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^{13}C-CP/MAS NMR Examination of Some Australian Woods and Their Chemical and Biochemical Degradation Residues

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ABSTRACT

Solid-state ^{13}C-CP/MAS NMR has been used to obtain structural information on three Australian sapwoods (two hardwoods and one softwood), their residues after chemical treatment, and the frasses obtained after digestion by wood-eating termites and beetles. Whole wood spectra indicate substantial differences in both the lignin and hemicellulose components between the hardwoods and the softwood.

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Spectra of residues clearly show that the harsh chemical treatment causes significant structural changes in the wood components, especially the lignins. Finally, examination of the frasses demonstrates the much greater ability of termites to digest carbohydrates to the extent that frasses are largely the lignin component of the woods. These lignins, in contrast to chemically derived lignins, appear to be structurally unchanged compared with that of the parent woods.

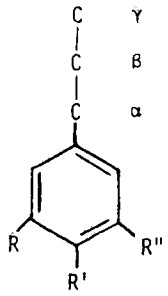
INTRODUCTION

In recent years solid-state high resolution ^{13}C-NMR has been shown to be a powerful tool for structural analysis of insoluble organic materials. The ability to obtain high resolution ^{13}C spectra of solids was facilitated by the development of the cross-polarization (CP), high power ^{1}H decoupling, and magic angle spinning (MAS). Some of the types of materials which have been studied with success include fossil fuels, soil organic matter, polymers, and woods. For the case of wood and its major constituents, i.e., cellulose, lignins, starch, and hemicelluloses, traditionally structural analysis has been necessarily carried out by tedious and often ambiguous wet chemical methods. The limited number of solid-state ^{13}C-NMR studies available to date well demonstrate the ability to provide structural information in a less tedious and unambiguous manner.

The first relevant application of solid-state ^{13}C-NMR was to cellulose by Maciel [1, 2] and VanderHart [3, 4]. In these studies it was shown that the C_4 and C_6 carbons exhibit two distinct resonances, each with the relative intensities within each pair variable with the origin of the cellulose. As in the case of both C_4 and C_6, one of the peaks was considerably broader, it was concluded that the two peaks arose from crystalline and amorphous regions in the solid state. Further, it was determined that these regions were in fact cellulose units in the interior and at the surface, respectively, of the cellulose fibrils. The surface units apparently experience a more variable local environment leading to a larger shift dispersion. Furthermore, the less crystalline C_6 signal exhibits a shorter ^{13}C T_2 than other resonances due to increased internal molecular motion resulting from less hydrogen bonding of the attached hydroxyl group when on the surface of the fibrils [3].

Hatcher [5, 6] and Maciel [7] have also examined whole woods and extracted lignins, initially as a part of a study of the formation and further coalification of lignites. In a typical spectrum of whole wood, resonances assignable to carbohydrates, and in particular cellulose which can constitute 40-50% of dry weight, dominate. However, resonances assignable to lignins, which can account for 15-30% of weight, and to hemicelluloses are evident. These resonances serve

to characterize to a significant extent the structural differences between different types of woods. As lignins in woods can differ quite significantly, the techniques have also been used to study such extracts. Unlike the other major constituent of wood, cellulose, lignins are quite heterogenous and variable in structure and are largely constructed of the phenylpropane units



R = H or OCH_3

R' = -O-

R" = OCH_3 or H

with substituents at C_3 and C_5, and linkages to other units via the C_4 oxygen atom. Maciel has studied a series of solvent-extracted lignins [8, 9] both in solution and as solids, and showed the variability in structure and the close correspondence between solution and solid-state results.

The work reported here extends these studies of woods by examination of examples of Australian hardwoods and softwoods. Further, studies have been conducted on residues after selective extractions of wood components in order to obtain information on individual components and, by comparison with whole wood spectra, to determine if standard wet chemical treatments result in substantial structural modification. Finally, frasses obtained after digestion of the woods by wood-eating beetles and termites have been analyzed in order to study the biochemical degradation processes of woods.

EXPERIMENTAL

Solic-state ^{13}C-NMR spectra were obtained at 75.46 MHz on a Bruker CXP-300 spectrometer using an Andrews type, single coil magic angle spinning probe. Rf fields of 12 and 48G for ^{1}H and ^{13}C, respectively, single 1 ms contacts, 2 s recycle time, and spin temperature alternation were used. Samples were spun at 2.8-3.0 kHz in boron nitride/Kel-F rotors with the magic angle being set by observation of the ^{79}Br resonance of a small amount of added KBr [10]. Spinning sidebands were removed from spectra by use of the Dixon TOSS sequence [11]. While intensities of peaks within any spectrum are not deemed to be quantitative in an absolute sense, it is felt that

comparison on a relative basis is reasonable for spectra obtained under identical conditions. One obvious consequence of the use of the TOSS sequence, which involves five spin-echo periods, is that resonance with short ^{13}C T_2 values will be attenuated by loss of signal during this echo period. For example, the signals from methoxyl resonances and the amorphous C_6 resonance of cellulose, both of which have reduced T_2 values due to internal motion, have been observed to be attenuated in intensity in TOSS spectra relative to normal CP/MAS spectra. Interrupted decoupling or "depolar dephased" spectra were obtained by the insertion of a 50-μs delay with decoupling after the TOSS sequence and prior to acquisition [12]. Between 500 and 4000 scans were accumulated prior to Fourier transformation with an exponential line broadening to 20-50 Hz.

MATERIALS AND METHODS

Sapwood fractions of two hardwoods, Brachychition populenum (kurrajong) and Eucalyptus maculata (spotted gum), and one softwood, Araucaria cunninghamii (hoop pine) were used. The beetle frasses were from Lyctus brunneus (kurrajong and spotted gum) or Calymmaderus incisus (hoop pine), and the termite frasses from Cryptotermes brevis. With one exception all were collected from natural infestations within Queensland by the Queensland Department of Forestry. The termite frass from kurrajong was collected over 4 months in a "no choice" laboratory procedure using at least 50 termites.

