^{19}F Nuclear Magnetic Resonance Spectroscopy for the Quantitative Detection and Classification of Carbonyl Groups in Lignins

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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 trifluoromethylating 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 (Jansherkar 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 Fuggerer, 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 *o*- and *p*-quinonoids, quinonemethides, and other extended conjugated enone systems, is responsible for not only the color of lignified plant tissue (Hon and Glasser, 1979; Lebo et al., 1990) but also sentisizing centers in the photoyellowing of lignocellulosic materials (Brunow and Eriksson, 1971). In general, the low content of these groups in lignins has made the elucidation of their role rather elusive. For example, quinones, which are present in wood and high-yield mechanical pulps (Argyropoulos et al., 1994; Argyropoulos and Heitner, 1994) in rather low amounts, only recently have been unequivocally shown to be responsible for the yellow color of photochemically reverted papers (Argyropoulos et al., 1994; Argyropoulos and Heitner, 1994; Lin and Kringstad, 1971; Forsskåhl et al., 1991; Castellan et al., 1993; Gellerstedt and Pattersson, 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 (Kolboe 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

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10.1021/jf980431p CCC: $18.00
Published on Web 12/12/1998

is limited and the various classes of carbonyl moieties cannot be differentiated.

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 proton (Lundquist, 1979a,b, 1980) and ^{13}C NMR spectroscopies (Lapierre et al., 1984; Robert and Brunow, 1984; Obst and Landucci, 1986) have prompted the examination of other NMR-active nuclei. These efforts have provided additional tools for obtaining fine structural details for these heterogeneous biopolymers. The determination of a variety of labile protons in lignins has been carried out by ^{29}Si-, ^{31}P- and ^{19}F-based NMR methods, for suitably silylated (Pan and Lachenal, 1994; Brezny and Schraml, 1987), phosphitylated (Nieminen et al., 1989; Archipov et al., 1991a,b; Argyropoulos et al., 1992, 1993; Argyropoulos, 1994a,b; Fillppov et al., 1991) and fluorinated (Manatt, 1966; Barrelle, 1995) lignins, respectively. Furthermore, Lebo et al. (1990) and, recently, Argyropoulos et al. (1995) have reported the detection of *o*-quinones in mechanical pulps. In particular, the latter group has actually managed to follow their formation during the process of light-induced yellowing using solid-state ^{31}P NMR spectroscopy (Argyropoulos et al., 1995).

The ^{19}F nucleus is 100% naturally abundant, and its high gyromagnetic ratio makes its NMR sensitivity nearly the same as that of a proton. Its chemical shift extends over a wide range providing adequate signal dispersion that may reduce signal overlap and aid interpretation. Attempts at determining the carbonyl content of lignin by ^{19}F NMR have actually been made previously after *p*-fluorobenzylation (Barrelle, 1993) of the lignin or its derivatization with *p*-fluorophenylhydrazine (Lachenal et al., 1995). In both cases the ^{19}F NMR signals overlapped over a relatively narrow range, thus diminishing the quantitative reliability of the techniques. Furthermore, the proposed methods were unable to distinguish among the different classes of carbonyl groups present in lignin.

In previous work, we developed (Ahvazi and Argyropoulos, 1996a) a selective and quantitative trifluoromethylation reaction for tagging the carbonyl groups in lignin model compounds, using trifluoromethyltrimethylsilane (TMS-CF_3) in the presence of tetramethylammonium fluoride (TMAF). A series of ketones, aldehydes, quinones, and dimeric lignin model compounds were quantitatively trifluoromethylated, and the resulting fluorine derivatives were analyzed by ^{19}F NMR. This effort allowed for a thorough understanding of the structure/^{19}F chemical shift relationships (Ahvazi and Argyropoulos, 1996b) of lignin-like moieties.

The present effort attempts to expand the application of quantitative trifluoromethylation to lignins, aimed at elucidating the nature and the quantity of the various carbonyl groups present in them.

EXPERIMENTAL PROCEDURES

Reactions. *Trifluoromethylation.* The following trifluoromethylation procedure was developed and applied to all lignin samples. One hundred milligrams of lignin was dissolved, under constant stirring, in 10 mL of dry tetrahydrofuran (THF) at room temperature. After 10 min of stirring, 600 μL of TMS-CF_3 was added. The mixture was cooled at 0 °C for 10 min followed by the addition of a catalytic amount (15 mg) of TMAF acting as the initiator. The reaction mixture continued to be stirred at 0 °C for 30 min and then at room temperature for 24 h. The intermediate trifluoromethylated siloxy adducts were then hydrolyzed by the addition of 50 mg of TMAF at room temperature for 24 h in THF. After the THF was evaporated under reduced pressure, the residue was washed and centrifuged thoroughly by 3 × 50 mL water. Finally, the isolated materials were dissolved in a mixture of dioxane/water (25:5, v/v) and freeze-dried under reduced pressure.

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 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.

Dakin Reaction. Lignin (200 mg) was suspended in a solution of 6.5 mL of *n*-propanol and 7.5 mL of water under constant stirring for 15 min. To this mixture was added 5 drops of a 0.5 M sodium hydroxide solution, causing the complete dissolution of the lignin. This was followed by the addition of 702 μL of 30% hydrogen peroxide. After the pH of the mixture was adjusted to 10.6 by 0.5 M NaOH, the reaction mixture was stirred at 50 °C for 4 h. The reaction was then neutralized by the addition of 1−2 drops of 25% sulfuric acid to pH 4.7. After the organic solvent was evaporated under reduced pressure, the lignin was precipitated by the addition of water. The precipitated lignin was washed with water and centrifuged three times with 15 mL of water. The isolated lignin was then dissolved in a mixture of dioxane/water (25:5, v/v) and freeze-dried.

Sodium Hydrosulfite Reduction of Lignin Model Compounds. Selected lignin model compounds (200 mg) were dissolved in 5 mL of dioxane, and a slow stream of nitrogen was bubbled through the solution for ∼30 min. To this solution was added 200 mg of sodium hydrosulfite ($Na_2S_2O_4$) 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 200 mg of sodium hydrosulfite 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.

Instrumentation. *Gas 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.

^{19}F NMR Spectroscopy. All spectra were recorded on a Varian Unity 500 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 an internal standard solution

(i.e., 1.69 g/mL 1.6:1, v/v, pyridine/$CDCl_3$ of 3,3′-bis(trifluoromethyl)benzophenone; the chemical shift was referenced to fluorotrichloromethane. Quantitative acquisitions were carried out in 5 mm tubes at room temperature with acquisition times of 0.64 s followed by a relaxation delay of 10 s. The number of scans acquired was 1000 per measurement. Pulse widths corresponding to a 45° flip angle and a line broadening of 2 Hz were used during acquisition and processing of the spectra.

