The DFRC Method for Lignin Analysis. 2. Monomers from Isolated Lignins

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Monomers released from derivatization followed by reductive cleavage (DFRC degradation) of lignins have been identified by mass spectra and/or comparison of their GC retention times with authentic compounds. The primary monomers from several isolated lignins representing softwoods, hardwoods, grasses, and dicots have been quantified by GC. Sources of the minor monomeric components have been rationalized on the basis of the DFRC mechanisms; products from cinnamyl alcohol endgroups, aldehydes, arylglycerols, and α-carbonyl units have been identified. From isolated lignins, the DFRC method produces its diagnostic primary monomers, the hydroxycinnamyl peracetates, with yields comparable to the production of monomers from other ether-cleaving analytical methods.

INTRODUCTION

Although significant advancements have been made in lignin research, lignin remains one of the most poorly understood natural products (Harkin, 1973; Sakakibara, 1991). Since the most frequent interunit linkages in lignin are arylglycerol-β-aryl (β-O-4) ethers (Adler, 1977), an important research target is to find mild, selective, and efficient methods for β-O-4 ether cleavage (Makino et al., 1990; Meshitsuka et al., 1987; Fukagawa et al., 1992; Shevchenko et al., 1991; Lapierre et al., 1983, 1985, 1991; Lundquist, 1992; Nimz, 1969, 1974). If β-O-4 ether linkages are cleaved cleanly and completely, the characterization of the degradation products such as monomers, dimers, and trimers provides valuable structural information about the initial lignin. Such cleavage is crucial either for an efficient degradation of polymeric lignins during chemical pulping or for the analysis of the various linkages present in lignins.

In a previous paper (Lu and Ralph, 1997b) we showed, using lignin models, that the method recently developed exploiting derivatization followed by reductive cleavage (the DFRC method) is highly efficient for cleaving lignin α- and β-aryl ethers. Since the DFRC yields of monomers from β-ether lignin models are close to quantitative, the method is potentially useful for lignin characterization.

In this study, several isolated lignins representing guaiacyl and guaiacyl−syringyl type lignins from a range of plant samples were used for testing the DFRC method and for identifying a range of minor lignin-derived products. Although the DFRC method works very well for lignin models, its utility as a analytical method for lignins and lignocellulosics must be demonstrated. The significant differences between models and real lignins and the potential of interfering products require that the DFRC procedure be optimized for these more complex substrates.

EXPERIMENTAL PROCEDURES

Materials and Reagents. Acetyl bromide (AcBr), dioxane, acetic acid, and zinc dust were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as supplied. Commercial analytical reagent grade solvents were used without further purification. All commercially obtained compounds mentioned below were from Aldrich. Lignins were isolated from pine (*Pinus taeda*), willow (*Salix*), aspen (*Populus*), bamboo (*Bambusa*), bromegrass (*Bromus*), and kenaf (*Hibiscus cannabinus Tainung*) according to methods described previously (Björkman, 1954; Ralph et al., 1994, 1995; Ralph and Hatfield, 1991). Basically, the plant material was first ground in a Wiley mill (1 mm screen), and soluble phenolics, carbohydrates, and other components were removed by successive extractions with water, methanol, acetone, and chloroform. The ground wood was then ball milled, treated with crude cellulases to degrade most of the polysaccharides, and extracted with 96:4 dioxane/water. Saccharides and metal ions were removed from the lyophilized crude lignin using 5 mM EDTA (pH 8) (Ralph et al., 1994). The final yields of such lignins were 15−30% of the original content. Compositions (w/w) of Klason lignin, uronics, and saccharides were, respectively, as follows: pine lignin, 86%, nd, 4%; willow lignin, 54%, 1.5%, 15%; kenaf lignin, 68%, 2%, 5.5%; bamboo, 77%, 1%, 10.5%; bromegrass, 78%, nd, 13% (nd, not determined)

Acetylation. Acetylations of synthetic or commercially obtained compounds, as well as DFRC products, were accomplished using Ac_2O/pyridine (1:1); workup was by coevaporation with ethanol under reduced pressure.

