^{13}C Nuclear Magnetic Resonance of Grass Lignins

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'Coastal' bermudagrass and Kentucky 31' tall fescue grass cut at 4 weeks of age and extracted with neutral solvents yield a milled lignin that is high in carbohydrate content. Further treatment of the residue with a mixture of dioxane, dry HCl, and 2,2-dimethoxypropane yields an acetal lignin. ^{13}C NMR spectra of acetal lignins of these grasses indicate they are relatively free from carbohydrate but still contain a carbohydrate component. This carbohydrate component appears to be hemicellulose that may be glycosidically linked to *p*-coumaryl and coniferyl esters that may be incorporated into the lignin polymer. The ^{13}C NMR spectra also indicate that warm-season grass lignins contain more *p*-coumaryl units than cool-season grass lignins at the same stage of maturity.

Lignin is considered to play a very essential role in the relative utility of grasses as forages for ruminant animals (Harkin, 1973). Although the amount of lignin in immature grasses appears to change very little among species, there exist significant differences in in vitro digestibilities between warm- and cool-season grasses (Barton, 1976, and references contained therein). In is highly likely that both the amount and the structural type of lignin influence this digestibility (Barton and Akin, 1977; Akin, 1979).

Because of the very complex nature of lignin, the study of its structure to determine how it may influence any chemical or biochemical process has been difficult. It is so difficult that no structural formulas have yet been proposed for the lignins of grasses. Formulas for hard and softwood lignin fragments have, however, been proposed (Nimz, 1974; Sarkanen and Ludwig, 1971). Lignins from these sources are considered to differ mainly in the relative amounts of the three alcoholic lignin precursors (*p*-coumaryl, coniferyl, and sinapyl alcohols). Some grass lignins are thought to contain mainly *p*-coumaryl units but other grass lignins appear to approximate the hardwood lignins, which contain primarily coniferyl and sinapyl units (Harkin, 1973).

The difficulty of isolation of relatively pure lignin from grasses has made progress in obtaining structural information on grass lignins slower than progress on wood lignins. The Björkman procedure (Björkman, 1956, 1957) has been extensively employed in the isolation of a milled wood lignin from plant tissue. This procedure has been successful in the isolation of relatively pure wood lignins. However when it is applied to other plant material having a lower lignin content, the results have not been as successful (Harkin, 1973). We have adopted an alternate procedure to isolate lignin relatively free of carbohydrate from immature warm- and cool-season grasses.

Since ^{13}C NMR has been shown to be a valuable tool for the classification of lignin (Lüdemann and Nimz, 1973, 1974a,b; Nimz, 1974; Nimz et al., 1974a,b, 1975; Nimz and Lüdemann, 1974, 1976), we have employed it to evaluate the structural characteristics of immature grass lignins and their associated carbohydrates.

MATERIALS AND METHODS

Isolation. 'Coastal' bermudagrass (*Cynodon dactylon* (L.) Pers.) and Kentucky 31' tall fescue (*Festuca arundinacea* Schreb.) grasses were cut at 4 weeks of age, fresh frozen, then freeze-dried, and ground in a Wiley mill to 20 mesh. Prior to lignin extraction 25-g samples of the ground grasses were preextracted with a benzene/ethanol (2.5:1 v/v) mixture, overnight, using a Soxhlet extractor. The residues were then treated with a 1% pepsin–0.1 N HCl solution according to the procedure of Routley and Sullivan (1958) for 48 h. These residues were then washed with *1* L of boiling water and *1* L of acetone and dried in a vacuum oven. The dried samples were suspended in toluene and ground to a 15-μm average size at O °C by using a Virtis Homogenizer. The toluene was decanted off, and the samples dispersed in a convenient amount of dioxane, and evaporated to dryness under vacuum at 35 °C.

Soxhlet extaction for 36 h with dioxane water (9:1 v/v) yielded a milled lignin, after precipitation of the lignin–carbohydrate complex with benzene and freeze-drying the resultant solution. The residue, in the Soxhlet cups, was then treated under nitrogen with a 0.2 N (dry) HCl in dioxane/2,2-dimethoxypropane solution (6.5:1 v/v) according to the procedure of Bolker and Terashima (1966), yielding an acetal lignin.

