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Nuclear Magnetic Resonance Studies of Cutin, an Insoluble Plant Polyester

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ABSTRACT: High-resolution ^{13}C NMR studies have been conducted for cutin, a biopolyester that forms the structural component of the protective cuticle in terrestrial plants. With use of cross polarization–magic angle spinning (CPMAS) methods, the cutin structure is found to include methylene, methine, alkene, arene, keto, and ester functional groups. In addition, several types of mobile aliphatic carbons are observed by direct polarization (DP) methods. Estimates of the relative composition of each carbon type are also available from appropriate comparisons of CPMAS and DPMAS spectra. A combination of ^{13}C T_1, ^{1}H $T_{1\rho}$, and line-width measurements suggests that cutin is a moderately flexible netting, with motional constraints at particular cross-link sites. A preliminary structural model is proposed to account for the compositional and dynamic information obtained from ^{13}C NMR experiments.

Introduction

Higher plants are encased in a cuticle that serves both as a protective barrier to the environment and as a regulator of molecular diffusion among various organs. Cuticular breakdown may occur through the action of pathogenic fungi, with potentially devastating agricultural and economic losses. Yet paradoxically, cutin's susceptibility to degradation by mammalian enzymes may also facilitate digestion of many waste materials that could be fed to livestock.[1,2]

In aerial organs of terrestrial plants the cuticle consists of a waxy lipid waterproofing and the cutin polyester structural component. Although this latter material has several known hydroxy, epoxy, and phenolic fatty acid constituents (Figure 1), the insolubility of the polymer has hampered investigations of how its monomeric units are linked together.

For polymers and biopolymers, detailed structural information has become available routinely from solid-state nuclear magnetic resonance (NMR) and from the cross polarization–magic angle spinning (CPMAS) technique in particular.[4,5] Maciel and co-workers have used ^{13}C NMR to examine molecular alterations that accompany wood processing treatments and to isolate spectral contributions from lignin and cell walls in a variety of herbage samples.[6] More recently, Lewis et al. have deduced chemical bonding information from ^{13}C NMR spectra of both synthetic and natural lignins.[7,8] For polyester copolymers (Hytrel elastomers), Jelinski, et al. have elucidated the dynamic behavior at each backbone site of the hard and soft segments by combining ^{13}C and ^{2}H techniques and evaluating the effects of spinning, cross-polarization, and nuclear relaxation on various chemical moieties.[9–11] Though these examples focus on materials related to plant cuticle, numerous other solid-state NMR investigations of polymers and biopolymers have been conducted.[4,5,12]

We report herein the first high-resolution ^{13}C nuclear magnetic resonance studies of intact cutin. Cross polarization–magic angle spinning (CPMAS) with high-power proton decoupling has been employed in order to identify and estimate the relative numbers of magnetically distinct carbon moieties. In addition, several types of mobile carbons have been observed by combining traditional direct signal acquisition with low-power decoupling and

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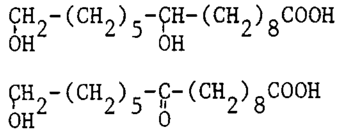


$$\underset{OH}{CH_2}-(CH_2)_5-\underset{OH}{CH}-(CH_2)_8COOH$$

$$\underset{OH}{CH_2}-(CH_2)_5-\underset{O}{\overset{\|}{C}}-(CH_2)_8COOH$$

Figure 1. Dihydroxypalmitic acid and hydroxyoxopalmitic acid, the major fatty acid constituents of lime cutin that are susceptible to depolymerization procedures.[3] Also present in minor amounts are several positional isomers and monohydroxy or trihydroxy derivatives. Other cutins also contain C_{18} constituents.

magic-angle spinning (DPMAS). Comparison of CPMAS and DPMAS results yields an approximate ratio of mobile to immobile methylene groups in the cutin polymer. Finally, preliminary ^1H and ^{13}C spin relaxation results have been used to identify mobile, rigid, and cross-linked spin groupings in the polymer structure. Results for cutin are also contrasted with prior NMR studies of synthetic polyesters.[10,11]

Materials and Methods

Isolation of the Biopolyester. Cutin was isolated from the skin of limes by using published methods.[13,14] Briefly, the procedure involved three steps: (1) removing peels from the fruit and disrupting the pectinaceous layer; (2) degrading residual pectin and carbohydrates enzymatically; and (3) extracting waxes repeatedly from the cutin matrix. A typical yield from 20 limes was 800 mg of powdered polymer.