Authentic Compounds. The *trans*-hydroxycinnamyl acetates P_t, G_t, and S_t were prepared from parent hydroxycinnamyl alcohols (Quideau and Ralph, 1992) by acetylation. Compounds **1−3**, **5**, **8−11**, **13**, and **16** were made by acetylation of commercially available parent phenols. Compound **4** was obtained from hydrogenation of compound **2** with Pd/C in ethanol. Compound **14** was from Friedel−Crafts acylation of guaiacol with propionic acid in polyphosphoric acid. Compound **22** was prepared by acetylation of 3,5-dimethoxy-4-hydroxybenzyl alcohol made from sodium borohydride reduction of the parent aldehyde in ethanol. Interesting new

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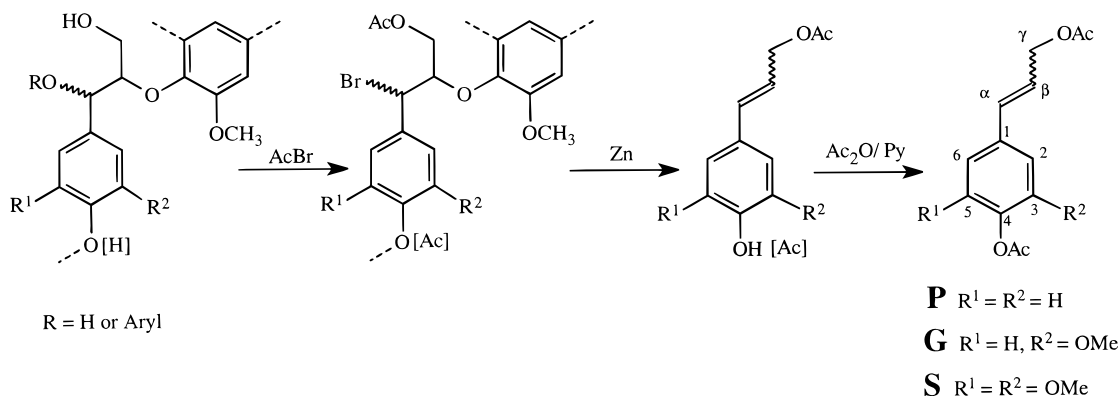
Figure 1. Selective ether cleavage in lignins by DFRC method, releasing diagnostic monomers **P**, **G**, and **S**.
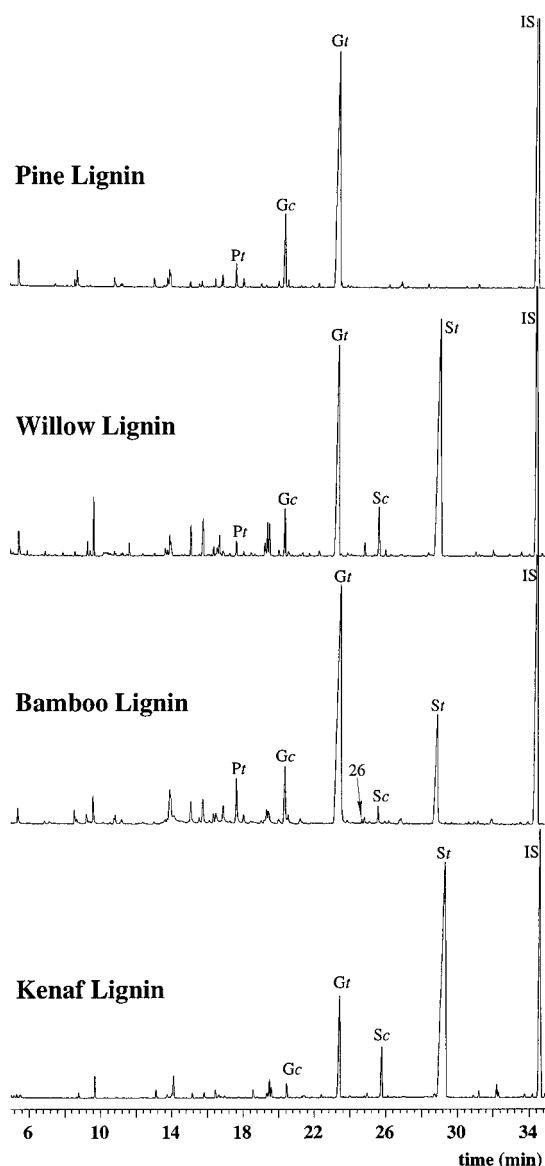

Figure 2. FID GCs of monomeric DFRC products from isolated lignins. *t = trans, c = cis.*

cyclopropanes (**7**, **15**, **17**, **24**, and **28**) have only just been identified. Their formal structural authentications will be detailed elsewhere. Other compounds were identified from their diagnostic mass spectral fragmentations.