^{13}C NMR Spectroscopy. The milled and acetal lignins were dissolved to the extent of 15% w/v in hexadeuteriodimethyl sulfoxide (Me_2SO-d_6) and ^{12}C-enriched hexadeuteriodimethyl sulfoxide (^{12}C-Me_2SO-d_6), respectively, with 1% tetramethylsilane (Me_4Si) added as the internal reference, and placed in a Wilmad small volume (435-μL) NMR sample tube. The ^{13}C NMR spectra of the lignins were obtained by using a JEOL PS/PFT 100 NMR spectrometer integrated to a Nicolet 1083 computer system equipped with a Diablo Model 31 disk drive. The spectra were observed at 25.2 MHz with a deuterium lock set at 15.4 MHz and with complete proton decoupling at 100.0 MHz. A pulse repetition time of 1.0 s with a 90° pulse angle (17 μs in width) and a 5-kHz spectral window were used to accumulate 100 000 spectra scans in 8K of computer core. JEOL's 0–180° phase shifter, together with Nicolet's systematic noise reduction, was used to improve the signal to noise ratio of the spectra by subtracting the spectral noise resulting from out-of-phase signal addition from the results of the in-phase signal addition. An automatic data reduction feature was employed to preclude dynamic range override. Prior to Fourier transformation the free induction decay signal was smoothed by an exponential function creating a 2.3-Hz peak broadening but also improving the signal to noise ratio. Pulse feed-through that was observed in the transformed spectra was subtracted out utilizing a computer substraction routine and the "spectrum" of a D_2O sample.

The ^{12}C-Me_2SO-d_6 was obtained from Merck and certified as containing approximately 99.9% ^{12}C and 98% ^{2}H.

RESULTS AND DISCUSSION

Figure 1 shows the ^{13}C NMR spectrum of the milled lignin from a 'Coastal' bermudagrass sample. The spectral

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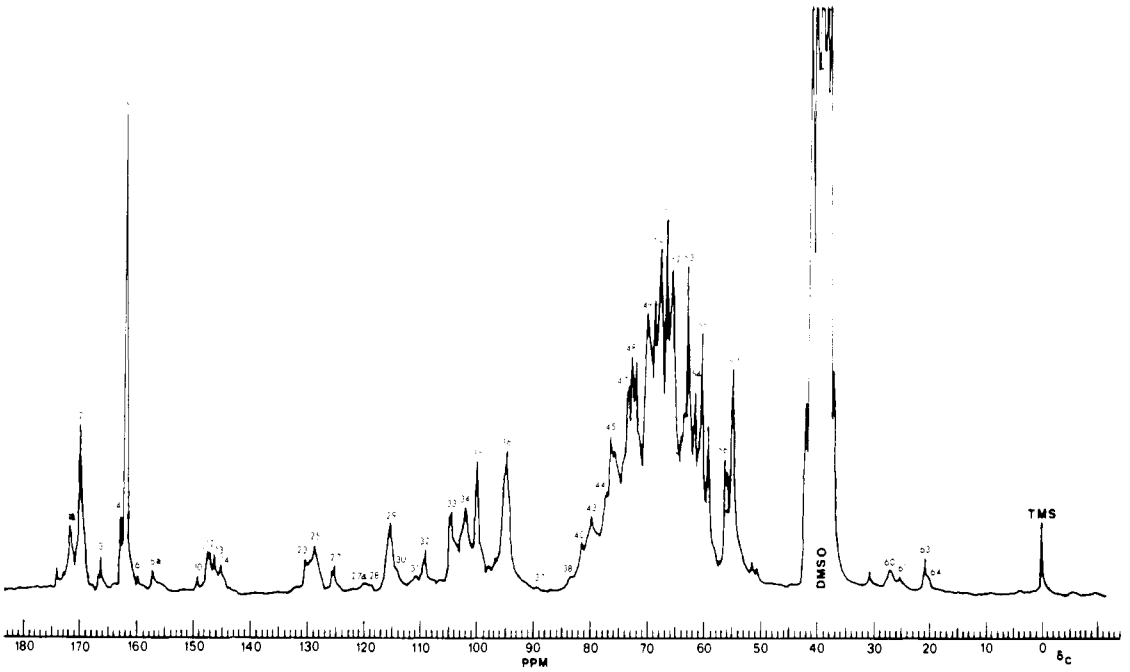

Figure 1. ^{13}C NMR spectrum of milled lignin from 'Coastal' bermudagrass. Signal numbers correspond to those in Table I for CBG and KY-31 acetal lignins.