Two-Dimensional NMR Spectroscopy (HMQC). Such spectra were acquired using 10−30 mg of sample dissolved in 0.6 mL of $CDCl_3$, DMSO-d_6, or acetone-d_6 on a Varian Unity 500 NMR spectrometer using a 5 mm inverse detection probe (DHP). The chemical shifts were referenced to Me_4Si and $CFCl_3$ for ^{13}C and ^{19}F, respectively. HMQC spectra were acquired over a 30 ppm window in F_2 (^{19}F) and 190 ppm in F_1 (^{13}C) with GARP-1 decoupling. The value of the coupling constant was adjusted to the estimated value for the ^2J C−F of 30 Hz; 2K × 256 increments were acquired. After F_1 zero-filling, Fourier transformation, and squared cosine bell apodization, the transformed data matrix was 1024 (F_2) × 512 (F_1) real points.

Characterization. *Characterization of Trifluoromethylated Model Compounds.* ***1***: ^1H NMR ($CDCl_3$) (TMS) δ 1.77 (s, 3H); 2.60 (s, 1H); 7.37−7.46 (m, 3H); 7.56−7.61 (m, 2H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −80.30 (s) ppm. MS *m/z* 190 (M^+), 151, 127, 121, 105, 91, 77, 51. Anal. Calcd for $C_9H_9F_3O$: C, 56.84; H, 4.77; F, 29.97. Found: C, 56.92; H, 4.69; F, 29.92. 96% yield.

2: ^1H NMR ($CDCl_3$) (TMS) δ 1.74 (s, 3H); 2.35 (s, 1H); 5.67 (s, 1H); 6.829 (d, 2H, J = 8.78 Hz); 7.432 (d, 2H, J = 8.58 Hz) ppm. ^{13}C NMR ($CDCl_3$) (TMS) δ 23.85; 74.54 (q, J_{C-CF} = 29.1 Hz); 115.11; 155.83; 129.36 (q, J_{C-F} = 284.9 Hz); 155.83. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −80.57 (s) ppm. MS *m/z* 206 (M^+), 188, 167, 149, 137, 119, 107, 91. Anal. Calcd for $C_9H_9F_3O_2$: C, 52.43; H, 4.40; F, 27.65. Found: C, 52.48; H, 4.49; F, 27.67. 98% yield.

3: ^1H NMR ($CDCl_3$) (TMS) δ 1.74 (s, 3H); 2.90 (s, 1H); 3.879 (d, 3H, J = 6.64 Hz); 5.84 (s, 1H); 6.86−7.13 (m, 3H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −80.38 (s) ppm. MS *m/z* 236 (M^+), 197, 167, 151, 124, 110, 69, 51. Anal. Calcd for $C_{10}H_{11}F_3O_3$: C, 50.85; H, 4.69; F, 24.13. Found: C, 50.91; H, 4.68; F, 24.19. 96% yield.

4: ^1H NMR ($CDCl_3$) (TMS) δ 1.73 (s, 3H); 2.54 (s, 1H); 3.879 (d, 6H, J = 10.26 Hz); 6.78 (s, 2H); 7.22 (s, 1H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −80.28 (s) ppm. MS *m/z* 266 (M^+), 227, 197, 181, 155, 123, 93, 69. Anal. Calcd for $C_{11}H_{13}F_3O_4$: C, 49.63; H, 4.92; F, 21.41. Found: C, 49.69; H, 4.85; F, 21.35. 95% yield.

5: ^1H NMR ($CDCl_3$) (TMS) δ 1.73 (s, 3H); 2.72 (s, 1H); 3.841 (d, 6H, J = 2.16 Hz); 6.825 (d, 1H, J = 8.48 Hz); 7.03−7.11 (m, 2H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −80.42 (s) ppm. MS *m/z* 250 (M^+), 211, 181, 139, 124, 107, 95, 77. Anal. Calcd for $C_{11}H_{13}F_3O_3$: C, 52.80; H, 5.24; F, 22.78. Found: C, 52.85; H, 5.28; F, 22.80. 95% yield.

6: ^1H NMR ($CDCl_3$) (TMS) δ 2.93 (s, 1H); 7.34−7.38 (m, 6H); 7.47−7.52 (m, 4H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −73.39 (s) ppm. MS *m/z* 252 (M^+), 233, 213, 183, 165, 127, 105, 77. Anal. Calcd for $C_{11}H_{13}F_3O_3$: C, 66.67; H, 4.40; F, 22.60. Found: C, 66.71; H, 4.32; F, 22.66. 94% yield.

7: ^1H NMR ($CDCl_3$) (TMS) δ 3.22 (s, 1H); 3.84 (s, 6H); 6.87−7.03 (m, 4H); 7.03−7.47 (m, 4H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −73.76 (s) ppm. MS *m/z* 312 (M^+), 273, 243, 212, 168, 135, 108, 77. Anal. Calcd for $C_{16}H_{15}F_3O_3$: C, 61.54; H, 4.84; F, 18.25. Found: C, 61.56; H, 4.88; F, 18.17. 96% yield.

8: ^1H NMR ($CDCl_3$) (TMS) δ 2.60 (s, 1H); 4.989 (q, 1H, J = 6.60 Hz); 7.38−7.48 (m, 5H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −78.680 (d, J_{F-H} = 6.1 Hz) ppm. MS *m/z* 176 (M^+), 159, 127, 107, 89, 79, 51. Anal. Calcd for $C_8H_7F_3O$: C, 54.55; H, 4.01; F, 32.36. Found: C, 54.58; H, 3.99; F, 32.40. 97% yield.

9: ^1H NMR (acetone-d_6) (TMS) δ 5.073 (q, 1H, J = 7.34 Hz); 5.30−6.30 (s, b, 1H); 6.658 (m, 2H); 7.359 (m, 2H); 7.80−8.90 (s, b, 1H) ppm. ^{19}F NMR (C_2D_6O) ($CFCl_3$) δ −77.745 (d, J_{F-H} = 6.1 Hz) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −77.901 (d, J_{F-H} = 6.1 Hz) ppm. MS *m/z* (silylated) 336 (M^+), 267, 249, 225, 197, 195, 151. Anal. Calcd for $C_8H_7F_3O_2$: C, 50.01; H, 3.67; F, 29.66. Found: C, 50.12; H, 3.71; F, 29.72. 98% yield.