DFRC Procedure. The DFRC method is essentially that described in part 1 of this series (Lu and Ralph, 1997b) and in the initial protocol (Lu and Ralph, 1997a).

AcBr Derivatization and Lignin Solubilization. Isolated lignins (5–10 mg) in AcBr stock solution (AcBr/acetic acid 8:92 v/v, 2.5 mL) were stirred at room temperature (~23 °C) overnight. Alternatively they were stirred at 50 °C for 1 h. During this time, complete solubilization was achieved. The AcBr and solvents were removed by rotary evaporation at 40 °C.

Reductive Cleavage. Reductive cleavage of β-aryl ethers was by dissolving the above product in dioxane/acetic acid/water (5:4:1) and adding zinc powder (50 mg), as described more fully in part 1 (Lu and Ralph, 1997b). Internal standard (tetracosane, 0.5 mg) was added before extraction of the products.

Acetylation. The residue was acetylated in dichloromethane (1.5 mL) containing Ac₂O (0.2 mL) and pyridine (0.2 mL) for 40 min followed by standard acetylation workup. The product was dissolved in 0.2 mL of methylene chloride and used for GC and GC/MS characterization.

GC and GC/MS. The degraded monomers from lignins were quantitatively determined by GC (Hewlett-Packard 5980, Atlanta, GA) using a 0.20 mm × 30 m (0.2 μm film thickness) SPB-5 (Supelco, Bellefonte, PA) column and a flame ionization detector with He as carrier gas (10 cm³ min⁻¹). GC conditions: initial column temperature, 140 °C, hold for 1 min, ramp at 3 °C/min to 240 °C, hold for 0.5 min, ramp at 30 °C/min to 310 °C, hold for 10 min; injector, 250 °C; FID detector, 300 °C; injection volume, ~2 μL.

The amounts of individual monomers (**P**, **G**, and **S**) were calculated using response factors (1.76, 1.85, and 2.06) determined by running mixtures of those pure monomers and tetracosane at the same GC conditions as used for lignin samples. A standard mixture with internal standard is recommended for determining response factors on researchers' instrumentation; such a standard is available from the authors.

EI-MS data were collected on a Hewlett-Packard 5970 mass selective detector with the same type of column using the same temperature program described above.

Thioacidolysis. Thioacidolyses were performed according to the published procedure (Rolando et al., 1992; Lapierre et al., 1986); optimization studies were not carried out. Response factors (1.5 for the G and S products) were used without authentication on our setup.

RESULTS AND DISCUSSION

The DFRC degradation method includes two key steps: (1) solubilization of lignin by bromination and acetylation with AcBr and (2) reductive cleavage with zinc dust (Lu and Ralph, 1998). Final acetylation is used to reduce the number of compounds to be quantitated because the etherified β-ether units give 4-hydroxycinnamyl acetates, whereas phenolic ones form 4-acetoxycinnamyl acetates (Figure 1). This distinction can be valuable to determine etherified vs free phenolic units in lignins but requires special care as some acetylation of phenols can occur in the reductive elimi-

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Table 1. Structures and Mass Spectral Data of DFRC Monomers from Isolated Lignins