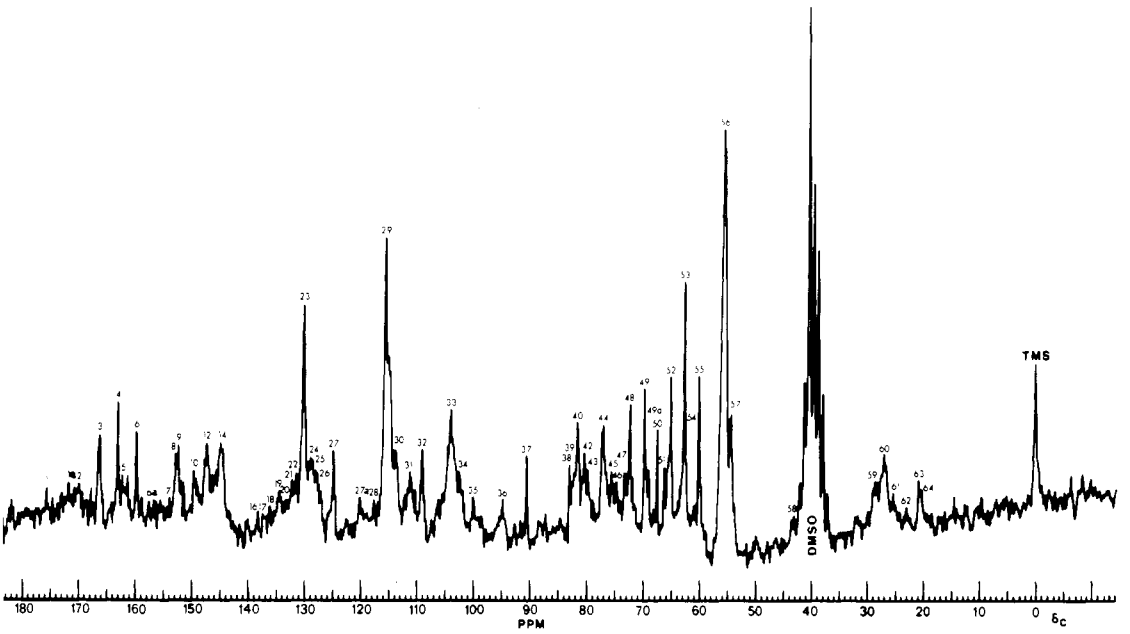

Figure 2. ^{13}C NMR spectrum of acetal lignin from immature 'Coastal' bermudagrass (see Table I for assignments).

region between 57 and 109 ppm indicates a large amount of carbohydrate was present in the milled lignin samples. This predominance of carbohydrate inhibits the analysis for lignin in most of the aliphatic and part of the aromatic region of the spectrum. The region from 160 to 175 ppm shows two rather large resonances at 162 and 170 ppm that, because of their intensities, are probably not due to lignin. The presence of these peaks makes any assignment of resonances to lignin, in this region of the spectrum, very tenuous. Only the region from 109 to 160 ppm is amenable to assignment as lignin. Since it was the intent of our study to classify the lignin structures, we choose not to use the spectra of the milled lignins of these grasses for that purpose. However, we have, after assigning signal numbers in Figures 2 and 3, assigned the same numbers to the signals at the corresponding chemical shift in Figure 1. This showed that the milled lignin contained more of the hemicellulosic type of carbohydrates than the acetal lignins.

Figures 2 and 3 show the ^{13}C NMR spectra of the acetal lignins isolated from 'Coastal' bermudagrass (CBG) and Kentucky 31' tall fescue (Ky-31). There is a very noticeable reduction of the amount of carbohydrate in these spectra when compared to the NMR spectra of the milled lignins of these grasses. This result is supportive of Bolker and Terashima's concept of a transacetalation mechanism being operative in this isolation procedure (Bolker and Terashima, 1966). The signals in the ^{13}C NMR spectra of these acetal lignins were assigned (see Table I) primarily in accordance with those of Nimz (Lüdemann and Nimz, 1973, 1974a,b; Nimz, 1974; Nimz et al., 1974a,b; Nimz and Lüdemann, 1976) for hard- and softwood lignins, dehydrogenation polymers, and model mono- di- and trilignols. In addition we have incorporated the results of confirming