Preliminary structural characterization of cutin was accomplished via infrared spectroscopy of the solid polymer as well as the customary $LiAlH_4$ depolymerization.[13] The methylene chloride soluble alcohols resulting from this treatment were examined by ^{13}C NMR, then volatilized by reaction with either *N,O*-bis(trimethylsilyl)acetamide or *N,O*-bis(trimethylsilyl)trifluoroacetamide, and analyzed by gas chromatography–mass spectrometry (GC–MS) (Finnigan 3300). (Both NMR spectra and mass spectral fragmentation patterns indicate the presence of hydroxyl-substituted fatty-acid constituents in the original polymer, as expected from prior isolations of lime cutin.[3]) A substantial portion of insoluble (unidentified) material (~50% by weight) was resistant to the depolymerization procedure, again in agreement with earlier reports.[3,13]

Samples for NMR analysis consisted of 175 mg of polymer, ground with a mortar and pestle and packed into a 7-mm cylindrical Al_2O_3 rotor. Alternatively, up to 235 mg of powder was placed in 5- or 10-mm o.d. glass NMR tubes for *z*-axis spinning experiments (vide infra). Cutin samples obtained from independent isolations exhibited identical spectral characteristics.

NMR Spectra. ^{13}C spectra of the intact polymer were obtained at 4.6 T on an IBM Instruments WP-200 spectrometer (resonance frequency of 50.33 MHz for ^{13}C). This instrument was equipped with a solids accessory consisting of auxillary high-power amplifiers and a magic-angle spinning probe (Doty Scientific).

For CPMAS experiments, a standard sample of *p*-di-*tert*-butylbenzene (PDTBB) was employed to adjust the Hartmann–Hahn matching condition, magic angle, decoupler power, and reference frequency. ^{13}C NMR spectra were run at an ambient temperature of 300 K (unless noted otherwise) and with a coarse field-frequency lock (estimated drift <0.01 ppm/h). Samples were spun at 5.00 ± 0.01 kHz. High-power proton decoupling (dipolar decoupling, DD) was employed with a field of 48 kHz. The usual ^{13}C spectral width was 20 kHz, defined by 2K time-domain points and zero-filled to 4K points prior to Fourier transformation. Chemical shifts are quoted with respect to TMS, via PDTBB as a secondary substitution reference.

All cross-polarization pulse sequences included proton spin-temperature alternation and quadrature phase cycling[15] in order to avoid base-line distortions and other spectral artifacts. A recycle delay of 1–3 s was inserted between acquisitions to permit repolarization of the proton spin reservoir. Variation of the ^1H–^{13}C contact time provided the relative amounts and ^1H $T_{1\rho}$'s for each carbon type (vide infra).[4] Two spectral editing sequences were also employed: CP with delayed decoupling (50–100 μs) to allow dephasing and suppression of signals from rigid protonated carbons;[16,17] CP followed by delayed decoupling and a 0.6-s longitudinal relaxation period to additionally suppress signals from mobile CH and CH_2 carbons.[18]

For DPMAS experiments, the solid-state NMR apparatus could be used if the high-power ^1H amplifier was bypassed and data acquired from the Bloch decay following a single ^{13}C pulse. Alternatively, FT spectra and spin–lattice relaxation times for mobile carbons were measured in a high-resolution probe with *z*-axis spinning (20 Hz). Both measurements employed a 6 kHz decoupling field (scalar decoupling, SD). In this case, 4K data points defined a 10-kHz spectral width. Recycle delays of 0.2–2.0 s were used in these experiments. Sample temperatures were estimated (±1 K) from measurements of ^1H chemical shifts in ethylene glycol.[19]

Quantitation of Carbon Types. Measurements of integrated intensities as a function of ^1H–^{13}C contact time yielded semilog plots that were usually linear for times that varied between 750 and 5000 μs; zero-time extrapolation of these data provided quantitative comparisons of the signals from chemically diverse rigid carbon sites.[4] To estimate the relative numbers of rigid and mobile methylenes in the cutin structure, two experiments were performed on a single polymer sample in the MAS probe: (1) ^{13}C integrated intensities for rigid $(CH_2)_n$ groups observed by CPMAS with DD were extrapolated to zero contact time; (2) the ^{13}C integrated intensity for mobile $(CH_2)_n$'s was measured by DPMAS with SD. The times between spectral acquisitions were ≳6 ^1H T_1's and ~5 ^{13}C T_1's, respectively.