GC peak[a]	RRT	structure assignment[b]	molecular ion and prominent fragments, *m/z* (rel intensity)
1	0.1594	G−CH₃	180 (8), 138 (91), 123 (100), 95 (27), 77 (31)
2	0.2506	G−CH₂CH=CH₂	206 (9), 164 (100), 149 (26), 133 (24), 131 (23), 103 (15), 91 (25)
3	0.2550	G−CHO	194 (6), 152 (100), 151 (96), 123 (10), 109 (19), 79 (17)
4	0.2709	G−CH₂CH₂CH₃	208 (3), 166 (35), 137 (100), 105 (5), 94 (8), 77 (16)
5	0.2749	P−CH₂OAc	208 (17), 166 (100), 124 (77), 107 (100), 106 (80), 95 (26), 84 (36)
6	0.2808	S−CH₃	210 (6), 168 (100), 153 (33), 125 (12), 107 (10), 79 (8)
7	0.3145	G−cPr	206 (11), 164 (100), 149 (23), 133 (16), 131 (16), 103 (14), 91 (19)
8	0.3165	G−CH=CHCH₃	206 (10), 164 (100), 149 (31), 133 (19), 131 (18), 103 (22), 91 (40), 77 (35)
9	0.3275	G−CO−CH₃	208 (5), 166 (42), 151 (100), 123 (14), 79 (16)
10	0.3795	G−CH₂CO−CH₃	222 (5), 180 (28), 137 (100), 122 (6), 94 (8), 84 (16)
11	0.3975	S−CH₂CH=CH₂	236 (5), 194 (100), 179 (11), 163 (8), 147 (8), 131 (12), 119 (13), 91 (14)
12	0.4007	G−CO−CH=CH₂	220 (5), 178 (70), 151 (100), 123 (12), 108 (13), 79 (26)
13	0.4045	G−CH₂OAc	238 (6), 196 (100), 154 (65), 137 (82), 122 (17), 107 (10), 93 (14)
14	0.4060	G−CO−CH₂CH₃	222 (4), 180 (26), 151 (100), 123 (10), 108 (5), 79 (6)
15	0.4756	S−cPr	236 (14), 194 (100), 179 (19), 163 (35), 151 (13), 131 (16), 120 (25), 91 (22)
16	0.4814	S−CO−CH₃	238 (5), 196 (62), 181 (100), 151 (5), 137 (14), 119 (12), 79 (26)
P*c*	0.4524	P−CH=CHCH₂OAc	234 (19), 192 (100), 149 (85), 133 (54), 121 (44), 107 (28), 94 (29), 77 (24)
P*t*	0.5125		
17	0.5244	G−cPr−OAc	264 (8), 222 (48), 179 (100), 162 (22), 151 (46), 137 (12), 119 (56), 107 (8), 91 (37)
18	0.5366	S−CH₂CO−CH₃	252 (3), 210 (36), 181 (100), 153 (10), 138 (6), 109 (5)
19	0.5534	G−CH₂CH₂CH₂Br	288 (3), 286 (3), 246 (26), 244 (28), 137 (100), 122 (5), 107 (6)
20	0.5589	S−CO−CH=CH₂	250 (3), 208 (100), 181 (95), 165 (26), 153 (14), 133 (30)
21	0.5630	S−CO−CH₂CH₃	252 (3), 210 (36), 181 (100), 153 (10), 138 (6), 109 (5)
22	0.5665	S−CH₂OAc	268 (5), 226 (100), 184 (67), 167 (66), 152 (6), 123 (26)
23	0.5816	G−CHOAc−CO−CH₃	280 (4), 236 (8), 195 (27), 153 (100), 125 (8), 107 (4), 93 (12), 65 (8)
G*c*	0.5925	G−CH=CHCH₂OAc	264 (11), 222 (100), 179 (37), 163 (9), 151 (12), 131 (27), 119 (15), 91 (14)
G*t*	0.6797		
24	0.5970	G−cPr−OAc	264 (8), 222 (48), 179 (100), 162 (22), 151 (46), 137 (12), 119 (56), 107 (8), 91 (37)
25	0.6992	G−CO−CH₂CH₂Br	302 (4), 300 (4), 260 (43), 258 (40), 179 (6), 151 (100), 123 (10), 92 (5)
26	0.7165	S−CH₂CH₂CH₂Br	316 (6), 374 (60), 194 (5), 167 (100), 151 (11), 137 (6), 107 (6)
27	0.7208	S−CHOAc−COCH₃	310 (4), 268 (14), 225 (26), 183 (100), 155 (12), 123 (14), 95 (7)
28	0.7546	S−cPr−OAc	294 (8), 252 (41), 209 (100), 192 (28), 181 (40), 149 (58), 137 (12), 121 (20), 103 (15)
29	0.7677	G−CHOAcCH₂CH₂Br	346 (5), 302 (36), 245 (7), 224 (3), 195 (12), 153 (100), 131 (16), 91 (9)
30	0.7785	G−CHOAcCH₂CH₂OAc	324 (6), 282 (56), 222 (23), 179 (54), 163 (20), 153 (100), 131 (36), 107 (12), 93 (22), 73 (17)
31	0.7816	G′−CH=CHCH₂OAc	292 (10), 250 (21), 208 (72), 165 (21), 148 (100), 137 (19), 120 (22), 103 (12), 91 (20)
S*c*	0.7445	S−CH=CHCH₂OAc	294 (8), 252 (100), 209 (24), 193 (10), 181 (8), 161 (17), 149 (13), 133 (6)
S*t*	0.7578		
32	0.8516	G−CO−CHOAcCH₂OAc	338 (4), 296 (24), 210 (20), 193 (4), 178 (6), 151 (100), 137 (3), 123 (7), 93 (4)
33	0.8843	G−CHOAc−CHOAc−CH₂OAc	382 (6), 340 (10), 322 (3), 280 (52), 238 (8), 195 (18), 178 (56), 153 (100), 137 (6), 103 (7), 93 (9)
34	0.8887	S−CHOAcCH₂CH₂Br	376 (3), 332 (46), 330 (46), 292 (11), 290 (10), 273 (8), 183 (100), 167 (8), 155 (20), 123 (13), 91 (10)
35	0.9002	S−CHOAc−CH₂CH2OAc	354 (22), 312 (100), 252 (18), 209 (54), 193 (24), 183 (57), 161 (26), 133 (24), 103 (30), 87 (67)
36	0.9043	G−CHOAc−CHOAc−CH₂OAc	382 (6), 340 (10), 322 (3), 280 (52), 238 (8), 195 (18), 178 (56), 153 (100), 137 (6), 103 (7), 93 (9)
37	0.9291	G″−CH=CHCH₂OAc	322 (6), 280 (63), 238 (100), 195 (35), 178 (44), 167 (20), 147 (25), 135 (22), 118 (19)
38	0.9698	S−CO−CHOAcCH₂OAc	368 (5), 326 (20), 267 (6), 223 (6), 207 (12), 181 (100), 149 (8), 121 (12)
39	0.9869	S−CHOAc−CHOAc−CH₂OAc	412 (6), 370 (20), 327 (9), 253 (10), 208 (52), 183 (100), 167 (12)
40	1.0147	S−CHOAc−CHOAc−CH₂OAc	412 (6), 370 (15), 327 (8), 253 (10), 208 (50), 183 (100), 167 (12)