10: ^1H NMR (acetone-d_6) (TMS) δ 2.997 (s, b, 1H); 4.997 (m, 1H); 5.560 (m, 1H); 6.834 (m, 2H); 7.976 (s, b, 1H); 8.035 (S, b, 1H) ppm. ^{19}F NMR (C_2D_6O) ($CFCl_3$) δ −77.587 (d, J_{F-H} = 7.5 Hz) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −77.691 (d, J_{F-H} = 8.0 Hz) ppm. MS *m/z* (silylated) 424 (M^+), 409, 383, 356, 352, 283, 247. Anal. Calcd for $C_8H_7F_3O_3$: C, 46.17; H, 3.39; F, 27.38. Found: C, 46.25; H, 3.41; F, 27.37. 97% yield.

11: ^1H NMR ($CDCl_3$) (TMS) δ 2.59 (s, 1H); 3.89 (s, 3H); 4.925 (q, 1H, J = 6.78 Hz); 5.72 (s, 1H); 6.947 (d, 3H, J = 13.93 Hz) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −77.756 (d, J_{F-H} = 8.0 Hz) ppm. MS *m/z* 222 (M^+), 205, 183, 153, 125, 93, 65, 51. Anal. Calcd for $C_9H_9F_3O_3$: C, 48.66; H, 4.08; F, 25.65. Found: C, 48.70; H, 4.11; F, 25.69. 95% yield.

12: ^1H NMR ($CDCl_3$) (TMS) δ 2.18 (s, 1H); 3.85 (s, 6H); 4.924 (q, 1H, J = 6.58 Hz); 6.68 (s, 2H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −77.633 (d, J_{F-H} = 8 Hz) ppm. MS *m/z* 252 (M^+), 205, 183, 167, 155, 140, 123, 95. Anal. Calcd for $C_{10}H_{11}F_3O_4$: C, 47.63; H, 4.40; F, 22.60. Found: C, 47.62; H, 4.42; F, 22.58. 98% yield.

13: ^1H NMR ($CDCl_3$) (TMS) δ 2.50 (s, 1H); 3.87 (s, 6H); 4.943 (q, 1H, J = 6.64 Hz); 6.841 (t, 1H, J = 4.88 Hz); 6.976 (d, 2H, J = 3.42 Hz) ppm. ^{13}C NMR ($CDCl_3$) (TMS) δ 55.73; 72.65 (q, J_{C-CF} = 32.1 Hz); 110.19; 110.91; 120.29; 124.30 (q, J_{C-CF} = 281.7 Hz); 126.44, 149.13; 149.99. ^{13}C NMR (DMSO) δ 24.05; 70.37 (q, J_{C-F} = 30.2 Hz); 114.89; 115.06; 118.85; 124.20 (q, J_{C-CF} = 282.3 Hz); 126.59; 144.95; 145.79. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −77.756 (d, J_{F-H} = 7.5 Hz) ppm. MS *m/z* 236 (M^+), 219, 197, 167, 139, 124, 108, 96. Anal. Calcd for $C_{10}H_{11}F_3O_3$: C, 50.85; H, 4.69; F, 24.13. Found: C, 50.92; H, 4.66; F, 24.11. 99% yield.

14: ^1H NMR ($CDCl_3$) (TMS) δ 2.97 (s, 1H); 4.58−4.66 (m, 1H); 6.20 (q, 1H, J = 9.46 Hz); 6.842 (d, 1H, J = 15.93 Hz); 7.31−7.44 (m, 5H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −78.229 (d, J_{F-H} = 7.5 Hz) ppm. MS *m/z* 202 (M^+), 184, 165, 133, 115, 91, 77, 55. Anal. Calcd for $C_{10}H_9F_3O$: C, 59.41; H, 4.49; F, 28.19. Found: C, 59.45; H, 4.52; F, 28.17. 99% yield.

15: ^1H NMR ($CDCl_3$) (TMS) δ 2.25 (s, 1H); 3.84 (s, 3H); 4.531 (t, 1H, J = 13.19 Hz); 5.65 (s, 1H); 5.961 (q, 1H, J = 9.12 Hz); 6.689 (d, 1H, J = 15.73 Hz); 6.82−6.88 (m, 3H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −78.241 (d, J_{F-H} = 6.1 Hz) ppm. MS *m/z* 248 (M^+), 219, 199, 179, 161, 147, 119, 91. Anal. Calcd for $C_{11}H_{11}F_3O_3$: C, 53.23; H, 4.47; F, 22.96. Found: C, 53.28; H, 4.44; F, 22.97. 99% yield.

16: ^1H NMR ($CDCl_3$) δ 2.42 (br, 4H); 6.19 (s, 4H,); 6.27 (s, 4H,) ppm. ^1H NMR (DMSO) δ 6.17 (s, 4H); 6.86 (s, 2H,) ppm. ^{13}C NMR (DMSO) δ 67.18 (q, J_{C-CF} = 29.4 Hz); 124.77 (q, J_{C-F} = 286.4 Hz); 129.16. ^{13}C NMR (acetone-d_6) δ 68.70 (q, J_{C-CF} = 30.3 Hz); 125.64 (q, J_{C-F} = 285.0 Hz); 130.26. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −79.80 (s, major); −79.72 (s, minor) ppm. MS *m/z* 179 ($M^+ - CF_3$), 159, 143, 110, 83, 69, 53. Anal. Calcd for $C_8H_6F_6O_2$: C, 38.73; H, 2.44; F, 45.94. Found: C, 38.75; H, 2.49; F, 45.96. 96% yield.

17: ^1H NMR ($CDCl_3$) δ 2.02−2.03 (d, 3H, J = 1.16 Hz); 2.30 (br, 2H); 5.98−5.99 (d, 1H, J = 1.72 Hz); 6.24-6.25 (t, 2H, J = 2.16 Hz) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −80.25 (s); −76.04 (s) ppm. MS *m/z* 193 ($M^+ - CF_3$), 173, 145, 124, 69, 51. Anal. Calcd for $C_9H_8F_6O_2$: C, 41.24; H, 3.08; F, 43.48. Found: C, 41.27; H, 3.15; F, 43.50. 95% yield.