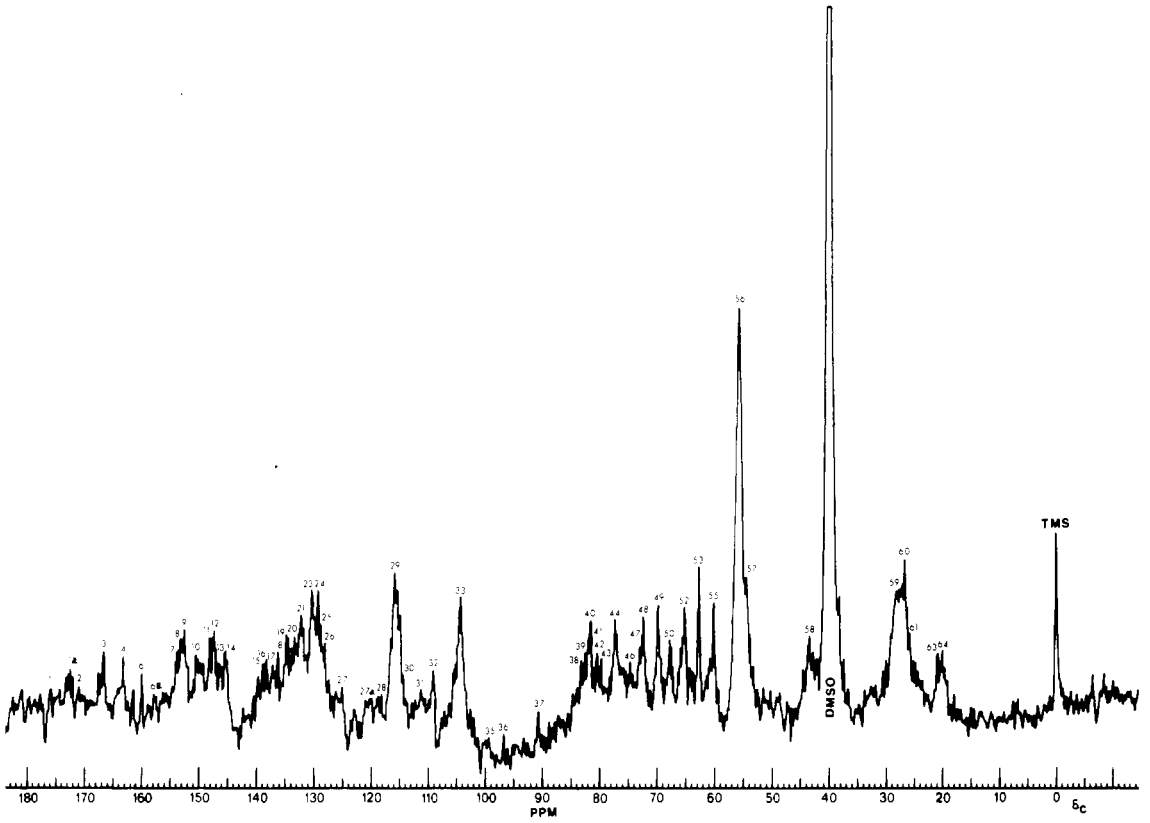

Figure 3. ^{13}C NMR spectrum of acetal lignin from immature Kentucky 31′ tall fescue grass (see Table I for assignments).

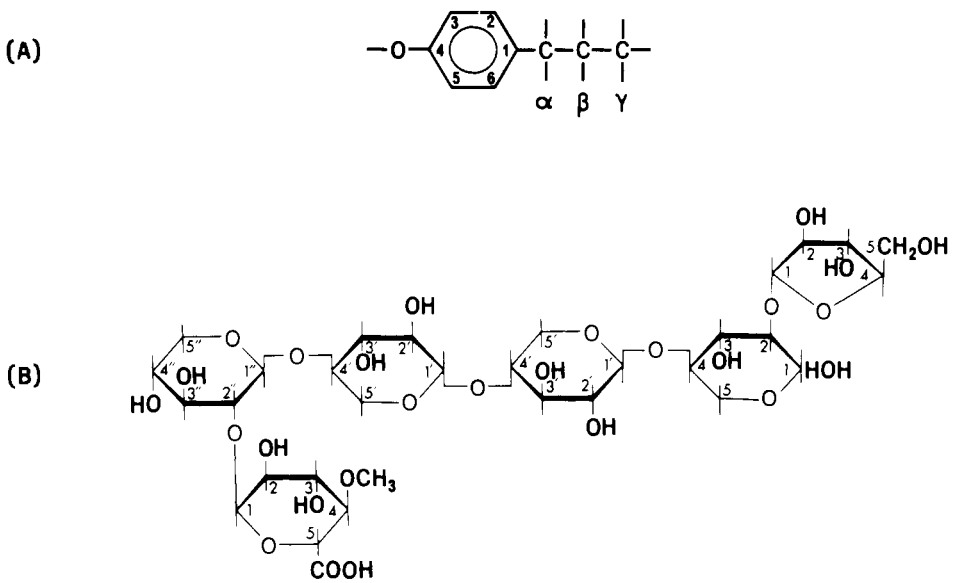

Figure 4. Numbered formulas showing the notation used for lignin (A), adapted from Sarkanen and Ludwig (1971), and carbohydrate (B), adapted from Bailey (1973) and Heyraud (1979).