[a] See Figures 2 and 3. [b] cPr, cyclopropyl.

P   R¹ = R² = H
G   R¹ = OMe, R² = H
S   R¹ = R² = OMe

G′   R= H
G″   R= OMe
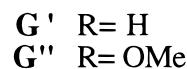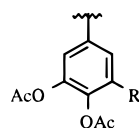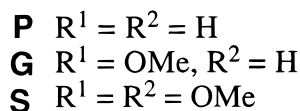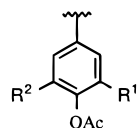
nation step. Procedures to allow this distinction are still under investigation. Therefore, the primary monomers derived from DFRC degradation of various softwood, hardwood, dicot, and grass lignins are essentially 4-acetoxycinnamyl acetate (*p*-coumaryl peracetate, **P**), 4-acetoxy-3-methoxycinnamyl acetate (coniferyl peracetate, **G**), and 4-acetoxy-3,5-dimethoxycinnamyl acetate (sinapyl peracetate, **S**) (Figures 1 and 2); trans-isomers predominate.

Identification of DFRC Monomers. The gas chromatograms of degradation monomers from several isolated lignins subjected to the DFRC method are shown in Figure 2. Table 1 lists the mass spectral data of DFRC monomeric products and their assignments. Peaks in those chromatograms were identified by comparison of their retention times with authentic compounds and/or from their mass spectra. From Figure 2, gas chromatograms of the monomers obtained from the DFRC method are simpler than the chromatograms

obtained from acidolysis or thioacidolysis (Lapierre et al., 1985; Rolando et al., 1992); although each method produces the primary monomers as isomeric mixtures, products from competing side reactions (such as elimination of hydroxymethyl groups) appear to be less significant in DFRC chromatograms.