Wood powders (<1 mm) were treated in the following manner to obtain the residues studied. Extractives were removed by repeated Soxhlet extraction using 2:1 benzene:ethanol azeotropic mixture. The residue was subsequently washed with ethanol and water and finally dried by suction. Starch removal was via a series of 1 h refluxing procedures with 5% calcium chloride solution until spot testing with iodine/iodine reagent gave a negative response. Hemicelluloses were removed by stirring the starch and extractive-free residue in 17.5% sodium hydroxide solution for 4 h. The remaining solid was washed with water and acetone. Finally cellulose was removed from the preceeding residue by treatment with 72% sulfuric acid for 2 h, diluted to 3% acid concentration, and refluxed for 4 h. The remaining resiude, which is a Klason lignin, was washed with water and dried at 70°C.

RESULTS AND DISCUSSIONS

Woods

The ^{13}C-CP/MAS/TOSS spectra of the two hardwoods, kurrajong and spotted gum, are shown in Fig. 1. The spectra exhibit many simi-

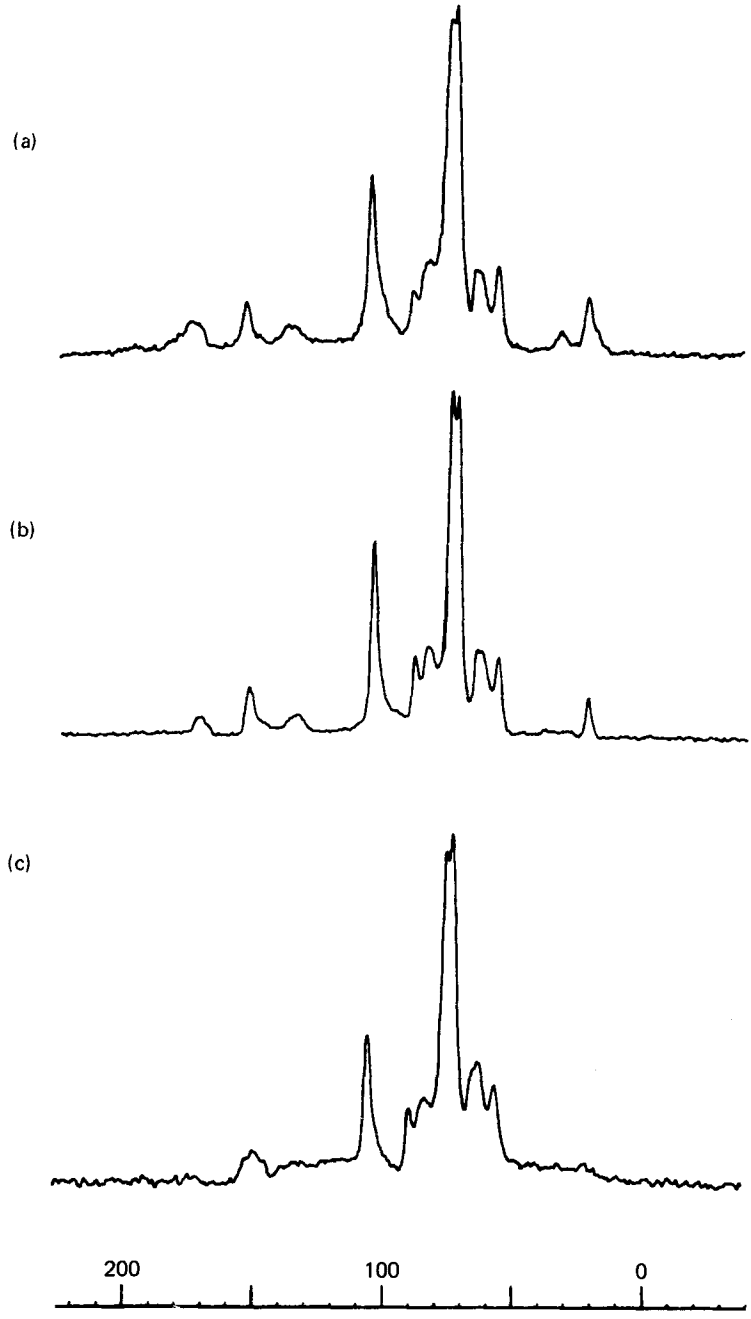


FIG. 1. ^{13}C-CP/MAS/TOSS spectra of (a) kurrajong wood, (b) spotted gum, and (c) hoop pine wood.

larities, and peaks can be largely assigned on the basis of previously published data on woods. Resonances from carbohydrates occur in the region 60 to 110 ppm and dominate the spectra. Cellulose accounts for ~40-45% of the wood by weight [13] and many of the peaks evident in this region of the spectrum can be assigned directly to this. The intense peaks at 74 and 72 ppm can be assigned to the C_2, C_3, and C_5 carbons according to the work of Maciel and Vander-Hart [1-4]. In high crystallinity samples of cellulose, three or more peaks have previously been resolved in this region. However, exact assignments to the three carbons have not been possible. The anomeric carbon, C_1 [1], resonates at 105 ppm and, in highly crystalline samples of cellulose, appears as a doublet. This splitting is not a morphological effect but is due to the presence of two types of glycosidic linkages. In the woods, two peaks are observed for C_4 and C_6 due to the morphological effect of cellulose fibrils. For C_4, a sharper signal is observed at 89 ppm and a broader signal at 83 ppm with the lower field signal due to C_4 carbons of interior anhydroglucose units in the more highly ordered environment.

As mentioned, C_6 shows similar but smaller morphological effects in cellulose samples with a sharp resonance at 64 ppm and a broad high field shoulder. In the hardwood spectra of Fig. 1, the C_6 peak is centered at 64 ppm but shows considerable high field asymmetry. Furthermore, overlap with the sharp signal at 56 ppm occurs.

