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Nuclear Magnetic Resonance Relaxation Studies of Plant Polyester Dynamics. 1. Cutin from Limes

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ABSTRACT: Magic angle spinning (MAS) ^{13}C NMR results are reported for intact lime cuticle and its two major components, cutin and wax. DPMAS and CPMAS experiments permit determination of the numbers of relatively mobile and immobile carbons in each biopolymer sample. $T_{1\rho}(C)$ and $T_1(C)$ relaxation experiments characterize kilohertz- and megahertz-regime motions, respectively; they indicate that motional restrictions are present at cross-links of the cutin polymer and along alkyl chains of the wax alone. Values of $\langle T_{1\rho}(C)\rangle$ and $\langle T_1(C)\rangle$, which differ significantly for analogous carbon sites of cutin and wax individually, approach common values for the two materials in the intact lime cuticle. These results, together with measurements of $T_{1\rho}(H)$, provide evidence for hydrophobic association within the plant cuticle of the long aliphatic chains of cutin and wax.

Introduction

It is well established that molecular motion provides a link between polymer molecular structure and bulk mechanical behavior.[1] Particular attention has been focused on local segmental reorientation, though overall conformational changes and chain translation also help to determine properties such as the modulus of a polymeric material. Magnetic resonance experiments have proven to be a rich source of such dynamic information about polymers.[2,3]

Much of the work in this area has focused on synthetic compounds. For example, efforts to understand the piezoelectric and mechanical properties of poly(vinylidene fluoride) have included ^1H and ^{19}F NMR relaxation studies of chain dynamics for both the crystalline and amorphous components of this material.[4] The mechanical impact strength of commercial polycarbonates and other glassy polymers has been rationalized in terms of mid-kilohertz main-chain motions, which can be studied via rotating-frame carbon relaxation ($T_{1\rho}(C)$) measurements conducted under cross-polarization magic angle spinning (CPMAS) conditions.[5] The thermoplastic behavior of Hytrel copolyesters has been examined by employing a battery of ^{13}C and ^2H solid-state NMR techniques to characterize the dynamics of individual backbone sites in both hard and soft polymer segments.[3]

The dynamics of natural polymers are no doubt important in understanding their properties as well, though the complexity of such materials has slowed their characterization. For intact plant materials such as lignin, cutin, and suberin, several recent solid-state ^{13}C NMR studies have identified and quantified the principal chemical moieties;[6-10] for cuticular support polymers such as lime cutin, segmental flexibility has been assessed from ^{13}C spin–lattice relaxation ($T_1(C)$) measurements.[9] We report herein an extensive dynamics study of lime cuticle along with its cutin and wax constituents, including determinations of $T_1(C)$, $T_{1\rho}(C)$, and $T_{1\rho}(H)$ and the proportions of liquidlike and solidlike carbons for each plant material. These results are interpreted in terms of the resiliency of the cuticle and its function as a barrier to invasion by fungal pathogens.[11,12]

Materials and Methods

Cuticle from Limes. Following published protocols,[13,14] the peels from 20 limes were treated to remove pectin and cellulose. The resulting cuticular material was either kept intact or treated further to separate the cutin and wax components. A typical preparation yielded 800 mg of cutin polymer and 80 mg of waxes. Samples for solid-state NMR (175–300 mg) were ground

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with a Wig-L-Bug amalgamator (Spex Industries) and packed into cylindrical double-bearing rotors made from Al_2O_3, BN, or Kel-F.

NMR Spectroscopy. Solid-state ^{13}C NMR spectra were obtained at ambient temperature (298 K) on two instruments: an IBM Instruments WP-200 (^{13}C resonance frequency of 50.33 MHz) equipped with high-power amplifiers and a probe supplied by Doty Scientific (Columbia, SC) and a homebuilt spectrometer (^{13}C resonance frequency of 31.94 MHz). Magic angle spinning speeds were 5.0 and 3.0 kHz, respectively. CPMAS ^{13}C NMR experiments used matched spin-lock cross-polarization transfers with 48–50-kHz B_1's and ^1H decoupling fields of 48–65 kHz (dipolar decoupling). ^1H–^{13}C contact times were varied between 0.10 and 12.0 ms in order to obtain values for $T_{1\rho}(H)$ and T_{CH} (RELAX(ation), 3M Co.) as well as information regarding chemical composition.[9,15] A recycle delay of 1 s was inserted between spectral acquisitions.

