independent techniques. The proximity of these data for three lignin samples qualifies the 19F NMR technique as a new analytical tool for detecting and determining carbonyl groups in lignins.

**LITERATURE CITED**


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**Table 6. Quantitative Analyses of Carbonyl Groups in Several Lignins by Using 19F NMR Spectroscopy**

<table>
<thead>
<tr>
<th>Lignin Sample</th>
<th>CO/C9 wt %</th>
<th>s</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrolin acid hydrolysis (bagasse)</td>
<td>0.12 ± 0.01</td>
<td>0.0097</td>
<td>1.89 ± 0.2</td>
</tr>
<tr>
<td>Alcell organosolv (mixed hardwoods)</td>
<td>0.11 ± 0.01</td>
<td>0.0086</td>
<td>1.56 ± 0.2</td>
</tr>
<tr>
<td>Steam explosion (yellow poplar)</td>
<td>0.13 ± 0.01</td>
<td>0.0100</td>
<td>1.57 ± 0.2</td>
</tr>
<tr>
<td>Dioxane acidolysis</td>
<td>0.15 ± 0.01</td>
<td>0.0095</td>
<td>2.27 ± 0.2</td>
</tr>
<tr>
<td>Kraft residuec</td>
<td>0.018 ± 0.005</td>
<td>0.0011</td>
<td>2.90 ± 0.2</td>
</tr>
<tr>
<td>Straw</td>
<td>0.018 ± 0.005</td>
<td>0.0011</td>
<td>2.90 ± 0.2</td>
</tr>
</tbody>
</table>

a x = mean value. b s = standard deviation. c The amount of CO/C9 is not reported because the C9 unit cannot be defined.

**Table 7. Determination of Total Amount of Carbonyl Groups in Lignins by Different Techniques**

<table>
<thead>
<tr>
<th>Lignin Sample</th>
<th>19F NMR</th>
<th>Oximation</th>
<th>UV–visa</th>
<th>MW</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrolin acid hydrolysis (bagasse)</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.12</td>
<td>0.03 ± NR</td>
<td>177.4</td>
<td>C9H8O2.3(OCH3)0.83</td>
</tr>
<tr>
<td>Alcell organosolv (mixed hardwoods)</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± NR</td>
<td>178.5</td>
<td>C9H7O1.8(OCH3)1.04</td>
</tr>
<tr>
<td>Steam explosion (yellow poplar)</td>
<td>0.13 ± 0.01</td>
<td>0.11 ± 0.04</td>
<td>0.09 ± NR</td>
<td>194.8</td>
<td>C9H7.5O2.5(OCH3)1.25</td>
</tr>
</tbody>
</table>

a Reduction with sodium borohydride followed by UV–vis [standard deviation was not reported (NR)].

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**Figure 8.** 19F NMR spectra of trifluoromethylated dioxane lignin (A), after Dakin reaction (B) and reduction (C) with sodium hydrosulfite.


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Kalboe, S.; Ellefsen, O. Infrared investigations of lignin; A discussion of some recent results. Tappi 1962, 45 (2), 163–166.


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