In addition to the major **P**, **G**, and **S** monomers that arise from β-ether units, many minor monomeric components originating from other structures were also found in DFRC products as shown in Figure 3, expanded FID chromatograms from pine and willow lignins. Basically those minor peaks can be divided into three groups according to their sources. Regrettably, these minor units in lignin do not react cleanly to give single products as do normal β-ether units. Quantitating such units will therefore be more difficult, as it is with other methods (Rolando et al., 1992). Qualitative comparisons remain valuable.
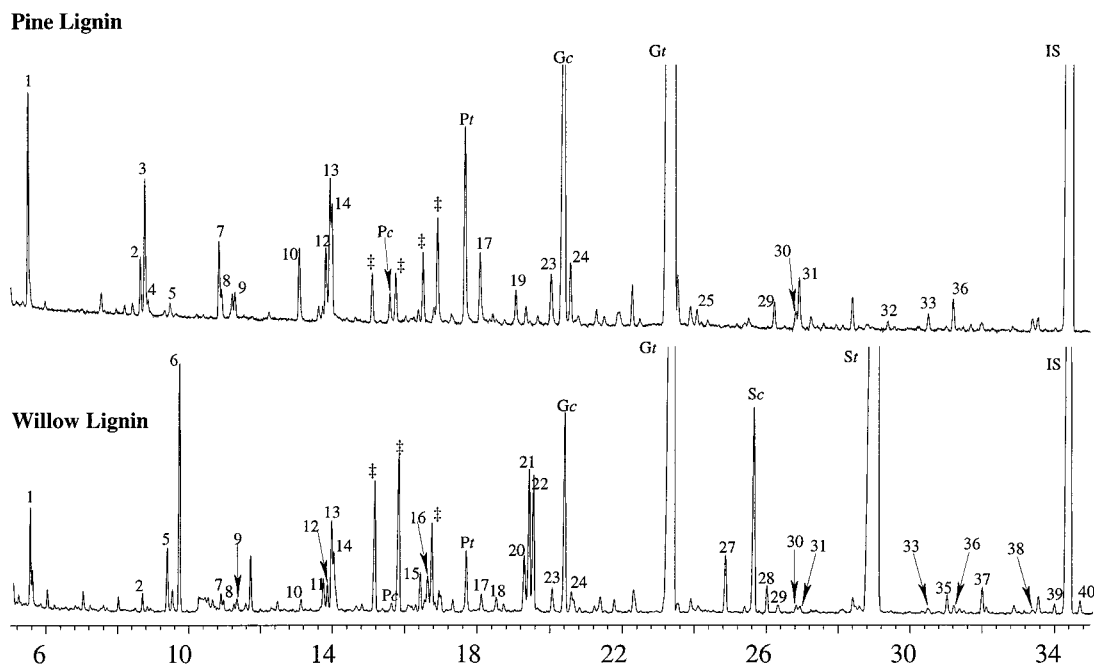
Pine Lignin

Figure 3. Vertically expanded FID GCs of monomeric products from pine and willow lignins, showing minor identifiable peaks (Table 1). ‡ = carbohydrate-derived peaks.

End Groups. Peaks 1, 3, 5, 6, 13, and 22, which possess C_6-C_1 skeletons, are from *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde endgroups connected by 4-O-β ether linkages. Subjecting vanillin and syringaldehyde to the DFRC procedure resulted in four classes of compounds: benzaldehydes, toluenes, benzyl acetates, and benzyl diacetates. 4-Acetoxybenzaldehydes (vanillin acetate **3**) result from acetylation of released 4-hydroxybenzaldehydes that survived the Zn step, whereas 4-acetoxytoluenes (**1** and **6**) are from benzaldehydes that were reduced by Zn or from benzyl alcohols (e.g. vanillyl alcohol) that brominate cleanly with AcBr and produce toluenes in the Zn step. 4-Acetoxybenzyl acetates (**5**, **13**, and **22**) result from benzaldehydes. As for 4-acetoxybenzyl diacetates, their formation may be through nucleophilic substitution by acetate anion of free phenolic benzylic α-bromoacetates formed during AcBr treatment of hydroxybenzaldehydes; they have not been found in DFRC products of lignins (where such units are etherified).