In cellulose samples the clear resolution of the two C_4 resonances can give a measure of degree of crystallinity [1-4]. For the two hardwoods, the higher field broad signal has far greater area than that at 89 ppm, suggesting a high degree of disorder in the cellulose fibrils of the woods. However, it must be considered in the case of woods that signals from other carbohydrates may occur in this region. For example, starch can be present in hardwoods to a level of 5-10% and hemicellulose can constitute up to 35% [13]. Starch can definitely interfere in the C_4 region. The two basic units of starch are amylose and amylopectin, and the ^{13}C-CP/MAS/TOSS spectrum of the former is shown in Fig. 2(a). Signals are present at 102 ppm (C_1), 82 ppm (C4), 72 ppm ($C_{2,3,5}$), and 62 ppm (C_6). The spectrum of a wood-derived cellulose is given in Fig. 2(b) for comparison. The other component, amylopectin, gives a virtually identical spectrum to amylose. No morphological splittings are observed for C_4, with a single broad resonance occurring at 82 ppm. Clearly, this would overlap with the lower crystallinity C_4 peak of cellulose, thus confusing the use of the C_4 peaks at 82 and 89 ppm in cellulose as a measure of crystallinity in whole woods.

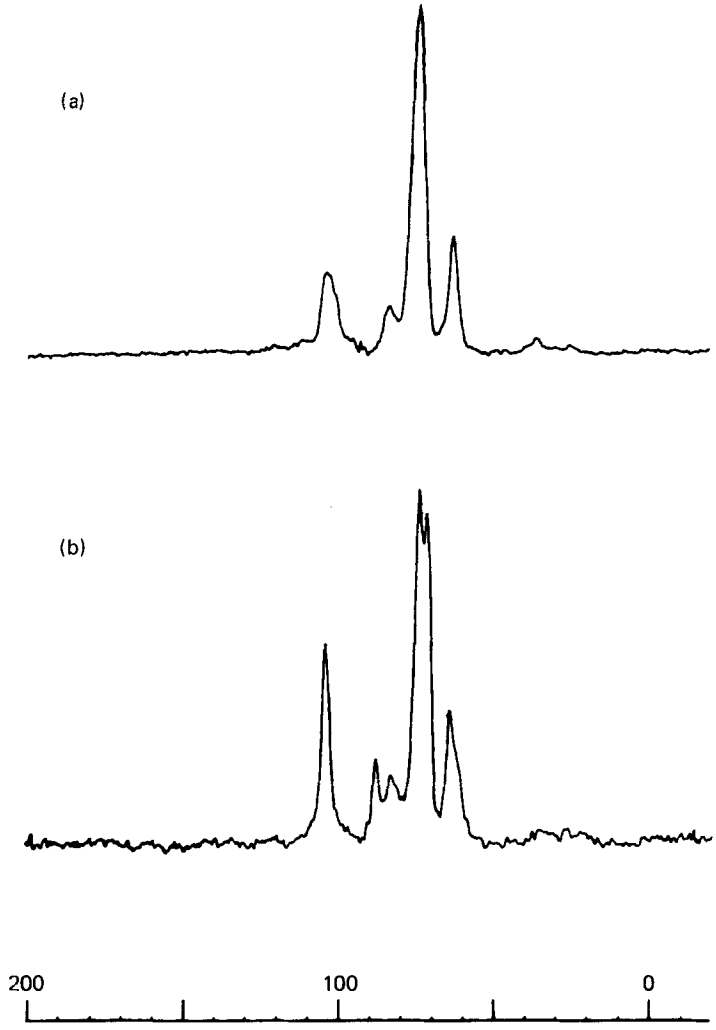


FIG. 2. ^{13}C-CP/MAS/TOSS spectra of (a) amylose and (b) a wood-derived cellulose.

Both hardwood spectra exhibit signals at 152, 135, and 56 ppm which are attributable to lignin. Those at 152 and 56 ppm are due to the aromatic and methyl carbons, respectively, of the aromatic methoxy groups known to be present in lignins. The resonance at 135 ppm is considerably broader and probably results largely from nonoxygenated aromatic carbons, both protonated and quaternary, present in lignins. The kurrajong would appear to be more highly methylated according to intensities of the aromatic and OCH_3 carbons.

Both hardwoods exhibit peaks at δ 172 and 20 ppm from acetyl groups. These groups are presumably present in the wood fraction termed hemicelluloses. Hemicelluloses covers low molecular weight alkali and/or water-soluble polysaccharides which are more loosely held than cellulose. These can comprise almost 30% hardwoods by weight [13]. The major component of hardwood hemicelluloses, the glucuronoxylans, consists of a β-(1-4) xylopyranose backbone with 4-O-methyl 2-glucuronic acid side chains at C_2. These methoxy groups presumably resonate at ~56 ppm along with those of lignin. A large proportion of the xylose units have O-acetyl groups at C-2 and C-3. Glucomannans can also be present in hardwoods up to ~5% by weight. However, it is uncertain as to how highly acetylated these are. One further broad peak at 30 ppm remains to be assigned. This can probably be assigned to polymethylene chain carbons presented as waxes or attached to some other components of the woods.

Softwood

The spectrum of the softwood examined, hoop pine, is given in Fig. 1(c). It consists of the same major peaks between 60-105 ppm assigned to carbohydrates for the hardwood spectra. The major difference to the hardwood spectra is in the peaks assigned to lignin and hemicelluloses. First, no peaks from acetyl groups of hemicelluloses are evident. Hemicelluloses can account for up to 27% by weight of softwoods with the major types being xylans, as in hardwoods, arabinogalactans, and galactoglucomannans [13]. However, the xylans are known not to contain acetyl groups. Conversely, the mannose units of the galactoglucomannans are supposedly acetylated at C-2 and C-3. Hence this latter class of hemicelluloses may not be a significant component in hoop pine. Clearly, as the majority of carbons in hemicelluloses resonate in the same region as cellulose, solid-state ^{13}C-NMR is only able to give information on the presence of acetylated hemicelluloses.