Following prior analyses of synthetic polymers,[10,20] it was assumed for $(CH_2)_n$'s that (1) a given population of carbons is observed in either CPDD or DPSD experiments, but not both, (2) center bands of the CPMAS spectra contain essentially all of the ^{13}C signal intensity, (3) the observed CP efficiency is $^3/_4$ of its theoretical value, (4) the $^{13}C\{^1H\}$ nuclear Overhauser effect $(1 + \eta)$ is 2.1 in the DP experiment. The validity of these assumptions is examined critically in the following section.

Polymer Dynamics. ^{13}C spin-lattice relaxation times (T_1's) were determined either by an inversion–recovery sequence[21] (for mobile carbons) or by a modified cross-polarization experiment[22] (for immobilized carbons). Longitudinally relaxed spectral editing runs also provided verification of the latter set of results. ^{13}C line widths (at half-height) were corrected for digital broadening. ^1H $T_{1\rho}$ values were derived for each spectral peak from the linear portion of a plot of ln (integrated intensity) vs ^{13}C–^1H contact time.

Results and Discussion

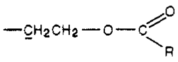
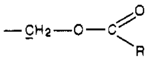
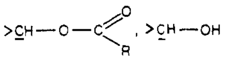
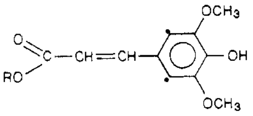
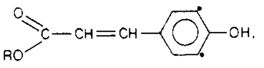
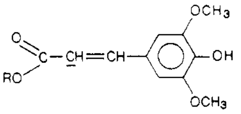
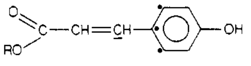
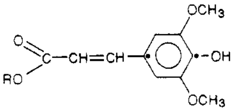
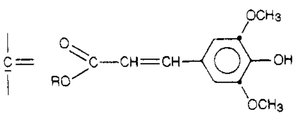
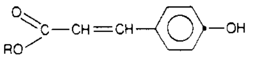
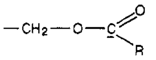
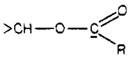
A. Identification of Carbon Types.

A typical ^{13}C CPMAS spectrum of intact lime cutin is shown in Figure 2. Table I summarizes chemical shift assignments for this material, based on ^{13}C NMR data for Hytrel,[10] other soluble polyesters,[23] depolymerized cutin, and numerous model compounds.[7,24–27]

Although chemical shifts observed in solution-state spectra must be used with caution in the interpretation of solid-state NMR results,[28] cutin exhibits numerous ^{13}C peaks expected for a polyester derived from hydroxylated fatty acids: bulk methylenes (29, 42 ppm), methylenes and methines shifted downfield by proximity to oxygens (64, 72 ppm) and carboxyl groups (168, 173 ppm). The presence of esters of both primary and secondary alcohols, as well as free secondary alcohol groups, has been deduced from chemical modification–depolymerization studies.[3,29] Assignments for these moieties are based on published chemical shift data and also ^{13}C T_1's found for specific sites in intact cutin (vide infra).

Several other ^{13}C resonances provide key structural information about lime cutin. First, evidence for ω-hydroxyoxopalmitic acid constituents (Figure 1) comes from the observation of a keto peak (209 ppm). Second, the presence of *p*-coumaric or related moieties[30]—from cutin or possibly lignin—is suggested by aromatic and olefinic peaks in the 105–150 ppm region.[7,27] (Aromatic derivatives do not appear among the soluble depolymerization products, however.[31]) These latter assignments are supported

Table I
^{13}C Chemical Shifts for Intact Cutin[a]

carbon type	shielding,[b] ppm
$-(\underline{C}H_2)_n-$	29 (has two shoulders)
	42
	64[c]
	72[c] (may be two peaks)
	105[d]
	115[d]
	120–135 (br, contribution from nonprotonated carbons)[c,d]
	
	
	147 (nonprotonated)[d,e]
	156 (nonprotonated)[d,e]
	168 (nonprotonated)[e]
	173 (nonprotonated)[e]
$>\underline{C}=O$	209 (nonprotonated)[e]

[a] From ^{13}C CPMAS spectra, obtained as described in Figure 2. [b] Referenced to external TMS (see Materials and Methods). [c] Ether or peroxide linkages are also possible.[1] [d] Assigned by analogy with lignin resonances.[27] [e] Determined from spectral editing experiments.

by delayed-decoupling editing experiments (Figure 3), though they must be considered tentative pending additional chemical evidence.