In addition to primary pairs of isomers (**P**, **G**, and **S**), several other pairs of isomers were identified in chromatograms from their mass spectral data (Table 1). Peaks 2, 7, and 8 have the same mass spectral patterns, suggesting that they are isomers. Compounds **2** and **8** are confirmed as eugenol and isoeugenol acetates, respectively, by comparison of GC retention times and mass spectra with the authentic compounds. Compound **7** has now been identified as guaiacylcyclopropane; model studies suggest that it comes from coniferyl alcohol endgroups. Compound **17** is an analogous aryl cyclopropyl acetate, which model studies show comes from coniferaldehyde endgroups. When coniferaldehyde was subjected to the DFRC procedure, the major products were 1-aryl-2-acetoxycyclopropanes **17** and **24**, isomers based on their mass spectra. Full authentication of the cyclopropanoid peaks (7, 15, 17, 24, and 28) will be forthcoming. Peaks 19 and 29, corresponding to bromo derivatives, and acetate **30** were also found in DFRC products of coniferyl alcohol. It is unclear at this point whether these minor bromides would eventually

become reduced in a more exhaustive reductive step. In the same way, peaks 11, 15, 26, 34. and 35 are presumably from sinapyl alcohol endgroups. Peak 28 is one isomer from sinapaldehyde DFRC products; the other isomer did not show up in chromatograms of lignin DFRC products as it has a retention time very close to that of $\mathbf{G}_t$—it can be found in selective ion spectra. Mechanisms for formation of these products resulting from hydroxycinnamyl alcohol or aldehyde endgroups and their confirmed identities will be reported separately.

α-*Carbonyl Units.* Keto units in lignin also cleave by more than a single pathway, as they do in thioacidolysis (Rolando et al., 1992). We have shown in a previous paper (Lu and Ralph, 1997b) that β-O-4 ether models possessing an α-carbonyl group are partially cleaved (~60%) under standard DFRC conditions, resulting in propiophenones (e.g. **14**, **21**), 1-acetoxy-1-phenylpropan-2-ones (e.g. **23**, **27**), and phenylacetones (e.g. **10**, **18**); such α-carbonyl models can be almost fully cleaved to just the former two products using a longer zinc treatment step (Lu and Ralph, 1997b). Thus, peaks 10, 14, and 23 come from guaiacyl α-CO β-ether units in lignin, whereas peaks 18, 21, and 27 are derived from the corresponding syringyl α-CO β-ethers. As noted in part 1 (Lu and Ralph, 1997b), examination of keto compounds is best done after an extended Zn treatment. Peak 4, guaiacylpropane, is a minor compound from α-CO β-ether units. Peaks 12 and 20 could be assigned to phenyl vinyl ketones, which together with peak 25 may also come from α-CO β-ether units. Peaks 9 and 16 are 4'-acetoxy-3'-methoxyacetophenone and 4'-acetoxy-3',5'-dimethoxyacetophenone, respectively, which have C_2 side chains. According to model studies and from the mechanism, the DFRC method should not artificially produce C_2 side-chain products. Although hydroxyacetophenones or their derivatives have been found in plants, whether peaks 9 and 16 belong to lignin or derive from residual extractives is still unclear at this time.

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Table 2. Yields of the Main P, G, and S Monomers Recovered from DFRC Degradation (Room Temperature) and Thioacidolysis of Various Isolated Lignins

lignin sample	DFRC yield[a] (% wt)	molar yield (μmol/g)		rel distribution[b] P:G:S
		DFRC	thioacid-olysis	
pine	17.13	651		0.03:1:−
willow	20.58	732	597	0.03:1:1.39 (−:1:1.54)
aspen	19.62	696		0.02:1:1.64
kenaf	36.43	1258	967	tr[c]:1:5.70 (−:1:5.08)
bamboo	21.18	781		0.11:1:0.44
bromegrass	22.04	800		0.04:1:0.72

[a] Yields are based on sample weights and are not corrected for lignin content. [b] Values in brackets are from thioacidolysis. [c] tr, trace.

