Carbon-13 Spectral Editing in Solid-State NMR Using Heteronuclear Scalar Couplings

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Abstract: The feasibility of spectral editing techniques making use of heteronuclear scalar couplings in solid-state CPMAS NMR is demonstrated for powder samples exhibiting line widths of several tens of hertz such as ordinary amino acids. These simple and robust experiments are insensitive to molecular motion, they work at high MAS spinning rates, and yield unambiguous identification of carbon types in natural abundance samples in a manner directly analogous to liquid-state NMR. Examples are given for some amino acids and for cholesteryl acetate.

1. Introduction

In powder samples, cross polarization (CP), magic angle spinning (MAS), and proton decoupling can yield high-resolution high-sensitivity NMR spectra of dilute spins such as carbon-13. However, in order for such spectra to be useful in the characterization of molecules in the solid-state, they must be assigned. Several methods have been proposed to obtain carbon−carbon correlation spectra using the through-space homonuclear dipolar interactions.[1−4] More recently, new techniques have been introduced to assign the carbon-13 spectra of ordinary powdered organic molecules using the homonuclear carbon−carbon scalar couplings.[5,6] However, both types of method are primarily of interest in carbon-13 enriched materials. Indeed, although it has been shown that correlation spectra can be obtained on small unlabeled molecules (three carbons),[6] for sensitivity reasons these techniques are not currently applicable to large natural abundance systems. Thus the assignment of high-resolution CPMAS spectra still represents a particular problem for natural abundance compounds.

In the absence of complete assignment methods, spectral editing techniques, which separate carbon-13 resonances according to their multiplicity, i.e., the number of directly attached protons, are a useful tool for the characterization of MAS spectra. In fact, spectral editing when combined with chemical shift analysis is often sufficient to provide an unambiguous characterization in medium-sized molecular systems. Several spectral editing techniques have already been proposed for solid-state CPMAS NMR of organic materials.[7−12] However, these techniques, usually based on differences in cross-polarization rates due to differences in average heteronuclear dipolar couplings, are sensitive to the presence of molecular motion and are thus limited to rigid compounds. In particular, although quaternary carbons and CH_3 groups can usually be easily identified, no satisfactory technique exists to clearly discriminate the CH and CH_2 groups in samples where some degree of motion may modulate dipolar couplings. Indeed, most organic materials are not sufficiently rigid to provide unambiguous identification using dipolar-based techniques. Additionally, dipolar-based techniques are only designed to work in the slow spinning regime, and at low magnetic fields.[12]

In analogy to the liquid-state case, spectral editing experiments using the multiplicity of carbon−proton *scalar couplings* in solids were suggested in the early eighties. However, their application has so far been limited to molecules of high mobility such as plastic crystals[13,14] or elastomers in amorphous phases,[15] in which rapid reorientational motion substantially reduces the effective dipolar couplings, and consequently results in carbon line widths of the order of a few hertz. In this article we show that using fast magic angle spinning together with an efficient high-power proton homonuclear decoupling scheme, the technique is applicable to ordinary organic solids with line widths of several tens of hertz. Particularly, the experiment, which is independent of molecular motion, provides a clear discrimination between CH and CH_2 groups.

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(1) Griffiths, J. M.; Lakshmi, K. V.; Bennett, A. E.; Raap, J.; VanDerWielen, C. M.; Lugtenburg, J.; Herzfeld, J.; Griffin, R. G. *J. Am. Chem. Soc.* **1994**, *116*, 10178−10181.

(2) Fujiwara, T.; Sugase, K.; Kainosho, M.; Ono, A.; Akutsu, H. *J. Am. Chem. Soc.* **1995**, *117*, 11351−11352.

(3) Sun, B. Q.; Costa, P. R.; Kocisko, D.; Lansbury, P. T.; Griffin, R. G. *J. Chem. Phys.* **1995**, *102*, 702−707.

(4) Lee, Y. K.; Kurur, N. D.; Helmle, M.; Johannessen, O. G.; Nielsen, N. C.; Levitt, M. H. *Chem. Phys. Lett.* **1995**, *242*, 304−309.