No spectral features are clearly attributable to contamination from cuticular waxes[31] or cell wall carbohydrates,[6,8] though small peaks would probably be obscured by the cutin itself. The observation of rather broad lines in the ^{13}C NMR spectrum is due to a dispersion of isotropic chemical shifts in environmentally similar methylene groups. It is also likely that lime cutin is an irregular polymer or a mixture of materials; no physical or spectroscopic evidence is currently available to demonstrate that its aliphatic and aromatic ester linked moieties are part of a single polymeric repeat unit.

B. Cross-Polarization Dynamics. It is well-known that the cross-polarization rate in a CPMAS experiment reflects the strength of static dipolar interactions, so that its value at a particular carbon site depends on both the distance from nearby protons and the local motional characteristics.[4,32] For cutin, both aliphatic and protonated aromatic signals achieve their maximum intensity within ~400 μs, whereas a 1-ms contact time is required for carboxyl and keto carbons at 173 and 209 ppm, respectively. These values exceed previously determined results for a variety of polymeric and crystalline solids,[32,33] though they are in rough agreement with cross-polarization rates for aromatic residues in lignin.[7,34] The carboxyl peak at 168 ppm does not appear to reach maximum intensity even after a CP contact time of 4 ms. Given the proximity of protons to each carbon in the polyester structure, long CP rates suggest attenuation of CH and HH dipolar interactions by molecular motion.

^1H $T_{1\rho}$ values (determined from ^{13}C signal decays) are presented for cutin in Table II. Both the long value determined for $CH_2O\underline{C}OR$ carbons, as well as the unequal values at other polyester sites, are notable. They suggest the presence of distinct polymer domains, for which the

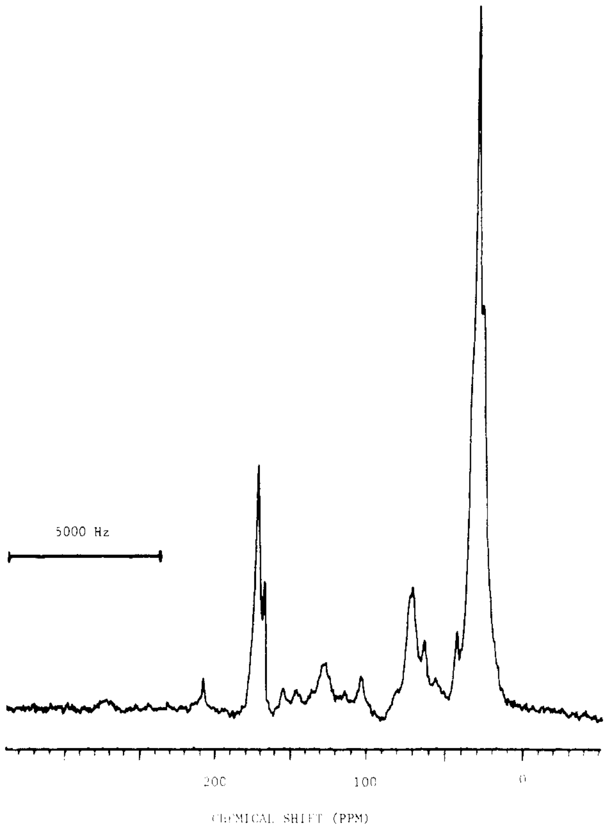


Figure 2. ^{13}C NMR spectrum of the cutin polyester, obtained with cross polarization (contact time 1.5 ms, repetition rate 1.0 s), dipolar decoupling (γB_2 = 48 kHz), and magic-angle spinning (5.0 kHz). This spectrum represents 6000 accumulations, processed with a line broadening of 20 Hz. The broad peak at 273 ppm is a spinning sideband of the carboxyl resonances.