The lignin resonances also show considerable differences to those in the hardwoods. First, the oxygenated aromatic carbon resonance is much broader, extending from ~142 to 152 ppm. Lignins are three-dimensional phenylpropane polymers linked by biphenyl, aryl-alkyl, or ether linkages and can be considered to consist of three substituted cinnamyl alcohol monomers, coniferyl, p-coumaryl, and sinapyl. Whereas p-coumaryl monomer has no substituents at C_3 and C_5, coniferyl monomer contains a single methoxy group at C_3 and sinapyl has a second such group at C_5. Two major types of lignins are found in woods, guaiacyl and cyringyl. The former is composed of all three monomers with a predominance of coniferyl and p-coumaryl. Syringyl lignin is also composed of all three monomers but with coniferyl and sinapyl monomers dominant. Solution state NMR studies have shown

that C_3 in coniferyl monomers resonates at 148 ppm while C_3 and C_5 in sinapyl groups generally resonate in the vicinity of 152 ppm [14]. The C_4 oxygenated carbon resonance shift is widely variable, depending on substituents and linkages to other phenylpropane units via this oxygen. In solution it can resonate over the range of ~135 to 160 ppm. Hence, the solid-state spectra indicate a substantial difference in the type of lignin between the hardwoods and the softwood. Allowing for the fact that the sinapyl monomer will yield double the signal intensity in the region due to the presence of methoxy groups at both C_3 and C_5, it appears that the hardwood lignins contain a much higher proportion of this monomer than the softwood. This is consistent with the fact that softwood lignins generally are of the guaiacyl type. Supporting this evidence of lower sinapyl monomer content in the softwood lignin is the lower relative intensity of the methoxy signal at 56 ppm due to the absence of a C_5-methoxyl group in the coniferyl monomer. The second major difference is the broad featureless resonances of other aromatic carbons between 105 and 140 ppm. Again this is indicative of a less homogeneously structured lignin.

Residues

Kurrajong and hoop woods were subjected to traditional wet chemical treatments in order to remove specific wood components selectively. ^{13}C-CP/MAS spectra were then obtained on the insoluble residues and are shown in Figs. 3 and 4, respectively. For kurrajong, extractives were removed by treatment with benzene/ethanol and water. The term extractives is used to cover soluble starch, pectins, tanins, lipids, etc. which can contribute up to 28% of wood weight [12]. Comparison of the spectrum after removal of extractives (Fig. 3a) with that of the wood (Fig. 1a) indicates that the only noticeable change is a relatively minor loss of intensity in the 60-85 ppm region consistent with the removal of soluble, low molecular weight sugars. This residue was subsequently treated with 5% calcium chloride solution in order to remove further starch. The spectrum shown in Fig. 3(b) exhibits substantial changes in the relative intensities of various peaks. A further reduction in intensity of peaks between 70 and 85 ppm is evident. Furthermore, subtraction from spectrum 3(a) reveals loss of signal in the region of 65 ppm and of a broad component in the region of ~100 to 105 ppm. Also, the sharp peak at 89 ppm, assigned to the crystalline C_4 resonance of cellulose, is larger relative to the broader C_4 peak at 82 ppm. Clearly, the changes in the spectrum are consistent with the removal of starch, particularly that in the C_4 region where starch only exhibits one C_4 resonance at ~82 ppm. In fact, subtraction of Fig. 3(b) from 3(a) yields a difference spectrum very simi-

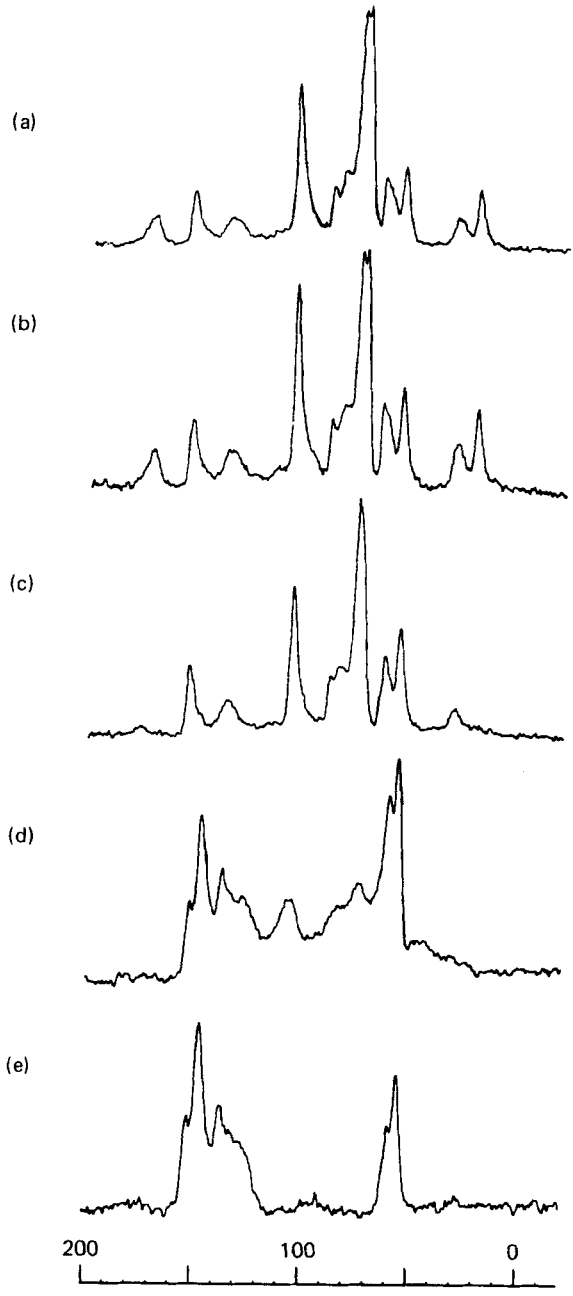


FIG. 3. ^{13}CP/MAS/TOSS spectra of kurrajong wood after treatment to remove (a) extractives, (b) starch, (c) hemicelluloses, (d) cellulose, and (e) as for (d) but with interrupted decoupling.