labeling and decoupling experiment assignments (Gagnaire and Robert, 1977). We have noted, as did Gagnaire and Robert, the 0.5–1.5-ppm upfield shift of resonances for samples run in Me_2SO-d_6 as opposed to the acetone-d_6/D_2O mixture employed by Lüdemann. Additionally we have utilized recently available ^{13}C NMR spectral data on a few carbohydrate compounds for the assignment of signals 1 and 3 and 32 through 55 (Gagnaire et al., 1976; Joseleau et al., 1977; Nunez et al., 1977; Inoue and Chûjô, 1978; Shashkov et al., 1978; Gagnaire and Robert, 1977; Heyraud et al., 1979; Himmelsbach and Barton, 1979). Here we have noted a 1.7-ppm downfield shift of resonances for samples run referenced to TSP in D_2O as opposed to those referenced to Me_4Si in Me_2SO-d_6. Since our spectra were referenced to Me_4Si in Me_2SO-d_6 all reported carbohydrate chemical shifts have been adjusted accordingly.

From comparison of the data contained in these references to our lignin spectra, we first noticed that carbonyl resonances from uronic acids and esters, in addition to cinnamic acids and esters, acetyl groups and other aliphatic esters, may contribute to signals 1–3. For example, C-6 in methyl uronates has been reported at 172.3 ppm (Shashkov et al., 1978).

Signals 32–55 have been mostly unassigned in previous ^{13}C NMR spectra of lignins; therefore a rather detailed