Direct-polarization magic angle spinning (DPMAS) ^{13}C NMR experiments monitored the signal following a 90° pulse, using a noise-modulated continuous-wave ^1H decoupling field of 5 kHz (scalar decoupling). Recycle delays of 2–5 s were used in these experiments.

^{13}C spin–lattice relaxation times ($\langle T_1(C)\rangle$) were measured from the recovery of ^{13}C magnetization that was inverted following cross polarization.[16] ^{13}C rotating-frame relaxation times ($\langle T_{1\rho}(C)\rangle$) were derived from measurements of the carbon signal that remained after a $T_{1\rho}(C)$ spin-lock of 0.05–12.0 ms following cross polarization. The spin-locking field, $B_1(C)$, was varied between 37 and 60 kHz in separate relaxation experiments. Average values $\langle T_1(C)\rangle$ and $\langle T_{1\rho}(C)\rangle$ were obtained from the initial decay of each carbon signal.[2,3]

Results and Discussion

A. Mobile and Immobile Carbon Types. Figure 1 compares CPMAS ^{13}C NMR spectra acquired for lime cutin and intact cuticle under identical experimental conditions. The principal new spectral peak from the wax component appears in the bulk methylene region, though some additional (unresolved) signal contributions from oxygenated carbons are suggested by both solution- and solid-state NMR of the wax alone (data not shown). The presence of both of these peaks is expected in light of prior reports that citrus cuticular wax is composed of C_{17}–C_{35} saturated aliphatic hydrocarbons, acids, and aldehydes.[18] Although the wax component is found to comprise roughly 10% by weight of the cuticle sample, deconvolution of the bulk methylene signals indicates that it contributes 20% of the CPMAS NMR signal intensity between 20 and 40 ppm. Waxes appear to be overrepresented primarily because methylene carbons predominate in this component and have a narrow dispersion of chemical shifts. For the intact lime cuticle, a distinct carboxyl carbon peak also appears at 168 ppm if the experiment is carried out with CP contact times greater than 5 ms. This latter resonance may be attributed to exogenous oxalate (from the cutin isolation procedure) or to malate (secreted by the plant).

Even for solid polymer samples, carbon segments which reorient at rates exceeding 10^5 Hz may exhibit ^{13}C NMR signals in direct-polarization (DP) NMR experiments, as demonstrated previously for synthetic materials[19,20] and for lime cutin.[9] The dynamic situation for methylene carbons of intact lime cuticle and its two constituents is reflected in Table I, which estimates the proportion of such carbons that exhibit enough motional freedom to isotropically average most CH dipolar interactions. Only for the cutin polymer alone is there a substantial proportion of very mobile carbons; for both the wax and the cuticular assembly, the immobilization of most methylene–carbon segments is sufficient at room temperature to allow cross-polarization transfer and to require high-power decoupling in ^{13}C NMR spectra. The latter situation is also found for CH_2's of suberin, a biopolyester that grows within the cell wall of wound-healing plant tissues.[21]