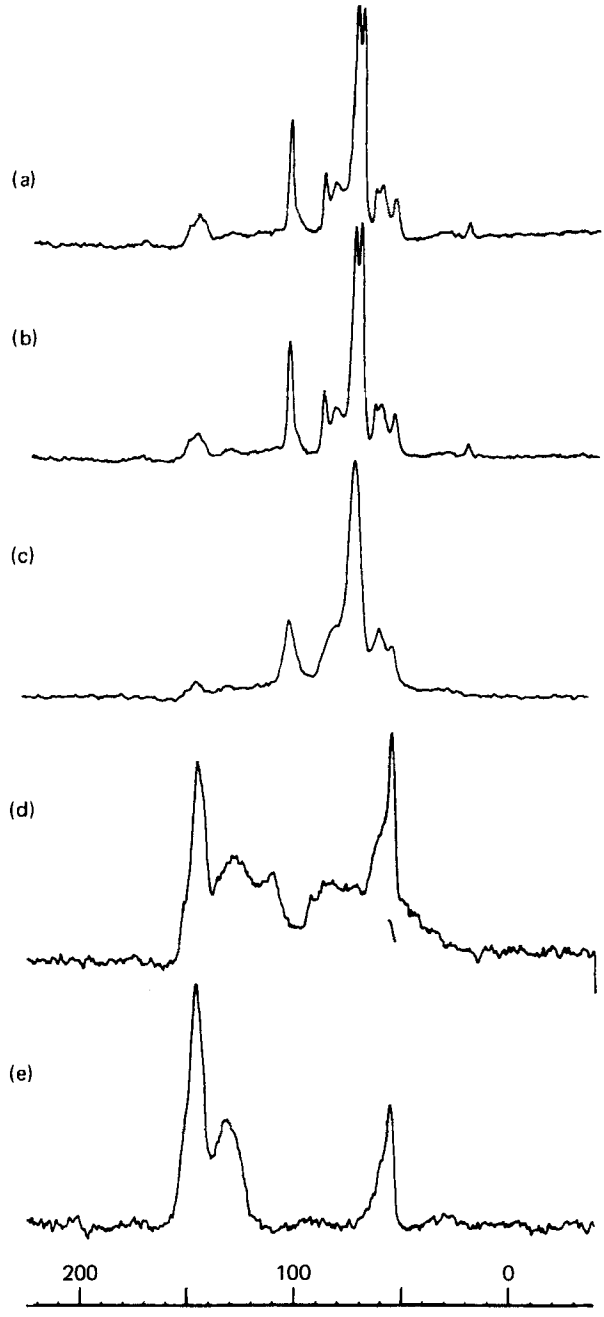


FIG. 4. ^{13}C-CP/MAS spectra of hoop pine wood after treatment as for Fig. 3.

lar to that shown for starch in Fig. 2(a). As a result of the extraction of starch, the peaks assigned to lignins and hemicelluloses are more prominent in Fig. 3(b).

After starch extraction, the residue was treated with sodium hydroxide to remove hemicelluloses. The resultant spectrum is shown in Fig. 3(c) from which it is evident that the peak at 20 ppm has totally disappeared while that at 174 ppm is significantly reduced as expected. Clearly, acetylated hemicelluloses have been largely removed with the remaining signal at 174 ppm possibly due to acetyl groups in the lignins. A number of other interesting changes have occurred. First, there is a dramatic change in the resonances at 74 and 72 ppm which are due to C_2, C_3, and C_5 in cellulose. The "doublet" observed in the wood and preceding residues has disappeared leaving only one broad signal centered at 74 ppm of increased intensity relative to the C_1 resonance of cellulose. Evidently, the NaOH treatment has resulted in a small shift to lower field of the 72 ppm resonance. Likewise, in the C_6 cellulose region, intensity has shifted from ~64 to ~62 ppm. Finally, the C_1 resonance at 105 ppm appears to have broadened slightly. These changes are consistent with the differences observed between spectra of highly crystalline and amorphous cellulose. Hence, treatment of the extracted kurrajong wood with NaOH appears to have severely affected the crystallinity or morphology of the cellulose in the wood. This is probably not unexpected given the harshness of the treatment. What is perhaps surprising is the lack of significant change in the relative intensity of the two C_4 resonances at 89 and 82 ppm. For cellulose the lower field resonance was not observed in the amorphous samples. No obvious explanation is available to explain this discrepancy. Treatment with NaOH does not appear to have affected the evident lignin peaks or the broad signal at 30 ppm from polymethylene carbons.

Finally, the NaOH-treated residue was subjected to treatment with sulfuric acid in order to remove remaining carbohydrates, which are presumably largely cellulose. The spectrum (Fig. 3d) shows that this step has been very successful in removing the remaining carbohydrates by the loss of signals at 105 ppm and in the region of 70-75 ppm. Hence, the residue should be the Klason lignin fraction of the wood. The remaining signals in the region of 60-105 ppm are consistent with the proposed structures of lignin. The residual signal between 60 and 90 ppm can be assigned to oxygenated methine and methylene carbons of the propyl chains in the phenylpropane units. The broad signal centered at 106 ppm is most likely due to aromatic carbons ortho to oxygenated aromatic carbons (i.e., phenolic, methoxylated) rather than the C_1 carbons of the remaining cellulose. The broad signal between 120 and 140 ppm, with maxima at 127 and 137 ppm, indicates the presence of carbons more remote than ortho from methoxylated carbons. Signs of nonoxygenated aliphatic carbon are

present in the broad low intensity signal between 20 and 50 ppm. The spectrums also displays two peaks which can be assigned to methoxylated carbons at 152 and 147 ppm with the latter being significantly more intense. This is in sharp contrast to the whole wood and preceding samples shown in Fig. 3. In these, the lower field signal is quite intense with a weak shoulder at 147 ppm. Clearly, the lignin extraction process has resulted in either modification of lignin or preferential extraction of one component of the lignin. The two peaks can be assigned to the two major types of lignin monomers, sinapyl and coniferyl, with the latter giving the higher field signal and hence predominant in this extract. The absence of a C_5 methoxyl group in coniferyl lignin can also explain the large intensity of signal in the 120-140 region in that there is an extra protonated aromatic carbon per ring. The methoxyl region also exhibits an extra peak at 60 ppm not evident in the wood spectrum although it may be hidden by overlap with the broad C_6 cellulose signal at 62 ppm. The shift could also be consistent with a hydroxy methylene carbon in the γ-position of C_3 propyl units. This signal is not evident in the preceding spectrum of the kurrajong wood and residues. This is presumably because it is hidden by the overlap of carbohydrate resonances, in particular, the C_6 CH_2OH resonances of carbohydrates. Furthermore, it will be less intense, relative to the resonance at 56 ppm, in the wood because the lignin isolation process has resulted in a loss of methoxy groups as evidenced by the $C_{3,5}$ aromatic resonances.