18: ^1H NMR ($CDCl_3$) δ 1.37−1.41 (d, 9H, J = 8.60 Hz); 3.19−3.30 (d, 2H, J = 21.23 Hz); 7.52−7.60 (m, 3H); 7.89−8.03 (m, 4H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −77.85 (s); −77.69 (s) ppm. MS *m/z* 335 ($M^+ - CF_3$), 320, 266, 251, 209, 181, 152, 112. Anal. Calcd for $C_{20}H_{18}F_6O_2$: C, 59.41; H, 4.49; F, 28.19. Found: C, 59.38; H, 4.46; F, 28.22. 94% yield.

19: ^1H NMR ($CDCl_3$) δ 1.05 (s, 9H); 1.25 (s, 9H); 2.45 (s, 1H); 3.05 (s, 1H); 5.19 (s, 1H); 6.06 (s, 1H) ppm. ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −68.78 (s); −74.86 (s) ppm. MS *m/z* (silylated) 504 ($M^+ - CF_3$), 489, 399, 379, 327, 285, 259, 239. Anal. Calcd for $C_{16}H_{22}F_6O_2$: C, 53.33; H, 6.15; F, 31.63. Found: C, 53.40; H, 6.10; F, 31.59. 89% yield.

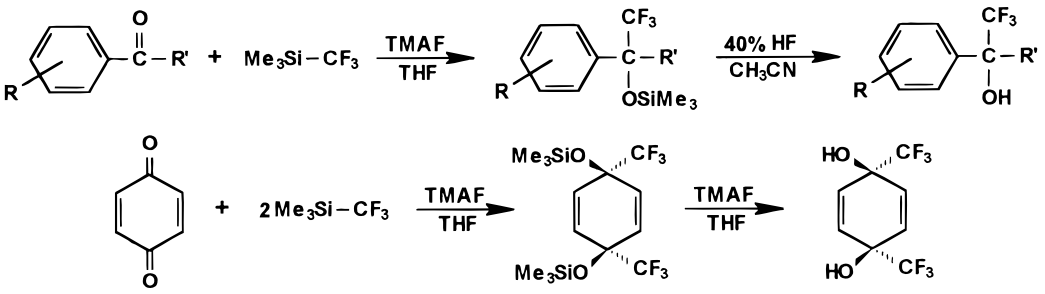


Figure 1. Trifluoromethylation of carbonyl-containing (including quinones) lignin-like model compounds.

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 ($M^+ - C_8H_6F_3O$), 152, 105, 77, 51. Anal. Calcd for $C_{16}H_{12}F_6O_2$: C, 54.87; H, 3.45; F, 32.54. Found: C, 54.91; H, 3.50; F, 32.58. 98% yield.

21: ^{1}H NMR ($CDCl_3$) (TMS) δ 3.63 (s, 1H); 3.78 (s, 3H); 3.863 (d, 6H, J = 5.40 Hz), 4.07−4.12 (m, 2H); 4.449(t, 1H, J = 1.80 Hz); 5.57 (s, 1H); 6.692 (q, 1H, J = 3.60 Hz); 6.77−6.83 (m, 2H); 6.865 (d, 1H, J = 5.10 Hz); 6.95−6.99 (m, 1H); 7.193 (d, 1H, J = 4.80 Hz); 7.294 (d, 1H, J = 1.2 Hz) ppm. ^{13}C NMR ($CDCl_3$/) (TMS) δ 55.85; 56.04; 56.17; 61.36; 79.59 (q, J_{C-CF} = 27.4 Hz); 82.64; 109.87; 110.48; 112.07; 118.25; 120.72; 121.66; 124.62; 124.97 (q, J_{C-F} = 286.59 Hz); 128.93; 146.18; 148.56; 148.89; 151.53 ppm. ^{19}F 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. Calcd for $C_{19}H_{21}F_3O_6$: 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 3.84 (s, 3H); 3.87 (s, 3H); 3.90 (s, 3H), 3.93 (d, 1H, J = 3 Hz); 4.24 (d, 1H, J = 12 Hz); 4.640 (d, 1H, J = 12 Hz); 5.865 (d, 1H, J = 3 Hz); 6.84−7.12 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$) (TMS) δ 56.11; 56.19; 56.64; 71.68; 75.62 (q, J_{C-F} = 28.65 Hz); 108.78; 110.06; 111.44; 113.40; 120.41; 121.80; 122.95; 125.60 (q, J_{C-F} = 286.59 Hz); 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* 372 (M^+), 303, 248, 235, 217, 189, 180. Anal. Calcd for $C_{18}H_{19}F_3O_5$: C, 58.06; H, 5.14; F, 15.31. Found: C, 58.21; 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 = 9 Hz); 4.12 (d, 1H, J = 5.99 Hz); 4.33 (s, 1H); 5.60 (s, 1H,); 5.70 (s, 1H); 6.55−6.64 (m, 3H); 6.94 (d, 1H, J = 5.40 Hz); 7.10 (d, 1H, J = 4.80 Hz); 7.311 (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_{C-CF} = 28.9 Hz); 82.64; 109.59; 112.95; 113.93; 118.43; 121.01; 122.19; 125.07 (q, J_{C-F} = 286.59 Hz); 128.71; 134.83; 143.81; 145.62; 146.35; 151.36 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. Calcd for $C_{19}H_{21}F_3O_6$: C, 56.72; H, 5.26; F, 14.16. Found: C, 56.78; H, 5.19; F, 14.18. 93% yield.

Characterization of Trifluoromethylated Lignins. Dioxane lignin: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −67.71 to −67.79 (m); −72.70 to −72.99 (b); −74.23 (s); −74.54 (s); −75.20 (b); −75.83 (s); −76.87 to −77.11 (b); −77.78 (d, J_{HF} = 6.6 Hz); −78.21 to −78.26 (b); −78.78 (d, J_{HF} = 7.0 Hz); −82.66 (s); −84.25 (d, J_{HF} = 5.2 Hz) ppm.

Kraft lignin: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −73.78 (s); −74.29 (s); −74.48 (s); −74.92 (s); −76.06 (s); −76.40 (s); −77.61 (b); −77.78 (d, J_{F-H} = 6.6 Hz); −79.14 (d, J_{F-H} = 6.6 Hz); −79.24 (d, J_{F-H} = 7.1 Hz); −83.42 (s); -84.22 (d, J_{F-H} = 4.2 Hz) ppm.

Sucrolin acid hydrolysis: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −74.28 (s); −75.77 (s); −75.81 (s); −76.53 (b); −76.64 (s); −77.65 (d, J_{F-H} = 6.6 Hz); −77.76 (d, J_{F-H} = 7.5 Hz); −77.89 (d, J_{F-H} = 7.0 Hz); −79.13 (d, J_{F-H} = 6.6 Hz); −84.32 (s); −85.35 (b) ppm.