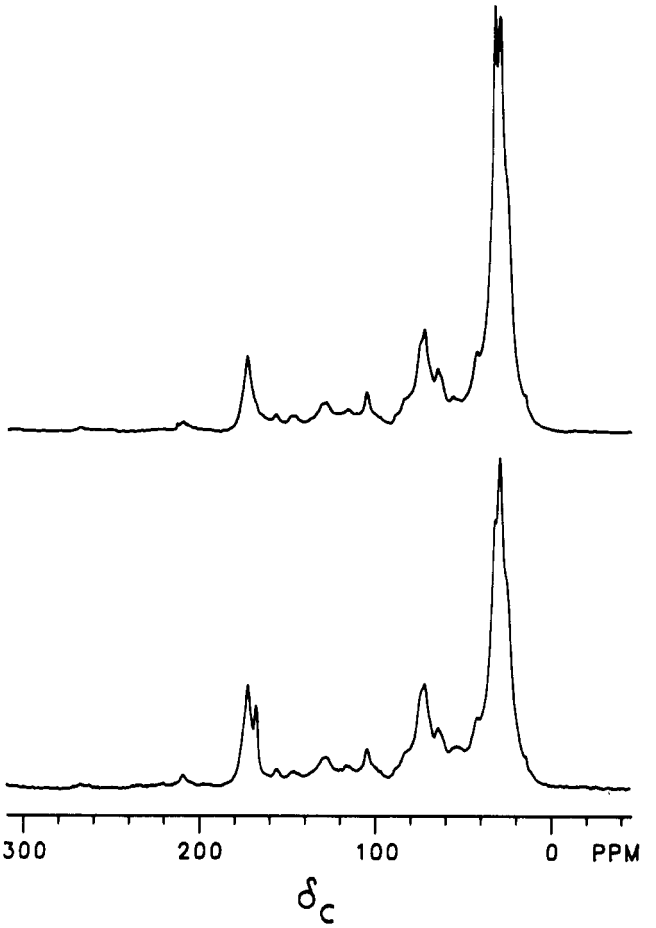

Figure 1. 31.94-MHz ^{13}C NMR spectra of cuticular material from limes, obtained with cross polarization (contact time 1.0 ms, repetition rate 1.0 s), magic angle spinning (3.0 kHz), and dipolar decoupling ($\gamma B_2/2\pi$ = 65 kHz). Each spectrum is processed with a digital line broadening of 20 Hz. Chemical shift assignments are summarized in Table II. Top: intact cuticle; 237 mg, 60 000 transients. Bottom: cutin; 286 mg, 50 000 transients. Spectra are plotted with corresponding 70 and 105 ppm signals set to the same height (a somewhat different format was presented previously[17]).

Table I
Methylene Carbons[a] in Lime Cuticle

sample[b]	motionally averaged carbons,[c] %
cutin	59[d]
cutin–wax	22[e]
wax	19

[a] Bulk methylenes between 20 and 40 ppm; oxygenated methylenes at 64 ppm exhibit qualitatively similar trends. [b] Preparation methods are described in Materials and Methods (above, ref 9 and 10). [c] From comparison of integrated intensities for DPMAS and CPMAS ^{13}C NMR experiments, with ^1H decoupling fields of 5 and 65 kHz, respectively. DP measurements are adjusted for the $^{13}C\{^1H\}$ NOE; CP measurements are adjusted for the CP enhancements (2.5) and extrapolated to zero CH contact time. (See ref 9.) Based on the uncertainties of these procedures, error limits are estimated to be 15%. [d] Reported (incorrectly) as 41% in ref 9. [e] Partially overlapped cutin and wax peaks have been treated as a single entity.

B. Megahertz Motions for Those Carbons Which Cross Polarize. The bulk modulus and other mechanical properties of solid polymeric materials are thought to depend on segmental reorientation occurring on the megahertz time scale,[1] rendering such behavior amenable to study via measurements of spin–lattice NMR relaxation times $T_1(C)$. For the solidlike carbon moieties of

Table II
^{13}C Spin-Lattice Relaxation Parameters for Solidlike Carbons in Lime Cuticle

carbon type	chemical shift, ppm	$\langle T_1(C)\rangle$,[a] ms: Hytrel:[b] 50 MHz	cutin: 32 MHz	cutin: 50 MHz[c]	cutin–wax: 50 MHz	wax: 50 MHz
$(CH_2)_n$, cutin	29	160–200	200	190	120	
$(CH_2)_n$, wax	33				160	4700
CH_2OCOR	64	190–200	240	190	100	
CHOCOR, CHOH[d]	72		(5000)	(5600)	(2000)	
COO[e]	174	>10000	(4600)	(2300)	(~1100)	

[a] From cross polarization–inversion recovery pulse sequence. Average values $\langle T_1(C)\rangle$ are derived from signal intensities at 0.0–0.1 or 0.0–0.25 s. The longer values in (parentheses) are derived from signal intensities at 0.25–2.5 s, though a rapidly recovering component also appears to be present. Uncertainties in the measurements are ~20%. [b] Range of T_1 values corresponding to a hard-segment mole fraction of 0.80–0.96. From ref 22. [c] Data from ref 9 have been analyzed as described above. [d] This peak remains in the spectrum after exhaustive treatments with cellulase, pectinase, and hemicellulase.[21] [e] Predominantly CHOCOR.

Table III
^{13}C Rotating-Frame Relaxation Parameters for Carbons in Lime Cuticle

carbon type	$\langle T_{1\rho}(C)\rangle$,[a] ms: 37 kHz cutin	37 kHz cuticle	37 kHz wax	44 kHz cutin	44 kHz cuticle	44 kHz wax	50 kHz cutin	50 kHz cuticle	50 kHz wax	60 kHz cutin	60 kHz cuticle	60 kHz wax	average[b] cutin	average[b] cuticle	average[b] wax
$(CH_2)_n$, cutin	2.4	2.3		2.8	2.6		3.3	2.8			3.3		2.8	2.6	
$(CH_2)_n$, wax		2.5	3.1		3.2	5.1		3.7	6.1		4.5	9.5		3.5	6.0
CH_2OCOR	3.4	2.8		4.0	3.4		5.3	3.6			4.5		4.2	3.3	
CHOCOR, CHOH	6.7	5.3		8.7	7.9		12.4	7.5			9.9		9.3	6.9	