Table I. Assignment of Signals in ^{13}C NMR Spectra of CBG and KY-31 Lignins

signal no.	CBG δ^a (int)[b]	Ky-31 δ^a (int)[b]	assignment[c]
1	176.2 (46)	176.2[d]	CO in aliphatic esters or acetyl, C-6 in uronic acids and esters, C-γ in cinnamic acids and esters
1a	171.5 (118)	172.4 (36)	
2	169.8 (107)	170.0	
3	166.1 (160)	166.6 (40)	
4	162.8 (152)	162.9[d]	C-γ COOR and C-4 in *p*-coumaryl with α-CO
5	162.0 (91)	162.0[d]	C-4 in *p*-coumaryl with C-4 etherified or unsubstituted and CO in acetyl
6	159.6 (101)		
6a	157.8[d]	157.8[d]	
7	155.3 (50)	155.2	
8	152.4[d]	153.8 (33)	C-4 in coniferyl and α-CO and C-3/5 in sinapyl etherified
9	152.1 (211)	152.5 (148)	
10	149.3 (172)	150.5 (109)	C-4 in etherified coniferyl, C-3 in coniferyl, C-3/5 in sinapyl, C-1 in biphenyls and C-4 in coniferyl etherified with carbohydrate
11		148.1 (56)	
12	147.2 (225)	147.3 (76)	
13		146.4 (36)	C-3 in coniferyl with carbohydrate on C-4, C-4 in phenylcoumarans, C-α in cinnamic acids and esters with C-4 etherified or unsubstituted
14	144.7 (329)	145.3 (59)	
15		138.9 (17)	C-1 in coniferyl etherated with α-CHO or α-CH_2R, C-4 in sinapyl with C-4 unsubstituted, C-3 in phenylcoumarans
16	138.3[d]	138.3 (23)	
17	137.5[d]	137.2 (46)	
18	137.2[d]	136.3 (23)	
19	134.2 (90)	134.8 (82)	
20		133.4 (125)	
21	132.1 (118)	132.2 (181)	C-β in cinnamaldehyde and C-1 in coniferyl with α-CO
22	131.3 (105)		
23	130.0 (411)	130.5 (188)	C-2/6 in *p*-coumaryl α,β-unsaturated and C-α in cinnamyl alcohols
24	129.5[d]	129.3 (85)	C-2/6 in *p*-coumaryl and C-β in cinnamyl alcohols
25	128.7 (326)	128.8 (79)	
26	127.5[d]	128.0 (17)	
27	124.7 (116)		C-1 in cinnamyl alcohols, acids, and esters
27a	120.3	121.0[d]	
28	117.4 (90)		C-5 in coniferyl, C-3/5 in *p*-coumaryl with α β unsaturated and C-β in
30	113.7 (151)		
31	110.0 (183)	111.6 (17)	C-2 in coniferyl with α,β-unsaturated
32	108.8 (126)	109.0[d]	C-1 in arabinans and C-2/6 in sinapyl
33	103.8 (476)	104.3 (194)	C-1 in β-D-glucopyranosiduronate, C-2/6 in sinapyl etherated at C-4 and C-1 in β-glycosides
34	102.3 (120)	102.5[d]	
35	100.2[d]	99.2[d]	C-1 in methyl α-D-glucopyranoside, α-D-glucopyranosiduronate or acid and C-1 in glucopyranoside of coniferyl
36	94.5[d]	96.5[d]	C-1 in β(1 → 4)-glucan
37	90.3 (48)	91.9[d]	C-1 β in acylated glycopyranosides, C-1 α in D-glucose and in glucan, and C-1 in β-D-arabinopyranose
38	82.8 (50)	83.2 (17)	C-β in β-OAr ethers and C-4 in α-L-arabinofuranosides
39		82.5 (27)	
40	81.3 (258)	81.6 (95)	C-β-OAr ethers, C-4 in 4-*O*-methyl-α-D-glucopyranosiduronate and C-2 in α-L-arabinofuranosides
41		80.9 (20)	C-4′ in β(1→4)-glucan
42	80.2 (142)	80.5 (33)	
43	79.6 (90)	79.6 (26)	
44	76.8 (307)	77.3 (181)	C-4′ in β(1 → 4)-xylan, C-α in β-O-ethers and C-3/5 in β-D-glucopyranosiduronate
45	75.3 (93)		C-5′ in β(1→4)-glucan and C-3′ in β(1→4)-xylan
46	74.5 (95)	74.7 (27)	
47	73.1 (32)		C-2 in β-D-glucopyranosiduronate, C-3 in 4-*O*-methyl-α-D-glucuronate and C-2′ in β(1→4)-xylan
48	72.1 (165)	72.4 (165)	C-2 in 4-*O*-methyl α-D-glucopyranosiduronate, and C-2′ in β(1→4)-glucan
49	69.6 (166)	69.7 (109)	C-α in coniferyl with β-OAr and β-Ar and C-4 β in xylose or C-4′′ in β(1→4)-xylan and C-5 in 4-*O*-methyl-α-D-glucopyranosiduronate
49a	69.0 d		
50	67.3 (86)	67.8 (63)	C-5 β in xylose or C-5′′ in β(1→4)-xylan and C-5′ in α(1→5)-arabinan
51	66.0 (54)		
52	64.9 (241)	65.2 (138)	C-γ in coniferyl with β-Ar and α-OAr and C-5′ in β(1→4)-xylan
		64.4 (23)	
53	62.5 (375)	62.8 (102)	C-5 in methyl α-L-arabinofuranoside and C-α in phenylcoumarans
54	60.3 (66)		C-γ in coniferyl alcohol with β-O-4 and α,β-unsaturated or C-6 in β(1→4)-glucan and 4-*O*-methyl-α-D-glucopyranosiduronate
55	60.0 (157)	60.1 (125)	
56	55.4 (1000)	55.8 (1000)	OCH_3 in coniferyl and sinapyl
57	54.1 (192)	53.8 (26)	C-β in *p*-coumaryl and coniferyl with β-Ar and α-OAr and C-β in phenylcoumarans
58	41.8 (49)	43.4 (115)	C-α methines with aliphatic substitution
59	28.4 (192)	28.6 (17)	C-α and C-β methylenes
60	26.6 (286)	26.7 (192)	
61	25.1 (88)	25.1 (17)	
62		24.8 (43)	
63	22.7 (71)	21.0 (30)	CH_3 in 2,2-dimethoxypropane acetals
64	20.6 (126)	20.0 (66)	CH_3 in acetyl

[a] δ (chemical shift) in ppm from internal Me_4Si in Me_2SO-d_6. [b] Number in parentheses is intensity of signal relative to signal 56 = 1000. [c] α, β, γ are used to denote side-chain carbon atoms when referring to phenylopropanoid units (common notation). Unprimed numbers for carbohydrates represent reducing end units in polysaccharides, primed represent internal units, and double primed represent nonreducing units. All other nomenclature is in accordance with IUPAC rules. [d] Calculated signal position with no relative intensity value provided by computer printout due to threshold level.

explanation of this region will be attempted here. Reference to Figure 4 may be helpful to the reader in following this discussion.