Table II
Spin Relaxation Parameters for Immobile Carbons of Cutin

carbon type	^1H $T_{1\rho}$,[a] ms	^{13}C T_1,[b] ms
$(\underline{C}H_2)_n$	5	145 ± 22[d]
$\underline{C}H_2CH_2OCO$		192 ± 38
$\underline{C}H_2OCOR$	5	122 ± 18
$\underline{C}HOCOR$, $\underline{C}HOH$	5	>7000
aromatics, alkenes	4[c]	~1000
$CH_2O\underline{C}OR$	35	~100
$CHO\underline{C}OR$	6	~1700
$\underline{C}O$	7	

[a] From ^{13}C signal heights vs contact time (0.4–4.0 ms). Accuracy of the measurements is 20–40%. [b] From cross polarization–inversion recovery sequence. [c] Average of values for peaks at 105, 128, and 156 ppm. [d] For the major resonance at 29 ppm; shoulder peaks at 25 and 33 ppm yield T_1's of 115 and 164 ms, respectively.

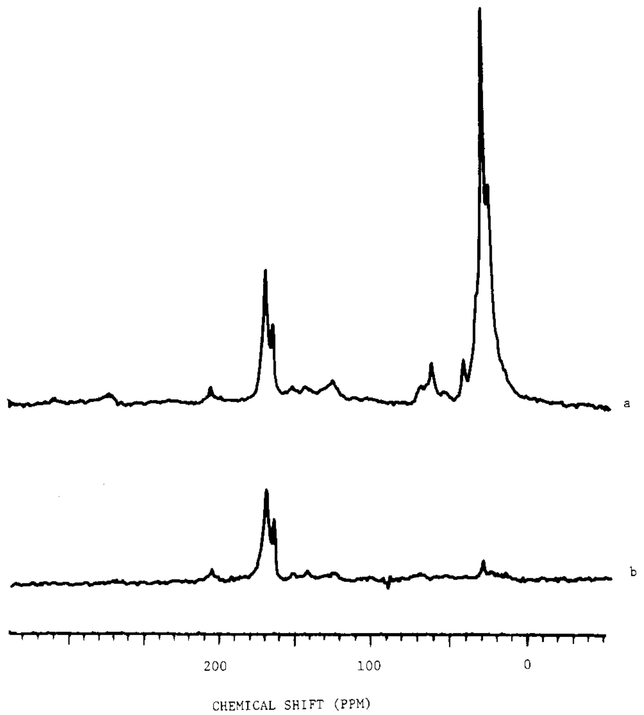


Figure 3. ^{13}C CPMAS spectra of cutin, obtained with spectral editing pulse sequences: a, ^1H decoupling delayed by 50 μs; b, delayed decoupling and 0.6-s longitudinal relaxation period.

efficiency of ^1H spin diffusion is reduced by molecular motion (see also ^{13}C T_1 results below). The fairly short $T_{1\rho}$ values, as compared with other polymeric materials,[4,33,34] indicate that cutin motions include significant spectral density contributions at frequencies near γB_2 (~50 kHz). In addition, rapid relaxation of protons in the rotating frame complicates the choice of an optimum τ_{CP} for methylene groups.

C. Polymer Dynamics: CP T_1 and Spectral Editing Experiments. Segmental motions play a major role in defining the physical properties and function of polymeric materials,[35] with NMR relaxation providing, in principle, a measure of the frequency and angular distribution of these motions. Included in Table II are ^{13}C spin–lattice relaxation times found for "solidlike" carbons which cross polarize readily. We assume in this preliminary analysis that chemical shift anisotropy does not contribute significantly to the spin relaxation.[36] Although the available signal-to-noise ratio and spectral resolution limit the accuracy with which individual ^{13}C T_1's may be determined, these values are nevertheless quite informative regarding the dynamics and structure of lime cutin.

First, average T_1's for the bulk methylene carbons are somewhat shorter than values reported previously for synthetic Hytrel polyesters,[11] indicating that in both materials substantial molecular motion occurs in the megahertz frequency regime. Similar dynamic properties are exhibited throughout the primary alcohol ester moiety, viz., $(\underline{C}H_2)_n$, $\underline{C}H_2CH_2OCOR$, $CH_2\underline{C}H_2OCOR$, and $CH_2CH_2O\underline{C}OR$ sites. These observations are confirmed in spectral editing experiments (Figure 3), where a longitudinal relaxation period of ~4 ^{13}C T_1's serves to suppress the protonated carbons of this set quite effectively.

Second, the estimated T_1 value for aromatic and alkene carbons is suggestive of a moderately rigid environment—more restricted dynamically than the aliphatic ester chain but less so than hard segments in a synthetic polyester.[11] (We assume that reorientational correlation times lie on the slow side of the T_1 curve.[24]) The ^{13}C T_1 value estimated from our experiments argues against incorporation of a coumaric acid moiety between two ester groups, though further structural inferences are unwarranted.