As methoxy carbon resonances, both in solution and solid, vary very little from 56 ppm, that latter explanation of a hydroxymethylene assignment seems more probable. However, a spectrum obtained with a 50-μs interrupted decoupling period (Fig. 3e) prior to acquisition disputes this assignment. Such an experiment should yield a spectrum containing only quaternary and methyl resonances due to the weaker ^{13}C-^{1}H dipolar couplings for such carbons. In the former case the dipolar coupling is small due to the large ^{13}C-^{1}H internuclear separation, while in the latter case the coupling is reduced due to internal motion. The 60-ppm resonance is still very evident in Fig. 3(e). Variable interrupted decoupling period studies on a wide range of materials have shown that a 50-μs period is more than sufficient to produce a spectrum devoid of resonances from rigid, protonated carbons. Hence, for this resonance to be assigned to the C_γ hydroxymethylene carbon, this group would have to be undergoing some form of internal motion. It is quite conceivable that a CH_2OH group should be undergoing internal rotation as exhibited by methyl groups. However, such a group would have to be in such an environment so as to be free of steric or hydrogen bonding interactions. This is certainly not the case for the CH_2OH group in carbohydrates such as starch and cellulose where considerable hydrogen bonding is conceivable,

and we have not observed a persistence of this resonance in interrupted decoupling experiments. However, we do favor the assignment of this resonance in lignin to the γ-CH_2OH carbons. Its persistence in interrupted decoupling experiments implies considerable internal motion. The reason for this must lie in the heterogeneity of the structure of lignins. Some fraction of the terminal γ-CH_2OH carbons must be in sterically unhindered and hydrogen-bond-free regions of the macromolecule.

The interrupted decoupling experiment also shows that quaternary aromatic carbons are confined to shifts above 120 ppm. From the relative reduction of signal intensity in the 120-137 region, it is evident that significant signal in this region results from protonated aromatic carbon. The quaternary signal at 137 ppm may largely result from oxygenated aromatic carbons. The C_4 carbon of syringyl lignin resonates at this shift in solution. The corresponding signal in coniferyl monomer of guaiacyl lignin resonances at ~150 ppm in solution and would be difficult to distinguish from methoxylated C_3 and C_5 resonances in solid-state ^{13}C spectra.

A similar treatment series on hoop pine was carried out and the spectra are shown in Fig. 4. Treatment to remove extractives and starch again results in a relative increase in the peak at 88 ppm relative to other carbohydrate peaks as a result of the removal starch and possibly other carbohydrates. Also, NaOH treatment again appears to excessively disrupt the cellulose fibrils and to a greater extent than for kurrajong. All peaks due to cellulose are considerably broadened with the sharp peak at 88 ppm totally absent and the $C_{2,3,5}$ peaks converged at 74 ppm. The broad C_6 peak at 62 ppm also predominates over that expected at 64 ppm.

The lignin spectrum shown in Fig. 4(d) again indicates substantial differences from the corresponding signals evident in the whole wood. As with kurrajong, the main Ar–OH signal is at 147 ppm compared with the broad signal extending to 152 ppm observed in the wood. The 152 ppm is barely evident in the hoop pine lignin spectrum, presumably as a result of its lower intensity in the initial wood and a similar loss to kurrajong via the treatment used to produce the lignin. Hence, modification of the lignin with isolation is evident again. The other major peaks in the aromatic region are at 130 and 110 ppm. The interrupted decoupling experiment (Fig. 4e) shows a significant portion of the signal between 120 and 140 ppm is due to quaternary carbons. The absence of signal between 100 and 110 ppm in this spectrum again indicates the effective removal of carbohydrates while the complete spectrum shows the significant presence of oxygenated aliphatic carbon, due to C_3 propyl units, by the broad signal in the 60-90 range. The signal observed at 60 ppm in kurrajong is also evident for hoop pine in Figs. 4(d) and 4(e) but appears as a shoulder of lower intensity.

Hence, such γ-CH_2OH are of less importance in this modified hoop pine lignin.

Frasses

We have also examined the wood frasses of kurajong, spotted gum, and hoop pine after digestion and secretion by wood-eating beetles and termites. Detailed descriptions of the species and techniques used to obtain frasses are given in the Experimental section. Examples for each wood of spectra obtained for beetle-derived frasses are shown in Fig. 5. Comparison with the whole wood spectra of Fig. 1 do not reveal any major differences in the composition of the frasses. Integration of the spectra indicates that the only real difference is a slight loss of carbohydrate intensity relative to the lignin signals. The beetle, Lyctus brunneus, feeds on nonconiferous sapwood rich in starch and is known to feed on starch, monosaccharides, disaccharides, and short-chain oligosaccharides. It appears to have some preference for amylopectin over amylose. Starch is uaually only present to a level of less than 5%. Less is known of the habits of Calymmaderus incisus except that it feeds on conifers such as hoop pine and confines itself to the sapwood. As the beetles are unable to digest some hemicelluloses, cellulose, or lignin which are the major components of woods, the lack of change exhibited by the spectra of Fig. 5 is not surprising.

In contrast, termites are known to be more efficient with gut microorganisms capable of digesting a much wider range of carbohydrates. Cryptotermes brevis attacks both sapwood and truewood of low density woods with a preference for the former. Their gut flagellates are able to digest cellulose and hemicelluloses while the termite itself digests starch and proteins. There are also some suggestions that a fraction of the wood lignin can be digested. The ability to consume a wide range of carbohydrates is confirmed by the spectra of Fig. 6 where, for all three woods, substantial loss of carbohydrate is evident. The reduction of carbohydrate relative to lignin is about threefold for kurrajong and slightly greater for the other two woods. For spotted gum the reduction in methyl group intensity at 20 ppm indicates loss of acetylated hemicelluloses. For the other hardwood, kurrajong, such a loss is not evident.