[a] From short-time behavior (0.05–1.00 ms) of ^{13}C magnetization held in the indicated fields after spin locking and cross polarization from ^1H. These values, measured at a ^{13}C resonance frequency of 32 MHz, have an estimated uncertainty of 15%. [b] Arithmetic average of values observed with spin-lock fields of 37–50 kHz (for wax $(CH_2)_n$, 37–60 kHz).

lime cuticle and its two chemical constituents, this relaxation information is summarized in Table II.

As expected, the spin-relaxation parameters depend dramatically on which chemical group is examined. Bulk methylene carbons of the wax alone exhibit long $T_1(C)$'s, indicating surprising motional restrictions for its aliphatic chains. In contrast, the short $T_1(C)$'s found for the bulk methylenes of cutin indicate considerable segmental mobility. When the two components are present as a cuticular assembly, both types of methylenes become even less restricted motionally than the hard-segment CH_2's of Hytrel copolyesters.[22] As noted previously for lime cutin itself,[9] other methylene–carbon segments within the primary alcohol ester moiety (CH_2OCOR) also possess a substantial degree of reorientational freedom as judged from measurements of $T_1(C)$. The rather modest dependence of these short values on ^{13}C resonance frequency confirms the importance of motions occurring more rapidly than the nuclear precessional frequency.

For carbons that belong to esters of secondary alcohols (CHOCOR), the $T_1(C)$ values for both cutin and lime cuticle are much longer than would be predicted from the CH_2OCOR data, assuming that spin relaxation occurs via dipolar interactions with attached protons.[23] The CHOCOR groups are thus much more rigid than the long-chain aliphatic groupings described above, as expected if they are involved in a cross-linking network. The particular T_1 values reported here indicate that CHO segments in cutin are less restricted than those in cellulose[24] but comparable dynamically to the polysaccharides in plant cell walls.[21] COO segments (predominantly secondary alcohol esters) are also relatively immobile on the megahertz time scale but clearly less so than corresponding carbon segments in Hytrel.[22]

The impact of cuticle formation on local dynamics is reflected in the $T_1(C)$ values for corresponding carbon segments in different physical states. To properly interpret these T_1's, however, CPMAS and DPMAS signal intensities (section A) must also be considered. Since close to 20% of the wax carbons give NMR signals under DPMAS (low-power decoupling) conditions in both cases, the change in methylene $T_1(C)$'s from 4700 to 160 ms may be interpreted directly to indicate dramatically reduced local motional restrictions in the assembled cuticle. Similar comparisons of $T_1(C)$ for $(CH_2)_n$ groups in cutin also suggest, on first glance, that the intact cuticle exhibits enhanced segmental reorientation. However, Table I reveals a concomitant shift from liquidlike to solidlike bulk methylenes. Thus some of the very mobile carbon segments observed by DPMAS for the cutin polymer have been added to the population seen by CPMAS methods for intact lime cuticle. These latter carbon segments no longer experience sufficient motional averaging to give ^{13}C NMR spectra under low-power decoupling, but their contributions do shorten the average $\langle T_1(C)\rangle$. A similar degree of segmental mobility is found for the solidlike methylene groups of both wax and cutin constituents in the cuticular assembly.

Other carbon moieties of the cutin polyester contribute NMR signal intensity only to the CPMAS spectrum at room temperature, regardless of whether the polymer is present alone or in conjunction with wax. Thus the halving of $T_1(C)$ values observed for CH_2OCOR, CHOCOR, and COO groups of the intact cuticular assembly may be taken to indicate substantially enhanced rates of segmental reorientation.

C. Mid-Kilohertz Motions for Those Carbons Which Cross Polarize. A complementary view of cuticular dynamics is obtained from measurements of $T_{1\rho}(C)$, which are summarized in Table III and Figure 2. These results are expected to reflect the efficiency of motions on the kilohertz time scale.[5,22,25] In physical terms, the $T_{1\rho}(C)$ values provide information on the slower overall undulations of a solid, such as cooperative main-chain modes that might be linked to cuticular resiliency.