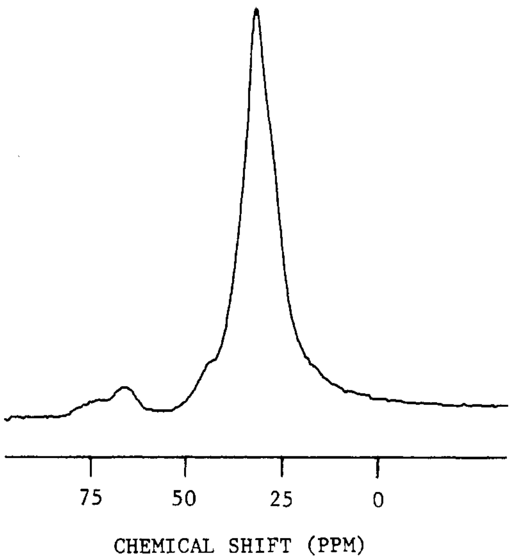


Figure 4. ^{13}C DP spectrum of cutin at 356 K, obtained with z-axis spinning and a 6-kHz decoupling field.

Finally, carbons of secondary alcohol ester groups ($\underline{C}HOCOR$ and $CHO\underline{C}OR$) are notable for their very long T_1 values. Spectral editing experiments again play a confirmatory role (Figure 3): delayed decoupling suppresses the 72 ppm $\underline{C}HOCOR$ signal because of the rigidity of this moiety, whereas the same procedure has little effect on the 64 ppm $\underline{C}H_2OCOR$ position. If $\underline{C}HOCOR$ groups constitute rigid cross-links in the cutin structure,[3,29] then it is expected that nearby $CHO\underline{C}OR$ sites will also exhibit long T_1's in the solid state.[37]

D. Polymer Dynamics: DP T_1 and Line-Narrowing Experiments. As reported previously for synthetic polyesters,[10] it is possible to observe ordinary ^{13}C direct-polarization spectra, with low-power ^1H decoupling, for those carbons in a polymer that reorient rapidly and over a large enough range of solid angle to average most chemical shift and dipolar interactions. For the natural polyester in the current study, these flexible moieties (Figure 4) may well impart resiliency to the plant cuticle.

Spin–lattice relaxation measurements for the mobile bulk methylene carbons in lime cutin yield a value of 225 ms at 305 K, which rises in an Arrhenius fashion to 420 ms at 356 K. Both the dependence on temperature and the particular T_1 values are in excellent agreement with prior results for the soft segment CH_2's of Hytrel copolymers.[10] It is probably reasonable to assume a dipole–dipole relaxation mechanism;[36] in this case the temperature dependence tells us that motions contributing to T_1 are on the fast side of the T_1 vs correlation-time curve.[24] Thus a typical methylene group in this mobile population reorients at much the same rate as Hytrel's soft-segment carbons, suggesting that it is found structurally within a long run of methylene groups with no intervening cross-links (see Figure 1).

Figure 4 and Table III compare direct-polarization spectra for cutin under contrasting conditions of temperature and spinning. At room temperature, enough motional averaging occurs to obtain a signal under conventional liquid-state acquisition conditions, but the nonspinning line width exceeds values for Hytrel[10] and styrene–isoprene copolymers[38] by factors of 5 and 3000, respectively. Since even crude analysis of the ^{13}C T_1 data gives reorientational frequencies much larger than the