In this range signals 32–34 have an equal probability of being due to C-2 and C-6 in sinapyl structures as they do of being due to C-1 in glycosides. Nimz (Nimz and Lüdemann, 1973) attributed these signals to sinapyl structures when there was a corresponding strong signal at 154.4 ppm for C-3 and C-5. In our grass acetal lignin spectra these resonances appear to occur between 147 and 153 ppm (signals 8–12). Their intensities, however, are not as great as those of signals 32–34. Thus it is probably safe to assume that signals 32–34 are partly due to carbohydrate. In support of this conclusion are the reports of C-1 in arabinosides occuring in the range between 107.5 and 108.6 ppm (Joseleau et al., 1977), C-1′ in β(1→4)-glucans between 103.2 and 103.4 ppm (Nunez et al., 1977; Inoue and Chûjô, 1978; Gagnaire et al., 1978), and C-1′ in β-(1→4)-xylans at 102.7 ppm (Himmelsbach and Barton, 1979) plus C-1 β-D-glucuronate at 104.6 ppm (Nunez et al., 1978).

Signal 35 is too far downfield to be assigned to free anomeric carbons of carbohydrates. However, this resonance corresponds well with those reported for C-1 in methyl α-D-glucopyranoside at 100.2 ppm (Gagnaire et al., 1978) and in the corresponding α-D-glucopyranosiduronic acid and its methyl ester at 100.8 and 100.7 ppm respectively (Nunez et al., 1977).

Signal 36 can be assigned a priori to C-1 of the β anomer of a hexopyranose or of a reducing end group of a hexopyranose in a polysaccharide.

Signal 37 could likewise be assigned to C-1 of the corresponding α anomer. Although the β anomers of hexopyranoses are generally considered to be the normal conformation in aqueous solution (Eliel et al., 1965), the α anomer predominates in Me_2SO solution and could account for a strong signal here. However, this signal is still upfield from where most α anomer resonances appear. It is more likely that this signal is due to C-1 in an acylated β-glycoside. For peracetylacted β-D-glucopyranose, Gagnaire reported C-1β to occur at 91.8 ppm (Gagnaire et at., 1976). Komura has also reported C-1β in tetraacetalides in the range 90.0–91.9 ppm (Komura et al., 1978). This signal may actually represent the glycosidic linkage of hemicellulose to *p*-coumaryl and coniferyl esters in grasses, such as those identified in wheat germ by Markwalder and Neukom (Markwalder and Neukom, 1976). The presence of this type of structure has also been verified by ultraviolet fluoresence microscopy in other Gramineae (Harris and Hartley, 1976). It may occur in both "lignified" and "unlignified" cell walls of grasses.

Signals 38 and 39 may arise from either lignin or carbohydrate sources. C-β in β-aryl ethers has been reported in this range by Nimz (Nimz et al., 1975) along with C-4 in α-L-arabinofuranosides at 84.6 ppm (Joseleau, et al., 1977); these may correspond to C-4 in α(1→5); α(1→3), or α(1→2)-arabinans. Signal 40 could be correlated with C-4 in 4-*O*-methylglucopyranosiduronates reported at 81.2 ppm (Shashov et al., 1978) or to C-2 in methyl α-L-arabinofuranoside as likewise reported by Joseleau. It may also be due to C-β in β-aryl ethers. Signals 41–43 appear to correspond only to C-4′ in β(1→4)-glucans reported at 80.5 for cellulose (Gagnaire, et al., 1978).

Signal 44, like signals 38–40, arises from both lignin and carbohydrate sources. Considering the relative intensity of these signals, they are probably primarily due to the β-aryl ether lignin structures. C-α in β-aryl ethers has been reported at 75.5 ppm (Nimz et al., 1977). C-4′ in β(1→4)-xylan occurs in this region also at 77.5 ppm, but signals 45–46 indicate a smaller contribution by β(1→4)-xylans and -glucans.

A stronger contribution by glucuronates is indicated by signals 47 and 48 where C-2 and C-3 are reported for methyl 4,6-di-O-methyl-α-D-glucopyranosiduronate at 72.2 and 73.95 ppm, respectively (Shashkov et al., 1978), and C-2, C-4, and C-5 for methyl (methyl α-D-glucopyranosid)uronate at 71.94, 72.4, and 71.87, respectively (Nunez et al., 1977).

Signals 49 and 49a can again be due to both lignin and carbohydrate. There is a strong probability that they are due to methylenes like C-5 in 4-*O*-methyl-α-D-glucopyranosiduronates reported at 70.25 ppm (Shashkov et al., 1978) owing to the other strong indications of glucuronates in signals 33–35, 40, 44, 47, 48, 54 and 55. Signals 50 and 51 appear to be due to the hydroxymethines of pentose sugars, as may be true for signals 49 and 49a also.