Alcell organosolv: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −66.19 (s); −66.23 (s); −72.84 (s); −73.20 (b); −73.65 (s); −74.27 (s); −74.81 (t, J_{F-H} = 13.2 Hz); −75.81 (s); −76.37 (b); −77.29 (d, J_{F-H} = 7.5 Hz); −77.35 (b); −77.46 (b); −77.54 (b); −77.65 (d, J_{F-H} = 7.1 Hz); −77.04 (b); −77.75 (s); −77.76 (s); −78.13 to −78.23 (b); −78.63 (s); −84.32 (s) ppm.

Steam explosion lignin: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −72.84 (s); −73.65 (s); −74.27 (s); −75.81 (s); −76.37 (b); −77.16 (d, J_{F-H} = 4.2 Hz); −77.29 (d, J_{F-H} = 7.5 Hz); −77.32 (b); −77.54 (b); −77.64 (d, J_{F-H} = 8.0 Hz); −77.75 (d, J_{F-H} = 7.1 Hz); −78.18 (b); −78.28 (d, J_{F-H} = 6.6 Hz); −78.63 (s); −78.76 (b); −79.13 (b); −79.22 (d, J_{F-H} = 3.8 Hz); −84.32 (s) ppm.

Straw lignin: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −67.67 (b); −74.26 (s); −75.15 (b); −75.80 (s); −76.52 (b); −76.64 (s); −77.04 (b); −77.25 (s); −77.69 (b); −78.19 (s); −79.05 (s); −79.15 (s); −83.27 (b); −83.95 (b); −84.39 (s) ppm.

Softwood Milled wood lignin: ^{19}F NMR ($CDCl_3$/pyridine) ($CFCl_3$) δ −74.23 (s); −75.55 (b); −76.41 (b); −76.64 (b); −78.18 (b); −79.13 (d, J_{F-H} = 6.6 Hz); −79.21 (d, J_{F-H} = 6.6 Hz); −79.80 (b); −84.20 (b) ppm.

RESULTS AND DISCUSSION

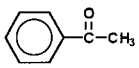
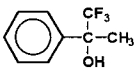
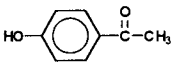
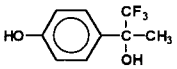
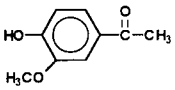
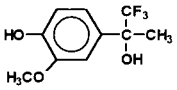
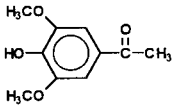
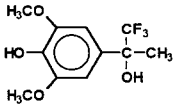
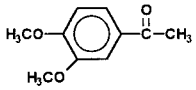
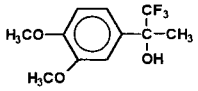
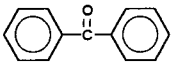
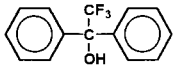
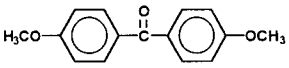
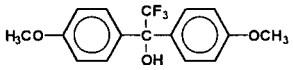
The detailed chemical reactions used to quantitatively trifluoromethylate the carbonyl groups (including quinones) that are present in lignin are shown in Figure 1. The precise trifluoromethylation conditions used for lignins were developed from an understanding of the reaction details for various model compounds (Ahvazi 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; Fillppov et al., 1991). For this reason all ^{19}F chemical shift values 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 $CFCl_3$), 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 (Ahvazi 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-

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

Entry	Precursor	Product	Overall % Yield	^{19}F NMR(ppm) $CDCl_3$	$CDCl_3$/Pyridine	GC-MS m/e
1)			96	-81.35	-80.30	MS m/z 190 (M^+), 151, 127, 121, 105, 91
2)			98	-81.76	-80.57	MS m/z 206 (M^+), 188, 167, 149, 137, 119
3)			96	-81.60	-80.38	MS m/z 236 (M^+), 197, 167, 151, 124, 110
4)			95	-81.47	-80.28	MS m/z 266 (M^+), 227, 197, 181, 155, 123
5)			95	-81.56	-80.42	MS m/z 250 (M^+), 211, 181, 139, 124, 107
6)			94	-74.79	-73.39	MS m/z 252 (M^+), 233, 213, 183, 165, 127
7)			96	-75.11	-73.76	MS m/z 312 (M^+), 273, 243, 212, 135, 108

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 trifluoromethylalted 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 ∼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 HMQC experiments were conducted on different carbonyl-containing lignin model compounds. These studies revealed that, during an HMQC experiment, minor variations in the selected *J*-coupling constants could have serious implications on cross-peak intensity. For example, Figure 7 shows HMQC 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 2. Fluoride Ion Induced Trifluoromethylation of Carbonyl Compounds of Aldehydes with Trifluoromethyltrimethylsilane