On the assumption that the spectra of Fig. 6 are representative of the wood lignin because termites are known to be unable to digest lignin to any significant extent, it is worthwhile to compare the spectra for kurrajong and hoop pine (Figs. 6a and c) with those of the whole woods (Fig. 1) and extracted lignins (Figs. 3 and 4). In both cases the major differences between the frasses and the extracted lignins are in the oxygenated aromatic carbon region. This is more clearly seen in the interrupted decoupling experiments (Figs. 6b and 6f). For kurrajong, the sinapyl-type lignin monomer resonance at 152 ppm is much larger than that for coniferyl at 147 ppm, a situation

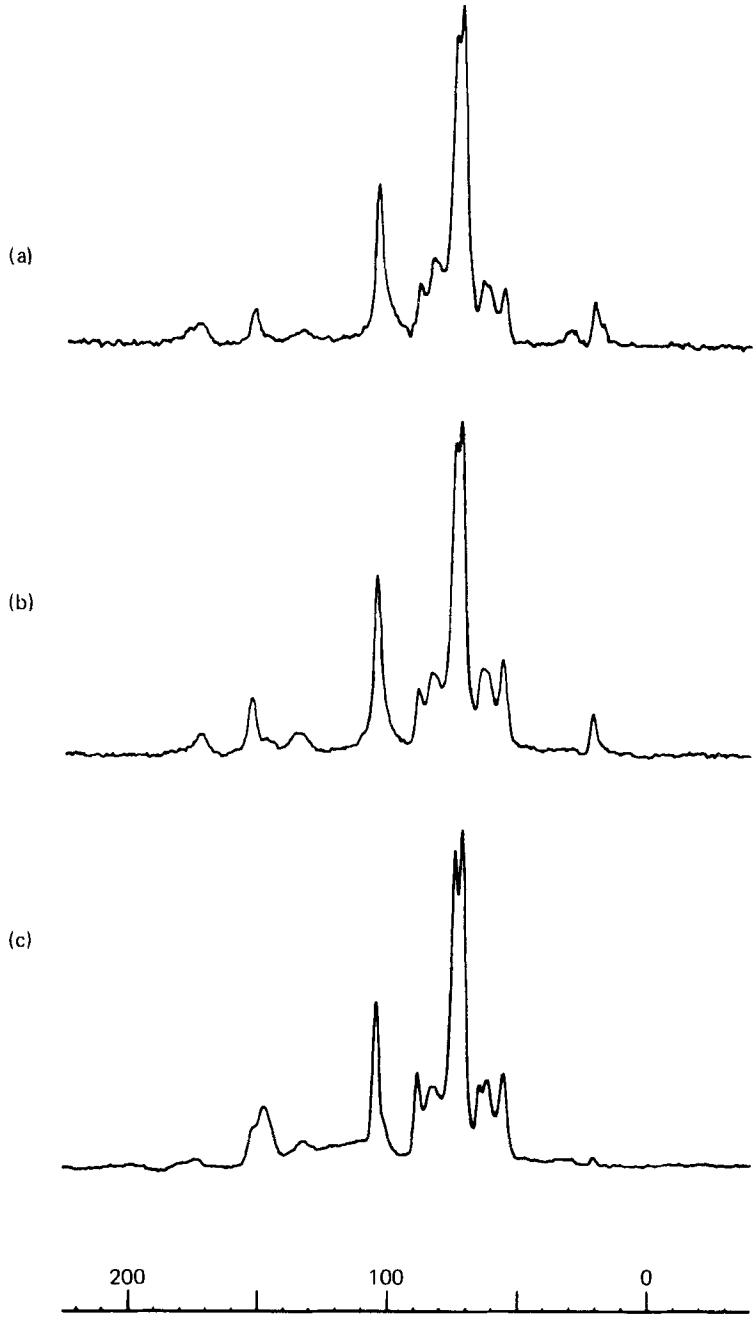


FIG. 5. ^{13}C-CP/MAS spectra of beetle frasses for (a) kurrajong, (b) spotted gum, and (c) hoop pine.

very similar to that observed for the whole wood. Likewise with hoop pine, while the higher field resonance dominates in the lignin preparation and the lower field resonance is barely evident, the latter is clearly visible with about two-thirds the intensity of the former in the frass. Again, the resonances are closer to that observed in the whole woods. Semiquantitatively, the hardwoods appear to consist of roughly 60% sinapyl monomer while the softwood contains approximately only 20%. This supports the classification of the softwood lignin as being of the guaiacyl type and that of the hardwoods as being of the syringyl type. Consequently, for both hard and soft woods, a more intense signal from methoxyl carbons should be observed in the frasses relative to the extracted lignins. This is confirmed by the greater intensity of this signal at 56 ppm relative to that at ~60 ppm in the interrupted decoupling spectra where this latter signal is only evident as a lower intensity shoulder on the methyl carbon resonance. However, it is still clearly observable in these spectra. Other comparisons between the termite frass and lignin spectra are hindered due to the residual carbohydrate in the former samples.

Finally, comparison of the two hardwood frass spectra reveals a high degree of similarity. The only really significant differences evident are the proportion of syringyl to guaiacyl-type lignin and the amount of acetylated hemicelluloses, both of which are slightly higher in kurrajong.

CONCLUSIONS

Solid-state CP.MAS ^{13}C-NMR of whole woods is able to provide semiquantitative information on and structural information about the major constituents of woods. For the woods studied, information is obtained on the presence of acetylated hemicelluloses and the structural characteristics of the lignins. The hardwoods are very similar in that they contain similar amounts of acetylated hemicelluloses and lignins of similar structure. The softwood differs in the absence of such hemicelluloses and in the structure of the lignins. The technique is also of value in structural studies of the separated components and in detecting morphological or structural changes induced by the chemical methods of isolating the components. In this study the techniques used for isolation have been shown to cause quite significant modification, thus indicating that relating information obtained by studies of these components to the structure of the woods must be done with great care. Finally, the solid-state ^{13}C-NMR method will have a significant role in studying the biochemical degradation of woods. Frasses from termites and beetles confirm the much greater ability of the former to utilize wood as a food source. In particular, the carbohydrates are very efficiently utilized whereas the lignins do not appear to be consumed or modified to any great extent.