For both the cutin polymer and the cuticular assembly, the efficiency of mid-kilohertz motions appears to be greatest for $(CH_2)_n$ groups in long acyl chains and least for CHOCOR carbons of the polymer cross-links. This trend follows that deduced for megahertz motions from

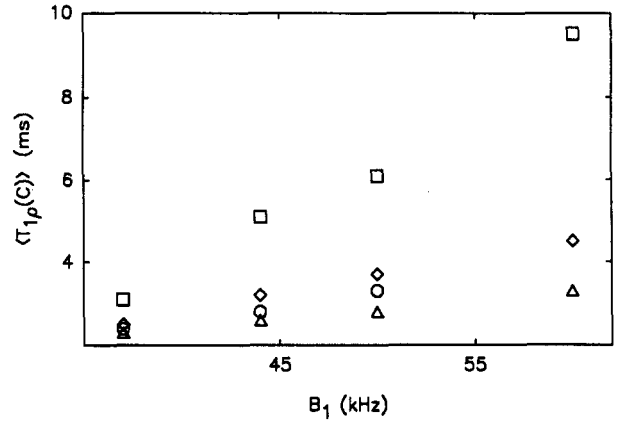

Figure 2. Average rotating-frame relaxation times ($\langle T_{1\rho}(C)\rangle$) for methylene carbons in lime cuticle samples. Cutin: alone (○) and intact cuticle (△). Wax: alone (□) and intact cuticle (◇). With a 37-kHz spin-locking field, the values of $\langle T_{1\rho}(C)\rangle$ are almost identical for cutin alone, cutin in cuticle, and wax in cuticle.

values of $T_1(C)$ (section B), though a much smaller spread of $\langle T_{1\rho}(C)\rangle$ values is found for both cuticular samples.

The magnitude of $\langle T_{1\rho}(C)\rangle$ for cutin $(CH_2)_n$ groups in both of these physical states is smaller and less dependent on spin-lock field strength (B_1) than in synthetic aliphatic–aromatic polyesters.[22] By contrast, $(CH_2)_n$'s of the wax component alone display $\langle T_{1\rho}(C)\rangle$ values that are longer and more sensitive to B_1; their relaxation behavior also resembles that of oxygen-bound methylenes in cutin and suberized cell wall.[21] Cutin CHOCOR groups appear to have much longer $\langle T_{1\rho}(C)\rangle$'s, but when the number of attached protons is taken into account their relaxation parameters approach those of the CH_2OCOR groups. Values of $\langle T_{1\rho}(C)\rangle$ for CHOCOR moieties are 2–3 times shorter in cutin than in butyral derivatives of poly(vinyl alcohol).[26] Cutin CHOCOR groups also exhibit a strong dependence of $\langle T_{1\rho}(C)\rangle$ on B_1, implying that those motions which contribute to the spin-relaxation process have mid-kilohertz to low-kilohertz frequencies. In each of these cases, plots of ^{13}C signal intensity vs spin-lock time are nonexponential, suggesting that a distribution of motional characteristics exists for structurally similar carbons within these heterogeneous polymeric materials.[5]

As with measurements of $\langle T_1(C)\rangle$, the impact of cuticle formation may be assessed by looking at values of $T_{1\rho}(C)$ for $(CH_2)_n$, CH_2OCOR, and CHOCOR groups that occur in the physical states represented by cutin, wax, and the cuticular assembly. Substantial convergence of both $\langle T_1(C)\rangle$ and $\langle T_{1\rho}(C)\rangle$ relaxation parameters is found to occur for $(CH_2)_n$'s of the intact cuticle (Figure 2), an observation consistent with hydrophobic association of the chain methylenes from each species. As compared with the individual constituents, a shortening of $\langle T_{1\rho}(C)\rangle$ for the lime cuticle suggests there is more mid-kilohertz spectral density for all three types of carbon functionalities. In fact, a qualitatively similar shortening effect has been observed in plasticized butyral derivatives of poly(vinyl alcohol).[26] Finally, the ratio $T_{CH}/\langle T_{1\rho}(C)\rangle$, which has been correlated previously with the impact strength of various synthetic polymers,[5] rises modestly for bulk methylenes of the wax when that component is within a cuticular sample (T_{CH} is ~70 μs in both physical states).