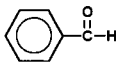
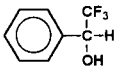
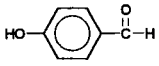
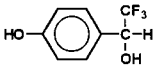
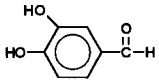
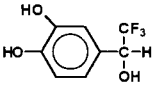
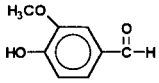
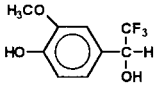
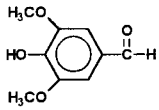
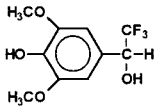
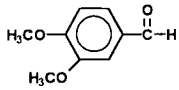
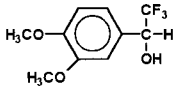
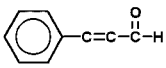
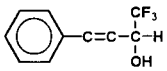
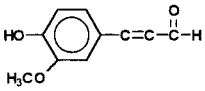
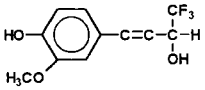
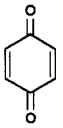
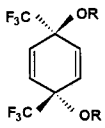
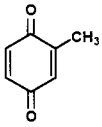
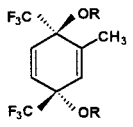
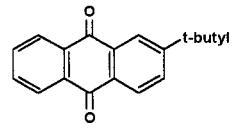
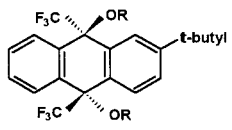
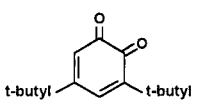
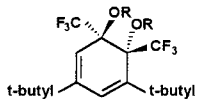
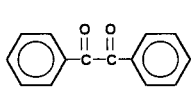
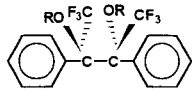
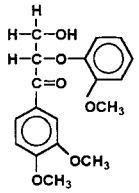
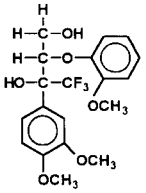
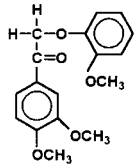
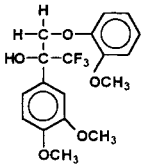
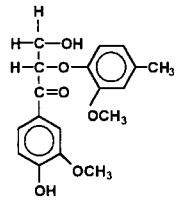
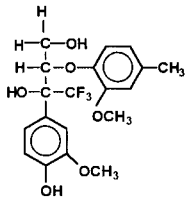
Entry	Precursor	Product	Overall % Yield	^{19}F NMR(ppm) $CDCl_3$	$CDCl_3$/Pyridine	GC-MS m/e
8)			97	-78.848 (d, J_{F-H} = 6.1 Hz)	-77.680 (d, J_{F-H} = 6.1 Hz)	MS m/z 176 (M^+), 159, 127, 107, 89, 79
9)			98	-77.745 (Acetone-D_6) (d, J_{F-H} = 6.1 Hz)	-77.901 (d, J_{F-H} = 6.1 Hz)	MS m/z (silylated) 336 (M^+), 267, 249, 225, 197, 151
10)			99	-77.587 (Acetone-D_6) (d, J_{F-H} = 7.5 Hz)	-77.691 (d, J_{F-H} = 8.0 Hz)	MS m/z (silylated) 424 (M^+), 409, 383, 356, 283, 247
11)			95	-78.981 (d, J_{F-H} = 6.1 Hz)	-77.756 (d, J_{F-H} = 8.0 Hz)	MS m/z 222 (M^+), 205, 183, 153, 125, 93
12)			98	-78.890 (d, J_{F-H} = 6.1 Hz)	-77.633 (d, J_{F-H} = 8.0 Hz)	MS m/z 252 (M^+), 205, 183, 167, 155, 140
13)			99	-78.916 (d, J_{F-H} = 6.1 Hz)	-77.756 (d, J_{F-H} = 7.5 Hz)	MS m/z 236 (M^+), 219, 197, 167, 139, 124
14)			99	-79.458 (d, J_{F-H} = 6.1 Hz)	-78.229 (d, J_{F-H} = 7.5 Hz)	MS m/z 202 (M^+), 184, 165, 133, 115, 91
15)			99	-79.607 (d, J_{F-H} = 6.1 Hz)	-78.241 (d, J_{F-H} = 6.1 Hz)	MS m/z 248 (M^+), 219, 199, 179, 161, 147

Table 3. Fluoride Ion Induced Trifluoromethylation of Quinones with Trifluoromethyltrimethylsilane

Entry	Precursor	Product	Overall % Yield	^{19}F NMR(ppm) $CDCl_3$	$CDCl_3$/Pyridine	GC-MS m/e
16)			96	16 -80.75, -80.81	16 -79.72 (minor), -79.80 (major)	MS m/z 179 (M^+- CF_3), 159, 143, 110, 83, 69
17)			95	17 -77.25, -81.21	17 -76.04, -80.25	MS m/z 193 (M^+- CF_3), 173, 145, 124, 69, 51
18)			94	18 -78.29, -78.61	18 -77.69, -78.85	MS m/z 335 (M^+- CF_3), 320, 266, 251. 209, 181
19)			89	19 -69.59, -75.87	19 -68.78, -74.86	MS m/z 504 (silylated), (M^+ - CF_3), 489, 399, 379, 327, 285
20)			98	20 -73.55	20 -75.66	MS m/z 175 (M^+- $C_8H_6F_3O$), 152, 105, 77, 51

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

Table 4. Fluoride Ion Induced Trifluoromethylation of Carbonyl Groups with Trifluoromethyltrimethylsilane

Entry	Precursor	Product	Overall % Yield	^{19}F NMR (ppm) $CDCl_3$	$CDCL_3$/Pyridine	GC-MS m/e
21)			95	-75.53	-76.03	Ms m/z (silylated) 402, (M^+), 302, 278, 248, 235, 221
22)			96	-77.63	-76.57	Ms m/z 372, (M^+), 303, 248, 235, 217, 189
23)			93	-75.48	-75.93	Ms m/z (silylated) 618, (M^+), 480, 451, 411, 365, 343

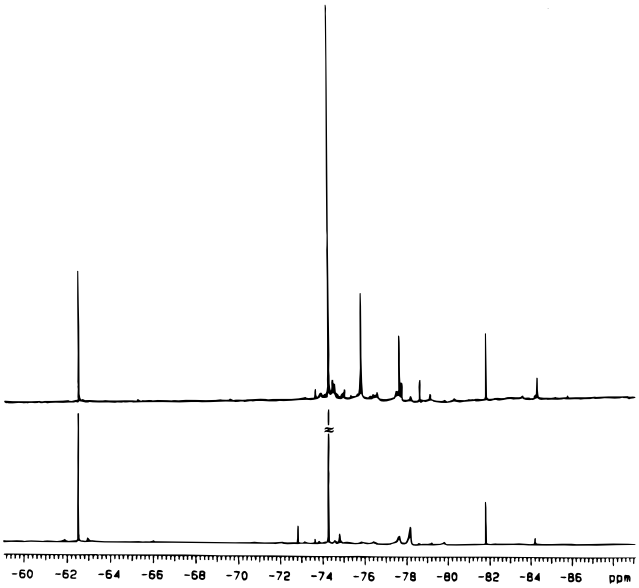


Figure 2. Quantitative ^{19}F NMR spectra of steam explosion yellow poplar (top) and black spruce milled wood (bottom) lignins.

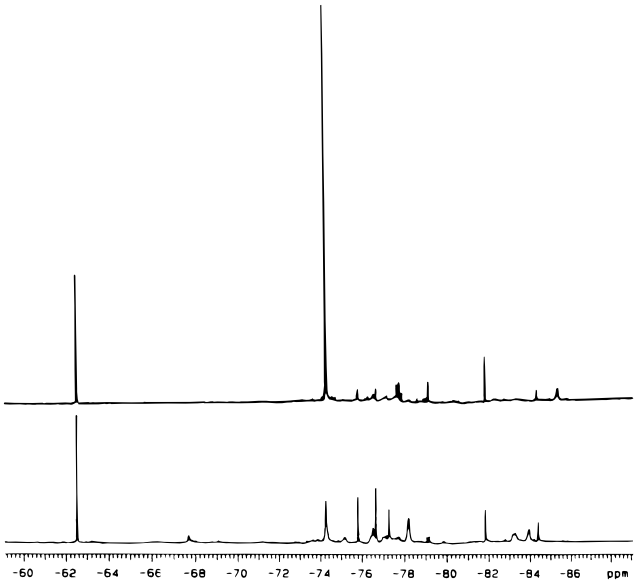


Figure 3. Quantitative ^{19}F NMR spectra of Sucrolin (top) and milled straw (bottom) lignins.