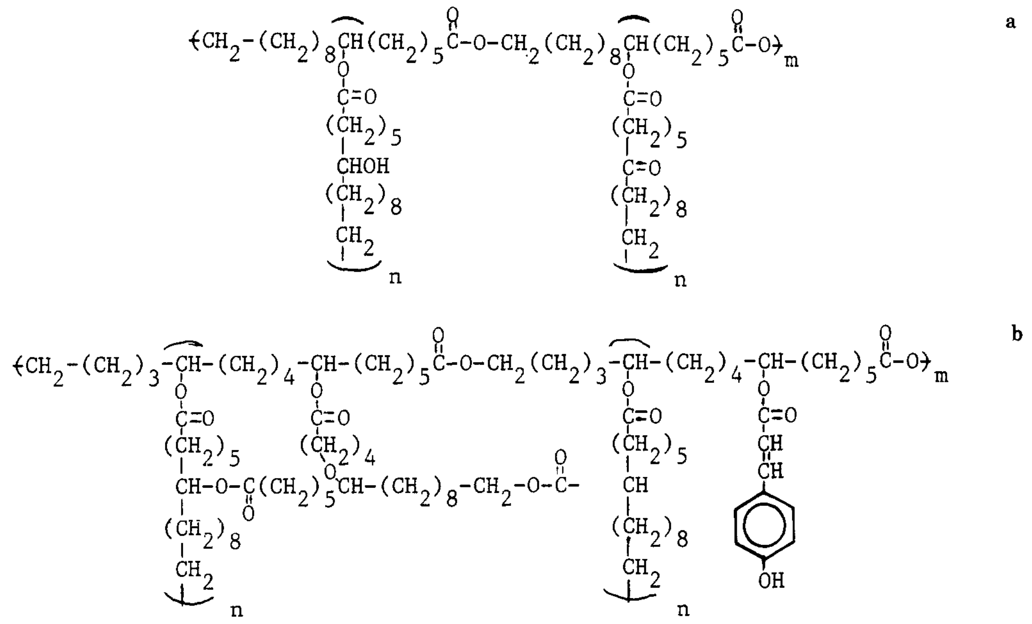


Figure 5. Structural models for lime cutin: a, aliphatic polymer with primary and secondary alcohol ester linkages; b, aliphatic–aromatic polymer with extensive cross-linking.

Table III
^{13}C NMR Line Widths for Mobile Methylene Groups of Solid Lime Cutin

expt[a]	temp, K	line width,[b] Hz
DP—no spin	304	828
DP—*z* spin[c]	304	804
DP—*z* spin[c]	356	550
DP—MAS	300	533

[a] A 6-kHz ^{1}H decoupling field was used in all experiments. [b] Full width at half-height, with no digital line broadening. Values are reproducible to within 10%. [c] Conventional spinning was about the axis of the B_0 field.

Table IV
Composition of Carbon Types in Intact Lime Cutin

carbon type	rigid moieties[a]	mobile moieties[b]
$(\underline{C}H_2)_n$[c]	51	36
$\underline{C}H_2OCOR$	1.9, 5.0[d]	*e*
$\underline{C}HOCOR$, $\underline{C}HOH$	8.1	*e*
aromatics, alkenes[f]	4.3	
$CH_2O\underline{C}OR$	(1)[g]	
$CHO\underline{C}OR$	5.5	
$\underline{C}O$	0.8	

[a] From extrapolation to zero contact time of cross-polarization signal intensities. [b] From comparison of integrated intensities for DPMAS and CPMAS experiments (see Materials and Methods). [c] Includes signals that span the chemical shift range of 25–33 ppm. [d] Derived from slopes of a biphasic decay. [e] Some signal intensity appears at elevated temperatures (see Figure 4). [f] Sum of extrapolated intensities for peaks at 105, 128, and 156 ppm. [g] Arbitrarily set to 1.

static dipolar interactions ($\lesssim 10^5$ Hz), it seems possible that some contributions to the nonspinning line width are due to a restricted angular range of motions that prevents complete averaging of chemical shift anisotropy and dipole–dipole terms. At elevated temperature the $(CH_2)_n$ peaks are narrowed, verifying that the motions are not in the extreme narrowing limit. We also observe two additional peaks at 66 and 72 ppm—presumably $\underline{C}H_2OCOR$ and $\underline{C}HOH$ moieties that are bonded to the mobile methylenes.

Magic-angle spinning at 5 kHz serves to narrow the mobile $(CH_2)_n$ resonance considerably, but its line width still exceeds 500 Hz (10 ppm). Residual dipolar broadening is not a likely explanation given the MAS conditions and T_1 results. Alternatively, it is possible to attribute the line width to chemical shift dispersion of methylene groups in an irregular polymer. It should be noted in this regard that CPMAS spectra with high-power decoupling yield a line width of 375 Hz for immobile $(CH_2)_n$'s, and solution-state ^{13}C spectra of aliphatic cutin monomers exhibit methylene chain peaks that span a chemical shift range of 1000 Hz (20 ppm).[31]

E. Quantitation of Carbon Types. One prerequisite of structural hypotheses for cutin is a determination of the relative numbers of each chemically distinct carbon atom. Estimates from ^{13}C NMR are summarized in Table IV, which includes moieties observed by both cross polarization–dipolar decoupling and direct polarization–scalar decoupling methods. As noted above, it is assumed that a given population of carbons is observed by one experiment or the other, but no carbons are counted twice. Indeed, we find that the immobilized carbons which can be cross polarized are virtually undetectable if low-power scalar decoupling is employed during signal acquisition.[39] The CP enhancement is estimated to be $^3/_4$ of the theoretically expected value of 4.[4] The $^{13}C\{^1H\}$ NOE for $(CH_2)_n$'s is measured to be 2.1.