D. Cutin–Wax Mixing. The relaxation parameter $T_{1\rho}(H)$, measured through the cross-polarization behavior of different carbon resonances, may also be used to characterize kilohertz motions in polymers. This relaxation time reflects the strength of ^1H–^1H dipolar interactions and indicates whether the various protons belong to a common spin reservoir. Thus the $T_{1\rho}(H)$ experiment also provides structural information, since the measured values depend on whether the protons of two solid materials are sufficiently rigid and close in space to be "intimately" mixed.[27]

Table IV
Rotating-Frame Proton Relaxation Times in Lime Cuticle

carbon type[a]	$\langle T_{1\rho}(H)\rangle$,[b] ms		
	cutin	cutin–wax	wax
$(CH_2)_n$, cutin	3.4	3.8[c]	
$(CH_2)_n$, wax		3.9[c]	4.2
CH_2OCOR	4.5	3.8	
CHOCOR, CHOH	4.6	4.0	
protonated aromatics[d]	3.9		
CH_2OCOR[e]	39	22	
CHOCOR	5.7	5.4	

[a] Chemical shifts are as noted in Table II. [b] Average values obtained from ^{13}C signal heights vs cross-polarization (CP) time (1.0–5.0 ms for aliphatics and aromatics; 5.0–12.0 ms for CH_2OCOR; 1.5–12.0 ms for CHOCOR). These measurements, conducted at 32 MHz, have an estimated uncertainty of 15%. [c] Despite some signal overlap, both peaks exhibit clearly parallel behavior as the CP time is varied. [d] Major peaks at 115 and 128 ppm. [e] May arise from oxalate or malate.

Table IV summarizes $T_{1\rho}(H)$ relaxation parameters for lime cuticle. Anomalously long $\langle T_{1\rho}(H)\rangle$ values are measured for the sharp CH_2OCOR resonances; these carbon moieties could be part of a separate cuticular domain, but it is more plausible to attribute them to secreted malate[12] or to oxalate used in the isolation procedure. As judged from measurements at the other carbon sites, the magnitudes of $\langle T_{1\rho}(H)\rangle$ are between 3 and 7 ms, shorter than for lignin, cellulose, and many synthetic polymeric materials[2,28] but comparable to values reported for suberized cell wall and intact herbage samples.[6,21] The values found for wax are more than 1 order of magnitude shorter than the 61-ms value measured for crystalline magnesium stearate. As noted previously for cutin itself,[9] the short relaxation times indicate the importance of motional frequencies near 50 kHz; their general trends also confirm the dynamic profile deduced from $T_{1\rho}(C)$ measurements (section C).

Nevertheless, both the absence of a common $\langle T_{1\rho}(H)\rangle$ and the fact that the values are generally longer for the proton-poor carbonyl sites indicate that spin diffusion is incomplete. The variances in $\langle T_{1\rho}(H)\rangle$'s are greatest for waxes alone, least for the intact cuticular assembly. In addition, biexponential relaxation curves are observed for many carbon types in each of the polymer samples, again demonstrating a distribution of $T_{1\rho}(H)$ values. Though it is possible to infer the presence of separate polymer domains in each of these samples, it is more likely, given the relaxation results of sections B and C, that spin communication is simply rendered inefficient by molecular motion.[27]

For bulk methylenes of the individual cutin and wax components, different values of $\langle T_{1\rho}(H)\rangle$ are observed (Figure 3, top). Yet for the cuticular mixture, a common value of $\langle T_{1\rho}(H)\rangle$ is measured (Figure 3, bottom), again providing evidence for close association between hydrophobic regions of the two components. The intact cuticle is distinguished, then, by the fact that both sets of methylene groups have common values of $\langle T_1(C)\rangle$, $\langle T_{1\rho}(C)\rangle$, and $\langle T_{1\rho}(H)\rangle$. By contrast, moieties from each chemical constituent in a suberin–cell wall assembly retain distinct values of these spin-relaxation parameters.[21]

Conclusions

Molecular motion is a key factor in determining the mechanical properties of solid polymers. CPMAS ^{13}C