(5) Baldus, M.; Meier, B. H. *J. Magn. Reson.* **1996**, *121*, 65−69.

(6) Lesage, A.; Auger, C.; Caldarelli, S.; Emsley, L. *J. Am. Chem. Soc.* **1997**, *119*, 7867−7868.

(7) Alla, M.; Lippmaa, E. *Chem. Phys. Lett.* **1976**, *37*, 260.

(8) Wu, X.; Zilm, K. W. *J. Magn. Reson. A* **1993**, *102*, 205−213.

(9) Wu, X.; Zilm, K. W. *J. Magn. Reson. A* **1993**, *104*, 119−122.

(10) Wu, X.; Burns, S. T.; Zilm, K. W. *J. Magn. Reson. A* **1994**, *111*, 29−36.

(11) Sangill, R.; Rastrup-Andersen, N.; Bildsoe, H.; Jakobsen, H. J.; Nielsen, N. C. *J. Magn. Reson. A* **1994**, *107*, 67−78.

(12) Zilm, K. W. *The Encyclopedia of NMR*; John Wiley & Sons: London, 1997.

(13) Terao, T.; Miura, H.; Saika, A. *J. Chem. Phys.* **1981**, *75*, 1573−1574.

(14) Nielsen, N. C.; Bildsoe, H.; Jakobsen, H. J.; Sorensen, O. W. *J. Magn. Reson.* **1988**, *79*, 555−560.

(15) Nielsen, N. C.; Bildsoe, H.; Jakobsen, H. J. *Macromolecules* **1992**, *25*, 2847.

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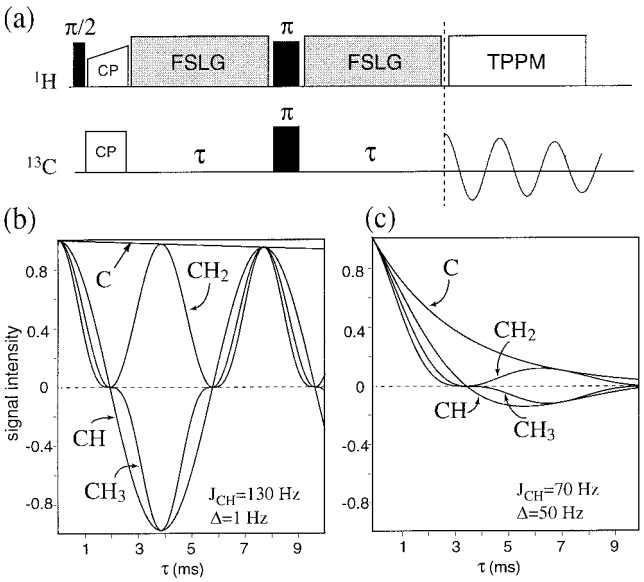

Figure 1. (a) The pulse sequence suitable for the solid-state attached proton test (SS-APT). See text for details. The theoretical evolution of carbon signal intensity is shown in b and c as a function of the delay τ. For each CH_n group ($n = 0, 1, 2, 3$), the curves were obtained using the expression:

$$I = I_0 \cos^n (2\pi J_{CH}\tau) e^{-2\tau/T_2}$$

where I_0 is the signal intensity after cross polarization and T_2 the transverse relaxation (dephasing) time during the period 2τ (equal to $1/\pi\Delta$). The calculations are shown for two different values of the line width Δ and J_{CH} coupling, 1 and 130 Hz respectively for panel (b), and 50 and 70 Hz respectively for panel (c), corresponding to the "ideal liquid state" case and to the equivalent solid-state case. Note that the line width Δ indicated in the figures is the full line width at half-height of the signals evolving during 2τ (i.e., the line width for a *single component* of the fine structure of the multiplet) and that this is different from the overall line width of the signals observed during acquisition. The calculations were done in the ideal case where all protonated carbon groups have the same heteronuclear scalar couplings; 130 Hz is a typical value for a one-bond J_{CH} coupling for an aliphatic carbon, and the value of 70 Hz corresponds approximately to a scaling factor of $1/\sqrt{3}$ due to the FSLG decoupling. These couplings are usually much larger than the two- or three-bond couplings, which can be neglected to first order. A τ value of $1/(2J_{CH})$ will produce a spectrum in which the C and CH_2 carbon resonances will be positive in sign, while the CH and CH_3 resonances will be inverted.