CF_3I is shielded by 0.149 ppm more for the $^{13}CF_3I$ isotopomer than in $^{12}CF_3I$ (Harris, 1983). This signal, however, was easily distinguished from the primary correlation because it was confined in the $-CF_3$ ^{13}C chemical shift region, and, in addition, it was slightly shifted (~0.1 ppm) from the parent ^{19}F 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 ^{19}F 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

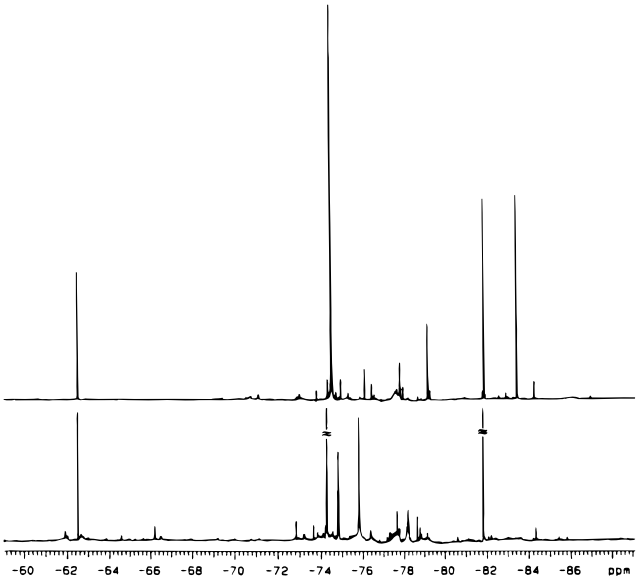


Figure 4. Quantitative ^{19}F NMR spectra of residual kraft (top) and Alcell organosolv (bottom) lignins.

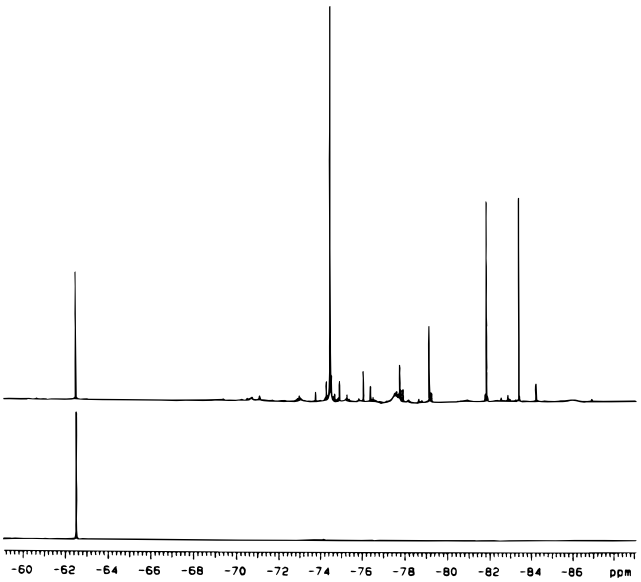


Figure 5. ^{19}F NMR spectra of trifluoromethylated kraft lignin before (top) and after (bottom) reduction of carbonyls by sodium borohydride.

Table 5. ^{13}Carbon NMR Chemical Shifts and Coupling Constants of Some Trifluoromethylated Model Compounds

	^{13}C NMR δ (ppm)		J_{C-F} (Hz)	
compound	CF_3	C−CF_3	^1J	^2J
dimer β-*O*-4 (**21**)	125.1	79.6	285	26
dimer β-*O*-4 (**22**)	125.1	79.8	285	27
dimer β-*O*-4 (**23**)	125.5	75.6	285	47
p-hydroxyacetophenone (**2**)	130.8	74.5	290	29
3,4′-dimethoxybenzaldehyde (**13**)	124.1	72.6	282	32
p-benzoquinone (**16**)	123.0	68.7	285	30

Pearl, 1965). α-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, α,β-unsaturated aldehydes react with alkaline hydrogen peroxide with the formation of the corresponding benzaldehydes and benzoic acids, whereas

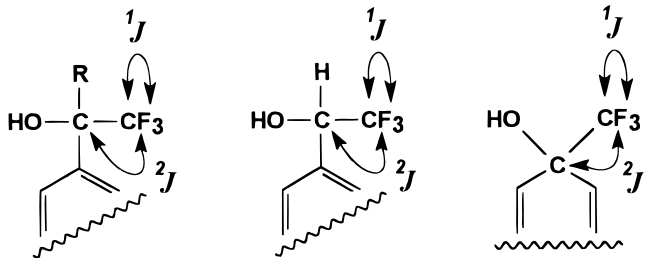


Figure 6. ^{19}F−^{13}C coupling constants for different classes of carbonyl groups.

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 α-carbonyls that bear free phenolic hydroxyl groups. The total concentration of etherified α-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 α-ketones would not be reduced by sodium hydrosulfite. More specifically, di-*tert*-butyl-*o*-quinone, *p*-quinone, acetovanillone, and syringaldehyde were reduced by sodium hydrosulfite. Both *o*- and *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}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}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 *o*- and *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 α-carbonyl-containing β-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 8). As such, this signal was assigned to be due exclusively to α-carbonyl groups of β-O-4 units bearing a free phenolic hydroxyl group *para* to the side chain.