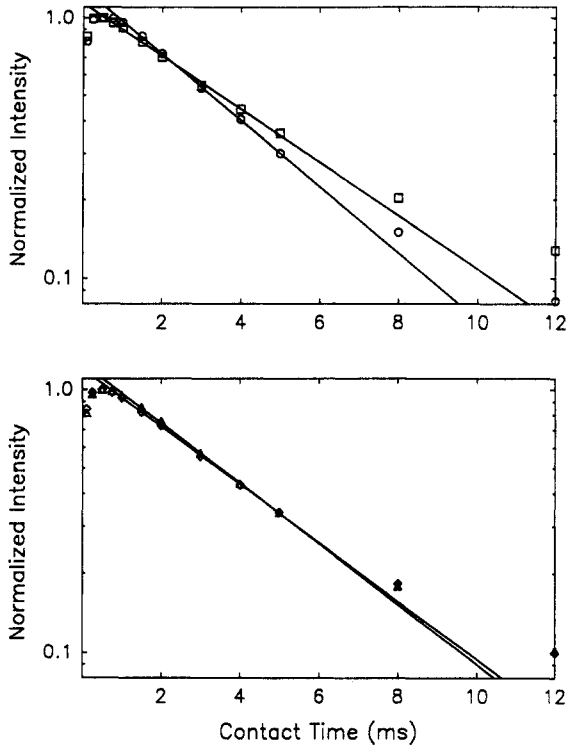

Figure 3. Plots of the bulk methylene carbon signal intensities as a function of CP contact time for cutin and wax. The straight lines represent least-squares fits of data acquired with contact times of 1.0–5.0 ms. Top: separate components. Bottom: intact cuticle. Cutin: alone (○) and intact cuticle (△). Wax: alone (□) and intact cuticle (◇).

NMR has proven to be an excellent and informative probe of molecular dynamics for the cutin and wax components of an intact biopolymer mixture. Moreover, detailed motional information is available for particular chemical groupings within each component. By combining the results of DPMAS, CPMAS, $T_{1\rho}$(H), T_1(C), and $T_{1\rho}$(C) measurements on lime cuticle and its constituents, we have established the efficiency of mid-kilohertz and megahertz motions in all three materials compared to related synthetic polymers and crystalline compounds. These data have also been used to develop a physical picture of hydrophobic association and resiliency in the cuticular assembly.

The segmental motions (megahertz time scale) that are thought to influence the bulk modulus of polymeric materials have been studied with measurements of T_1(C) and through the comparison of ^{13}C signal intensities in DPMAS and CPMAS spectra. Sufficient motional averaging occurs so that 60% of the methylene carbons in cutin are observed under low-power decoupling conditions (DPMAS); 80% of the wax and cuticle methylenes, plus essentially all other carbon moieties, benefit from cross-polarization enhancement and require dipolar decoupling. Among these latter solidlike carbon segments, $(CH_2)_n$'s of the cutin and cuticle acyl chains have sufficient spectral density at megahertz frequencies to produce efficient T_1 relaxation (100–240 ms), but $(CH_2)_n$'s of the wax and CHOCOR's of the rigid cutin cross-links relax much more slowly (up to 5.6 s). A qualitatively similar contrast between $(CH_2)_n$ and CHOCOR moieties is found for values of $\langle T_{1\rho}(C)\rangle$, which are sensitive to cooperative mid-kilohertz motions that may influence cuticular toughness and resiliency.

Solid-state ^{13}C NMR spectra and spin-relaxation measurements have also provided useful information about changes in resiliency and hydrophobic association that accompany cuticle formation. Although the presence of wax reduces the proportion of cutin methylenes that yield motionally averaged NMR spectra, it nevertheless enhances megahertz motions (for both constituents) as judged from a shortening of $\langle T_1(C)\rangle$ values. Thus, filling the cutin polymeric network with wax may serve to restrict the *range* of angular excursions for long-chain CH_2 groups but simultaneously enhance the *rate* of rapid segmental motion at these sites. Both the shortening of $\langle T_{1\rho}(C)\rangle$ (for all carbon sites) and the rise in the ratio $T_{CH}/\langle T_{1\rho}(C)\rangle$ (for wax $(CH_2)_n$'s) indicate more efficient mid-kilohertz motions and suggest that the cuticular assembly possesses greater resiliency or impact strength. Hydrophobic association of the cutin and wax methylenes within the lime cuticle is suggested by the establishment of a common $\langle T_{1\rho}(H)\rangle$ value for these two components and supported by convergence of both their $\langle T_1(C)\rangle$ and $\langle T_{1\rho}(C)\rangle$ values.

From a functional point of view, these studies suggest that, although the wax constituent may have waterproofing potential for the plant, it requires the cutin support in order to actually form a resilient cuticular barrier to the environment. The intimate mixing of the wax and cutin components is expected in light of their hydrophobic properties, and this mixture may also be viewed as a hydrocarbon "solvent" for the action of degradative enzymes such as fungal cutinase. The assessment of toughness in these biopolymer materials is also potentially significant, since the formation of cracks in the cuticular veneer is known to promote invasion of the plant by pathogens.[12]

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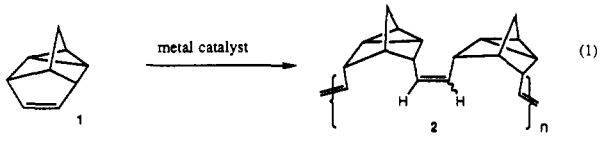
Communications to the Editor