Table 3. Yields (Weight Percent of Sample) of DFRC Main Monomers from Isolated Kenaf Lignin

	run no.[a]							
	1	2	3	4	5	6	7	av value
guaiacyl	4.91 4.87	4.97	4.91	4.92	4.95	5.00 4.93	5.02	4.95 ± 0.07
syringyl	31.47 31.28	31.19	30.93	32.07	31.87	32.00 31.27	32.15	31.67 ± 0.5

[a] Runs 1−3 and 4−7 were performed with the same lignin sample, but on two different days. Duplicate values for runs 1 and 6 correspond to results of duplicate GC injections of the sample.

Other Structures. Peaks 31 and 37, 3,4-diacetoxycinnamyl acetate and 5-acetoxyconiferyl acetate, respectively, were found in minor amounts compared to monomers **G** or **S** (≤1%). Although only trace quantities of demethylation products were detectable in model studies, we suspect that they do arise from minor demethylation reactions rather than from minor units in lignins, although such units can be found in some materials (Lapierre et al., 1988). Peaks 33 and 36 are assigned to two isomers of guaiacylglycerol peracetates, and peaks 39 and 40 are two isomers of syringylglycerol peracetates. They presumably come from glycerol endgroups connected by 4-O-β ether linkages. Glycerol units have been found in several lignins (Ede and Ralph, 1996; Kilpeläinen et al., 1994; Lai and Sarkanen, 1971). Peaks 32 and 38 can be assigned to 1,2-diacetoxypropiophenones. At the present time we do not know their origin.

Quantitation of the Primary Degradation Monomers P, G, and S. Since constituent components and interunit linkages are key elements for understanding polymer structure, a selective and efficient degradation method is essential to provide such reliable information. Thioacidolysis is probably the most structurally revealing method with wide application. To test the DFRC method, several isolated lignins representing guaiacyl and guaiacyl−syringyl lignins were subjected to the DFRC procedure. The results are listed in Table 2. It is observed that pine (softwood) lignin is more condensed than willow or aspen (hardwood) lignins. In other words, more monomers are released from willow or aspen lignin than from pine lignin. Kenaf lignin has an extremely high S/G ratio and a correspondingly high β-O-4 ether content (Ralph, 1996), which results in very high yields of monomers from DFRC analysis. For comparative purposes, our thioacidolysis yields from kenaf and willow lignins are also listed in Table 2. It appears that the monomer yield is higher from DFRC than from thioacidolysis, and this is in accord with the results from our previous model study (Lu and Ralph, 1997b). However, we caution that we did not carefully

optimize thioacidolysis and do not claim that our yields, obtained according to standard published procedures (Lapierre et al., 1986; Rolando et al., 1992), are optimal. Values in the literature for similar materials are higher but comparisons are valid only on identical samples. Without performing extensive comparisons, it remains clear that comparable yields are to be expected from the DRFC method.

For evaluation of the reproducibility of the DFRC method, a series of experiments were done with kenaf lignin sample on different days. As seen in Table 3, the results were as good as with any other analytical methods based on GC.

This work has shown that the DFRC method works well with isolated lignins. Since it is well-known that AcBr in acetic acid can fully solubilize lignocellulosics (Johnson et al., 1961; Dence, 1992; Morrison, 1972; Bagby et al., 1973; Iiyama and Wallis, 1988; Martin, 1967), it is reasonable to expect that DFRC will be suitable for lignocellulosic materials. We are working on optimizing or improving the DFRC conditions to make the method applicable to whole cell wall materials.

Conclusions. The DFRC method has been applied to isolated lignins representing softwood, hardwood, grass, and dicot lignins. Most of the monomers released have been identified and their sources rationalized. We have demonstrated that the DFRC procedure is a useful analysis method which can provide structural information similar to thioacidolysis. The method also forms the basis for more wide-ranging analyses that will be the topics of future papers. The DFRC method is reproducible, at least for isolated lignins, and is potentially suitable for lignocellulosic materials (in situ lignins). It is expected that the DFRC method will find application in fields related to lignin due to its simplicity, high selectivity, and use of relatively innocuous reagents.

LITERATURE CITED

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