2. Pulse Scheme

The pulse sequence for the experiments is shown in Figure 1a. The sequence is based on one of the simplest liquid-state spectral editing techniques,[16−19] the attached proton test (APT), and has been modified to be suitable for solids by the introduction of cross-polarization and homonuclear dipolar decoupling during the evolution periods.[13,14] For the spectra shown in this article, the proton−proton dipolar couplings were removed during the period 2τ by using frequency-switched Lee−Goldburg decoupling (FSLG),[20,21] but other techniques are equally applicable, as discussed further below. During the periods τ, the inhomogeneous interactions, i.e., the chemical shift and the heteronuclear couplings, are averaged by rapid magic angle spinning to their isotropic components, leaving the isotropic chemical shift and the scalar J_{CH} coupling. The 180° pulses applied simultaneously to proton and carbon in the center of the 2τ period have the net effect of refocusing the carbon chemical shift evolution while maintaining the evolution under the scalar J_{CH} coupling. Thus, after cross polarization, carbon coherences evolve only under the effect of a *scaled* heteronuclear scalar coupling. The scaling factor varies according to the dipolar decoupling sequence that is used; for the FSLG sequence the scaling factor is $1/\sqrt{3}$. During the acquisition period, on resonance continuous wave or TPPM[22] heteronuclear decoupling is applied.

According to the carbon multiplicity, the intensity of the observable magnetization will evolve differently as a function of τ. The calculated evolution curves are shown in Figure 1, b and c. For $\tau = 1/(2J_{CH})$ positive signals should be observed for C and CH_2 carbons, while CH and CH_3 groups should give negative resonances. Unlike the "ideal liquid-state" case shown in Figure 1b, the signal intensity will be strongly attenuated by transverse relaxation in the SS-APT experiment. However, if the FSLG sequence applied during the 2τ period is efficient enough to yield line widths comparable to the size of the scaled heteronuclear scalar coupling ($\Delta \approx 50$ Hz), then a significant signal should be observed, as illustrated in Figure 1c (under FSLG decoupling, a J_{CH} coupling of 125 Hz, which is a typical value for a sp^3 carbon in hydrocarbons,[23] will give an effective scaled coupling of 72 Hz). Note that the sign of the peaks is independent of the line width. Variations in line widths will simply lead to a modulation of the amplitude of the signals, but will not prevent distinction between positive (CH_2) and negative (CH) signals. Note especially that in cases where some groups are more flexible than others, partial averaging of the dipolar couplings through molecular motion will simply increase the signal amplitude (by decreasing the line width).

3. Experimental Section

The (natural abundance) samples were purchased from Sigma and used without further recrystallization. Approximately 10 mg of each sample was used. Except for cholesteryl acetate (Figures 5 and 6), the experiments were performed on a Bruker DSX 500 spectrometer (proton frequency 500 MHz) using a 2.5-mm double-tuned MAS probe (sample volume 12 mL). The spinning rate was 13 kHz. During acquisition continuous wave decoupling was applied with a proton field strength of $\omega_1/2\pi = 200$ kHz. During the τ delays homonuclear decoupling was achieved using the frequency-switched Lee-Goldburg (FSLG) scheme[20,21] with a radio frequency field strength of $\omega_1/2\pi = 125$ kHz. For the cross-polarization step, the radio frequency field was set to 80 kHz for carbon, while a ramped rf field was applied on protons,[24,25] and matched to obtain optimal signal. The 180° pulses on proton and carbon were set to 3.8 μs and 6.1 μs, respectively. For cholesteryl acetate, the experiments were done on a Bruker DSX 400 spectrometer (proton frequency 400 MHz) with a 4-mm triple resonance MAS probe. The sample volume was restricted to about 25 mL in the center of the rotor to increase the radio frequency field homogeneity. The spinning speed was 10.5 kHz. The proton rf field strength was set to 100 kHz during both the τ delays (FSLG decoupling) and during acquisition