Signals 52 and 53 appear to arise mostly from carbohydrate. C-γ in phenylcoumarans has been reported in a range between 61.7 and 74.5 ppm (Lüdemann and Nimz, 1974). C-5′ in β(1→4)-xylan is probably the primary contributor to signal 52 since it occurs at 64.1 ppm (Himmelsbach and Barton, 1979). C-5 in α-L-arabinofuranosides, reported at 62.4 ppm for methyl α-L-arabinofuranoside by Ritchie et al. (1975) and at 62.2 ppm (after adjusted to Me_4Si standard) by Joseleau et al. (1977), is most likely responsible for signal 53.

Signals 54 and 55 are due to C-6 in β(1→4)-glucan and the methoxyl in a 4-*O*-methylglucopyranosiduronate or C-γ in coniferyl alcohols.

From these data it appears that these acetal lignins are contaminated with a 4-*O*-methylglucronoarabinoxylan polysaccharide, hemicellulose, and possibly a glucan from a hemicellulosic or cellulosic source. Signals 3 and 37 indicate that these polysaccharides have most likely remained linked to lignin through a glycosidic bond to esterified phenylpropanoid units after the acidic acetal treatment. By comparison to the milled lignin it appears that accessible weak ether linkages, such as those in long-chain polysaccharides, have undergone cleavage, leaving short-chain polysaccharides attached to lignin.

In comparing the aromatic and vinylic portions of the two acetal lignin spectra, it is very obvious that the CBG lignin sample contains an appreciably greater amount of *p*-coumaryl units as indicated by signals 23 and 29. This appears to accompany larger intensities for signals 3 and 37 in the CBG lignin. This may indicate that the *p*-coumaryl groups are those primarily associated through ester bonds to the polysaccharides in this grass. This, however, remains to be substantiated by studies with synthetic models.

Comparison of the methylene region of the acetal lignin spectra, signals 59–62, indicated considerably more reduced unsubstituted α and β carbons in the Ky-31 lignin sample. This probably indicates that there are fewer lignin and lignin–carbohydrate bonds at these positions in this cool-season grass.

Signals 63 and 74 show that about the same amount of acetal has been incorporated into the sample. This appears to indicate that the positions that have undergone transacetalation are not a source of differentiation between the two grasses.,

Overall these spectra reveal that the acetal lignins of these grasses are primarily composed of *p*-coumaryl and coniferyl lignin units with some sinapyl units being incorporated into the structure. Some carbohydrate still remains attached in the acetal lignin isolation process and

appears to be glycosidically linked through ester groups to some of the phenylpropanoid units.

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Gas–Liquid Chromatography of Trimethylsilyl Derivatives of Sugars from Iraqi Dates

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A method for accurate measurement of sugars in date was developed, in which 95% ethanol was used for Soxhlet extraction of sugars from two varieties of Iraqi dates. Sugars wre converted to their trimethylsilyl (Me_3Si) ether derivatives before application to GLC. Arabinose was used as an internal standard, and the separation of the sugar derivatives was carried out on a 3% SE-30 column. Fructose, α- and β-glucose, and sucrose were the major sugars present. Sorbitol and sorbose, which were not previously reported in the literature, were found in small quantities. The identification of sorbose and sorbitol was based on retention data using three different chromatographic columns.

Most of the analyses which have been carried out on date sugars were concerned mostly with the major fractions, fructose, glucose, and sucrose. Sugar determinations are carried out normally by thin-layer chromatography (TLC) (Raadsveld and Klomp, 1971), paper chromatography (PC) (Chan and Cain, 1966), column fractionation (Thompson et al., 1962), and ion-exchange chromatography (Dubois et al., 1956). However, these techniques are time consuming and hard to quantitate.

A more reliable and accurate method is gas chromatography (GLC), which has been used successfully in the analysis of sugars in plant material (Ford, 1974; Phillips and Smith, 1973).

Since sugars comprise about 78% of the total dry weight in dates as reported by Cook and Furr (1953), therefore their detailed analysis would help in better understanding their nutritive value. Sugars can also be used as indicators for ripeness (Ricardo and Rees, 1970).

A detailed study of the individual free sugars present in dates was carried out in our laboratory by using GLC separation of trimethylsilyl (Me_3Si) ether derivatives according to Sweeley et al. (1963) and Richey et al. (1964). This technique was used in the analysis of carbohydrates in food by many workers (Brobest and Lott, 1966; Davison and Young, 1969; Mason and Slover, 1971; Reineccius et al., 1972; Schubert et al., 1973; Kovacs, 1974).

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