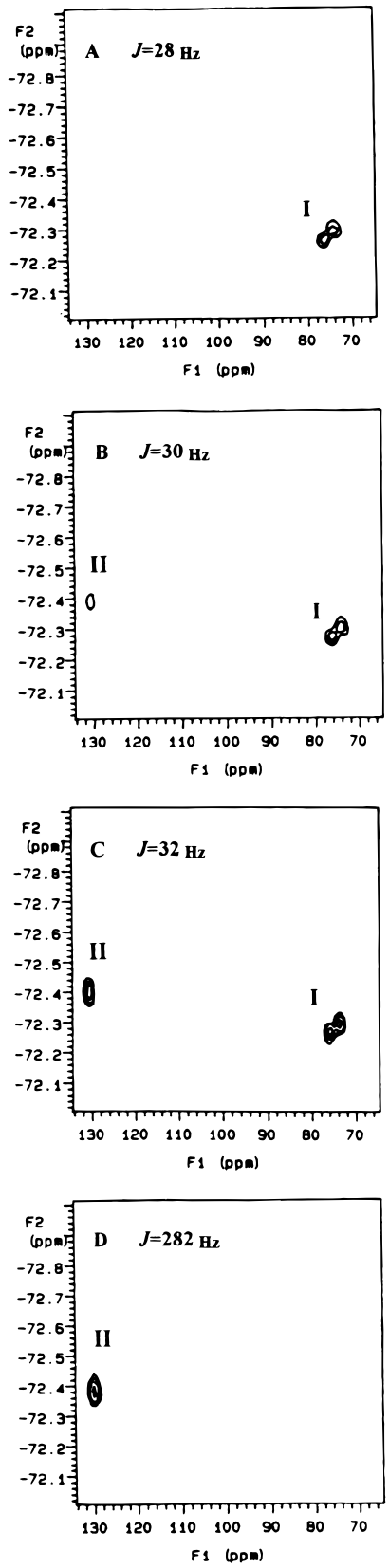


Figure 7. $^{19}F-^{13}C$ HMQC spectra of trifluoromethylated 3,4-dimethoxybenzaldehyde acquired by selecting different *J* values.

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 ^{19}F NMR signals of aldehydes, quinones, and also α-carbonyls could be all found in this region.

The last set of ^{19}F 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 CF_3 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 ^{19}F spin−lattice relaxation times for trifluoromethylated lignins and the internal standard. As anticipated, the longest T_1 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 ^{19}F 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 contens 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 ^{19}F 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 ^{19}F NMR technique as a novel and promising analytical tool for detecting and determining the

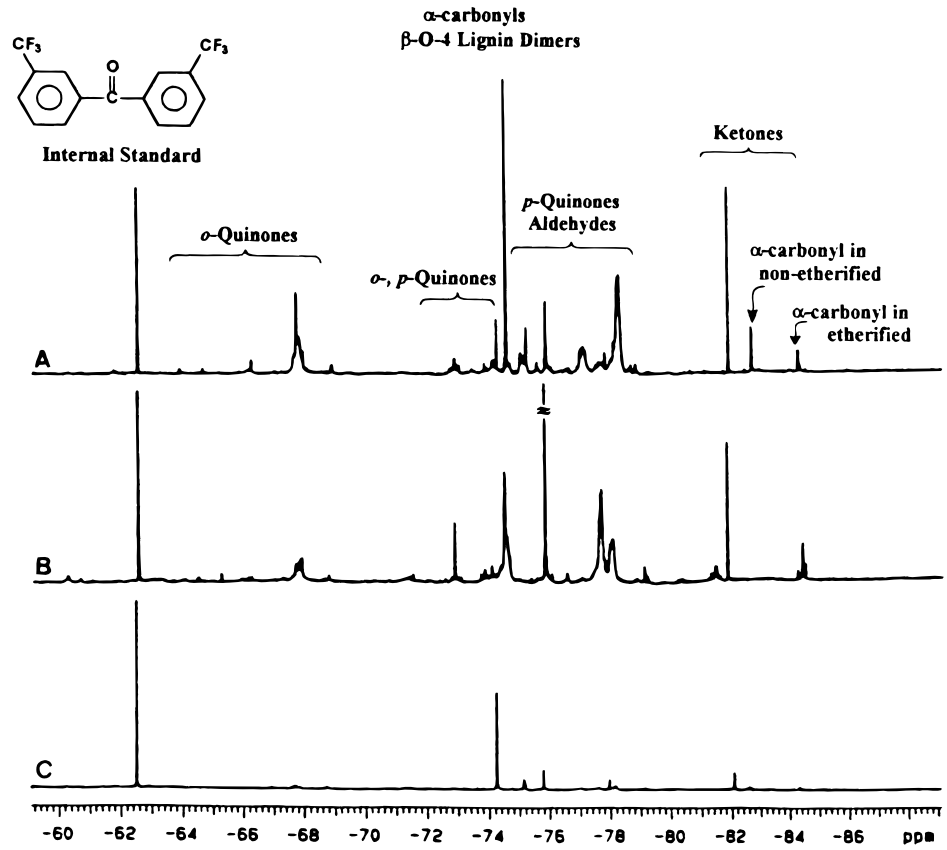


Figure 8. ^{19}F NMR spectra of trifluoromethylated dioxane lignin (A), after Dakin reaction (B) and reduction (C) with sodium hydrosulfite.

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

	CO/C9		wt %		
lignin sample	$\bar{x}$	s	$\bar{x}$	s	MW
Sucrolin acid hydrolysis (bagasse)	0.12 ± 0.01	0.0097	1.89 ± 0.2	0.15	177.4
Alcell organosolv (mixed hardwoods)	0.11 ± 0.01	0.0086	1.56 ± 0.2	0.13	178.5
steam explosion (yellow poplar)	0.13 ± 0.01	0.010	1.57 ± 0.2	0.18	194.8
dioxane acidolysis	0.15 ± 0.01	0.0095	2.27 ± 0.2	0.15	189.4
kraft residual[c]			2.90 ± 0.2		
straw	0.018 ± 0.005	0.0011	0.25 ± 0.05	0.012	201.1

[a] $\bar{x}$ = 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

	detection method				
lignin sample	^{19}F NMR	oximation	UV−vis[a]	MW	formula
Sucrolin acid hydrolysis (bagasse)	0.12 ± 0.01	0.12 ± 0.12	0.03 ± NR	177.4	$C_9H_{8.3}O_{2.2}(OCH_3)_{0.83}$
Alcell organosolv (mixed hardwoods)	0.11 ± 0.01	0.10 ± 0.0	0.11 ± NR	178.5	$C_9H_{7.7}O_{1.9}(OCH_3)_{1.04}$
steam explosion (yellow poplar)	0.13 ± 0.01	0.11 ± 0.04	0.09 ± NR	194.8	$C_9H_{7.8}O_{2.5}(OCH_3)_{1.25}$

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

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 ^{19}F 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 ^{19}F NMR technique as a new analytical tool for detecting and determining carbonyl groups in lignins.

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Received for review May 5, 1998. Revised manuscript received September 14, 1998. Accepted September 22, 1998.

JF980431P