(16) Freeman, R. *Spin Choreography*; Spektrum Academic Publishers: Oxford, 1997.
(17) LeCocq, C.; Lallemand, J. Y. *J. Chem. Soc., Chem. Commun.* **1981**, *119*, 150−152.
(18) Pei, F. K.; Freeman, R. *J. Magn. Reson.* **1982**, *48*, 318.
(19) Jakobsen, H. J.; Sorensen, O. W.; Brey, W. S.; Kanya, P. *J. Magn. Reson.* **1982**, *48*, 328.
(20) Bielecki, A.; Kolbert, A. C.; Levitt, M. H. *Chem. Phys. Lett.* **1989**, *155*, 341.
(21) Levitt, M. H.; Kolbert, A. C.; Bielecki, A.; Ruben, D. J. *Solid State NMR* **1993**, *2*, 151−163.
(22) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951.
(23) Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1988.
(24) Metz, G.; Wu, X.; Smith, S. O. *J. Magn. Reson. A* **1994**, *110*, 219−227.
(25) Hediger, S.; Meier, B. H.; Kurur, N. D.; Bodenhausen, G.; Ernst, R. R. *Chem. Phys. Lett.* **1994**, *223*, 283−288.

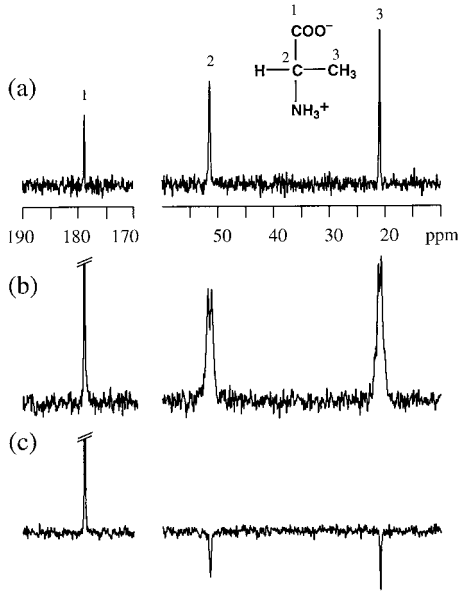

Figure 2. (a) One-dimensional cross-polarization spectrum of L-alanine acquired with continuous wave proton decoupling (4 scans). The contact time for CP was 500 ms. The other experimental parameters are described in the Experimental Section. (b) One-dimensional cross-polarization spectrum of L-alanine acquired with FSLG proton decoupling during acquisition (32 scans). (c) SS-APT spectrum of L-alanine (256 scans). This spectrum was recorded using the pulse sequence of Figure 1a with $\tau = 5.5$ ms.

(TPPM[22] decoupling). In both cases, an eight-step phase cycle was used (pulse program available on request). Synchronization of the τ delay with the rotor period was not found to be necessary.

4. Discussion

Until now it has not been possible to resolve scalar J_{CH} couplings in rigid organic solids having line widths of several tens of hertz. However, we have found that sufficient resolution can be achieved so as to observe these couplings when combining moderately fast magic angle spinning (13 kHz) and frequency switched Lee-Goldburg (FSLG) homonuclear decoupling. This is illustrated in Figure 2 for a sample of powdered L-alanine. Figure 2a shows the normal fully decoupled CP spectrum obtained with continuous wave decoupling during the detection period. Under our experimental conditions this compound, which is typical of "real" crystalline organic solids, has carbon line widths of 25 Hz for the carbonyl carbon (peak 1), 40 Hz for the α carbon (peak 2), and 30 Hz for the methyl carbon (peak 3). When FSLG homonuclear decoupling is applied during the detection period of a normal one-dimensional cross-polarization experiment (Figure 2b), the carbon−proton heteronuclear scalar couplings are clearly apparent. The methyl carbon shows a quartet splitting with the expected 1:3:3:1 intensity distribution (although the outer lines are barely resolved), and the α carbon displays a clear doublet splitting. As expected, the carbonyl carbon is not split, since it experiences no one-bond carbon-proton scalar couplings. The carbonyl peak is only slightly broadened (30 Hz), due to less efficient dipolar decoupling (in this case, the carbon−proton dipolar couplings are only removed by fast MAS) and also perhaps due to small long-range scalar couplings (a few hertz).