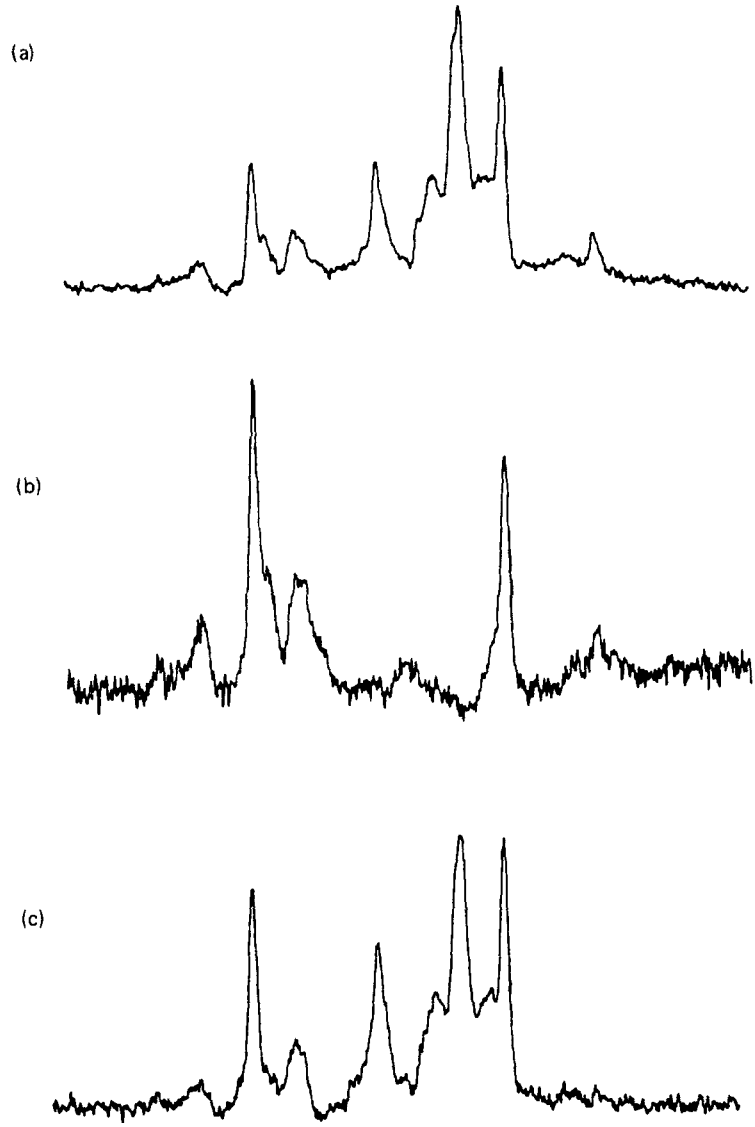


FIG. 6. ^{13}C-CP/MAS/TOSS spectra of C. brevis termite frasses for (a, b) kurrajong, (c, d) spotted gum, and (e, f) hoop pine. Spectra in (b), (d), and (f) were obtained with interrupted decoupling.

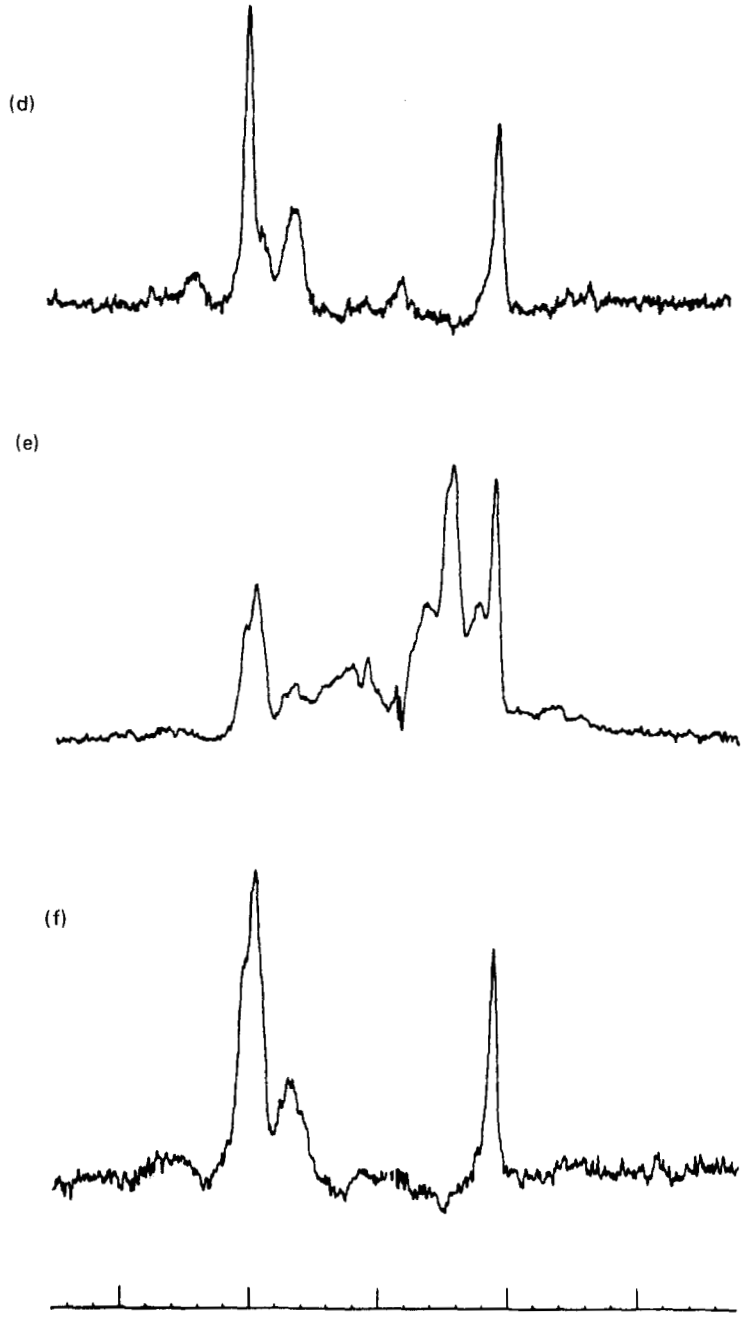


FIG. 6 (continued)

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