Synthesis and Epoxidation of Cis-Enriched Polydeltacyclene

The epoxidation of polyolefinic compounds is an area of current interest due to the ability to modify the physical properties of the polymer.[2] The chemical reactivity imparted by the highly strained epoxide is also of great importance. For example, the synthesis of epoxy resins provides commercially important materials with a diverse range of applications including their use as thermosetting resins, as protective coatings, and as matrices in composites. Polyepoxides are essential components in many adhesives and are used as stabilizers and plasticizers for vinyl resins.[3]

As part of our interest in the synthesis and utility of a new polymer, polydeltacyclene **2**, prepared via a ring opening metathesis polymerization (ROMP),[4] we have investigated the behavior of **2** toward epoxidizing agents in order to prepare highly strained and rigid polyepoxy polymers. In this article, we report the synthesis and characterization of cis-enriched polydeltacyclene and the reactivity of **2** and polynorbornene with dimethyldioxirane.

We have recently shown that deltacyclene, **1**, can be efficiently polymerized in the presence of ruthenium trichloride to yield polydeltacyclene **2** as a mixture of cis and trans isomers, eq 1.[5] This result is in contrast to



(1)

the behavior of norbornene, which gives predominantly the trans polyolefin in the presence of ruthenium trichloride.[6] Since our monomer had behaved quite differently compared to norbornene, it was of interest to examine the olefin stereoselectivity in the presence of other catalysts that have been successfully used to effect highly cis stereoselective polymerizations of norbornene.[4a]

Changing the catalyst from $RuCl_3$ to WCl_6/Ph_4Sn[7,8] effected a smooth polymerization of deltacyclene and provided a white solid in good yield (73%) and of moderate molecular weight M_w = 20 000. Analysis of the ^1H NMR spectrum indicated a more selective reaction had taken place, yielding **2**, with the cis geometry predominating. Three peaks at 5.46, 5.55, and 5.64 ppm corresponding to the isomeric olefinic protons were observed, and integration of these peaks indicated a 70:30 cis/trans mixture. Integration of signals at 2.47 and 2.18 ppm, which we assign to the allylic protons, provided a second means of measuring the stereoselectivity of the reaction. In the ^{13}C NMR spectrum, the signals at 11.1, 14.8, and 16.2 ppm are assigned to cyclopropane carbons while those at 35.8, 41.1, 43.7, and 48.2 ppm are assigned to aliphatic carbons. Expansion of the region between 129.4 and 132.8 ppm revealed five peaks, which are assigned to the olefinic carbons.

$ReCl_5$ was substantially more cis stereoselective than the tungsten or ruthenium catalysts. The reaction was most effectively carried out by addition of deltacyclene to a flask containing the catalyst in the glovebox at room temperature *in the absence of any solvent*. Under these conditions a solid was isolated in moderate yield (48%), which, by ^1H and ^{13}C NMR, was >95% *cis*-**2**. A low molecular weight material (M_w = 6000) was also obtained in ca. 20% yield.[9] The GPC of pure *cis*-**2** indicated that the polymer was of very high molecular weight (M_w = 986 000). The ^1H NMR spectrum showed a single resonance at 5.48 ppm, which was assigned to the cis olefinic hydrogens and a signal at 2.48 ppm for the allylic protons (Figure 1A). The ^{13}C NMR spectrum (Figure 1C) contains six peaks at 11.3, 16.3, 35.7, 40.7, 43.8, and 131.2 ppm, which indicates a highly stereoregular polymer was formed. It is interesting to note that *cis*-**2** is substantially less soluble in chloroform, THF, or benzene than is the cis/trans polymer. We have not yet found a catalyst that is highly trans selective to compare the properties of *cis*-**2** and *trans*-**2**.

We have also examined the reactivity of the *cis*-polydeltacyclene/*trans*-polydeltacyclene toward oxidizing agents with the goal of preparing a polyepoxide, Table I. The partially epoxidized material starting from *cis*-**2**/*trans*-**2** proved to be quite sensitive to one of the most commonly used epoxidizing agents, i.e., *m*-chloroperbenzoic acid (MCPBA).[3] The use of a two-phase buffered system[10] was also ineffective at inhibiting decomposition of the epoxidized polymer if greater than 10–15% of the olefins were epoxidized. Acid-catalyzed opening of the epoxide is a likely source of the instability.

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