The relative numbers of rigid groupings, as determined by extrapolating CPMAS line intensities to zero contact time, are subject to the following uncertainties: (a) $(\underline{C}H_2)_n$'s are underestimated by perhaps 10% since the peak at 42 ppm was not included in the calculation; (b) carbonyls and aromatics are underestimated by ~8% because even with motional averaging, MAS at 5 kHz may leave some signal intensity in spinning sidebands (see Figure 2); (c) aromatics and alkenes are poorly estimated due to limited signal-to-noise ratio.

Despite these uncertainties, we can use the information in Table IV and previously published hypotheses[1] to draw several conclusions about the cutin structure. First, the proportion of $(CH_2)_n$'s as compared with carbonyls or with aliphatics bonded to oxygen does fit reasonably with a C_{16} aliphatic polyester such as that shown in Figure 5a. Aromatic moieties could be present additionally as side chains or cross-links. However, the apparently large number of rigid secondary alcohol ester carbons (Tables II and IV) suggests a more heavily cross-linked structure (Figure 5b). Following this latter hypothesis, cross-linked regions are resistant to depolymerization and some monomers fail to appear among the soluble products.[3] The fragment in Figure 5b does not account for all of the compositional data in Table IV, however; the cutin polymer may also contain a variety of ester, ether, and peroxide linkages.[1]

Second, a polymer based on the constituents of Figure 1 is expected to exhibit at least as many mobile as immobile methylene groups rather than the opposite ratio found from our NMR measurements. A high degree of cross-linking could give these groups enough rigidity to permit cross polarization yet enough mobility to retain short ^{13}C T_1's. An alternative scenario includes highly substituted monomers—without long runs of $(CH_2)_n$'s and thus with fewer mobile groupings (Figure 5b). These latter constituents may well be resistant to chemical depolymerization methods.[3]

Summary

These results demonstrate the feasibility of ^{13}C NMR structural studies for the biopolyester cutin. CPMAS and DP techniques yield high-resolution spectra for relatively rigid and mobile carbons, respectively. Carbon chemical shift assignments indicate the presence of both aliphatic and aromatic constituents and verify that cutin contains ester linkages to both primary and secondary alcohols. Although aromatics are usually absent in cutin's soluble depolymerization products,[3,13] the intact polyester includes a fair number of such moieties.

Dynamic information about cutin is available from a combination of cross-polarization rates, ^{1}H $T_{1\rho}$'s, ^{13}C T_1's, line widths, and spectral editing experiments. For those carbons that cross polarize, significant molecular motion is indicated in both the megahertz and kilohertz frequency regimes. (^{13}C $T_{1\rho}$ measurements are in progress to characterize the low-frequency motions more completely.[39]) Inefficient spin communication is evident among aliphatic, aromatic, and carbonyl regions, though our molecular model for cutin is not refined enough to permit further interpretation. Bulk methylene and primary alcohol ester groupings exhibit short ^{13}C T_1's and extensive motions, but secondary alcohol esters appear to be involved in more rigid cross-links of the polymeric matrix. For those methylene carbons observed by direct-polarization methods, rapid motions over a restricted range of solid angle are implicated by ^{13}C T_1 and line-width behavior. In sum, this dynamic profile depicts the structural component of plant cuticle as a flexible netting, constrained only by specific cross-links (and/or the addition of lipid waxes). Both the resiliency of the cuticle and its susceptibility to fungal attack may be responsive to the number of cross-links in its structure.

On the basis of quantitative analysis of carbon signal intensities, analysis of chemical depolymerization products,[3,13,31] and prior suggestions,[1] we have proposed a working model for the structure of lime cutin. This model must be viewed as preliminary: it does not fit the data uniquely and we have not verified the identity of all proposed chemical linkages. In addition to investigations of intact cutin, ongoing work includes analysis of depolymerization-resistant materials and efforts to achieve selective depolymerization enzymatically. These experiments should provide important new information at the molecular level regarding the mechanism by which terrestrial plants interact with their environment.

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