Testing various homonuclear decoupling schemes, we found that the choice of decoupling scheme and the exact experimental set up were critical factors in obtaining spectra of this quality. We have obtained satisfactory results using the FSLG sequence, as reported above, but also using the (+X)(+Y)(−X)(+X)(−Y)(−X) sequence proposed by Hafner and Spiess,[26] which is an original homonuclear decoupling scheme suitable for high spinning speeds, and which we designate here HASP-1 (each parenthesis in the sequence represents a 90° pulse around the corresponding axis). On the other hand, schemes such as MREV8 or BLEW12 did not lead to nice multiplet patterns, but rather to broader peaks under our experimental conditions. Even when using FSLG or HASP-1, optimum performance of the decoupling sequences depends on the spinning rate. We find the sequences work well for spinning rates between about 10 and 16 kHz. All the spectra shown in this article were obtained at spinning rates of either 10.5 or 13 kHz. Indeed, we found that the performance of the decoupling sequences degrades at *both* higher (>16 kHz) and lower spinning speeds (<10 kHz). The degradation at higher speeds is probably due to the breakdown of the approximation that the cycle times for the two averaging processes (the sample rotation for the heteronuclear dipolar decoupling and the FSLG or HASP-1 scheme for the homonuclear dipolar decoupling) are independent, and thus we start to see interference effects. At lower spinning speeds, on the other hand, we assume that the averaging of the heteronuclear dipolar couplings by MAS is not efficient enough for the observation of resolved fine structures. As mentioned in the Experimental Section, the FSLG decoupling was applied using a field amplitude of 120 kHz. Attempts to increase the decoupling power in order to reduce the cycle time of the sequence (and thus allow faster spinning speeds) have so far not resulted in any improvement. This is probably because the performance of the FSLG sequence suffers from larger phase and frequency switching artifacts as we reduce the pulse lengths.[27] Applying a 4π rotation rather than a 2π rotation around the magic angle axis, thereby increasing the delay before phase and frequency switching, did not lead to significant improvement. Note that the better the decoupling sequence performs, the better the signal-to-noise ratio will be in the final edited spectrum, but that imperfect decoupling should not lead to ambiguous results.

Thus, we find that the J_{CH} couplings can be resolved by carefully adjusting the experimental parameters. From simulations, we find that the CH_3 peak in Figure 2b fits well to a quartet with individual line widths of about 45 Hz, and an effective J_{CH} coupling of about 70 Hz. From the curves of Figure 1b, we can expect that the SS-APT editing sequence will work reasonably well under these conditions, and this is indeed the case, as illustrated in Figure 2c which shows the SS-APT spectrum for L-alanine. As predicted the methyl and the α carbon resonances are both inverted, while the carbonyl resonance remains positive. For this spectrum, the efficiency of the SS-APT experiment, defined as the ratio of peak intensity in the normal CP experiment to peak intensity in the edited experiment, was around 10% for the CH_3 and CH groups. These values are in agreement with what we expected from calculations (note that using other values of τ, higher efficiencies can be obtained for each individual peak, as will be shown in Figure 4).

Figure 3 shows the SS-APT edited spectra obtained with samples of L-histidine (Figure 3A) and L-isoleucine (Figure 3B). These two model samples, for which the assignment is known from previous studies,[6,28] represent somewhat more complex systems than the L-alanine model. In both cases the technique

(26) Hafner, S.; Spiess, H. W. *J. Magn. Reson. A* **1996**, *121*, 160−166.
(27) Bielecki, A.; Kolbert, A. C.; deGroot, H. J. M.; Griffin, R. G.; Levitt, M. H. *Adv. Magn. Reson.* **1989**, *14*, 111.
(28) Gu, Z.; Ridenour, C. F.; Bronnimann, C. E.; Iwashita, T.; McDermott, A. *J. Am. Chem. Soc.* **1996**, *118*, 822−829.

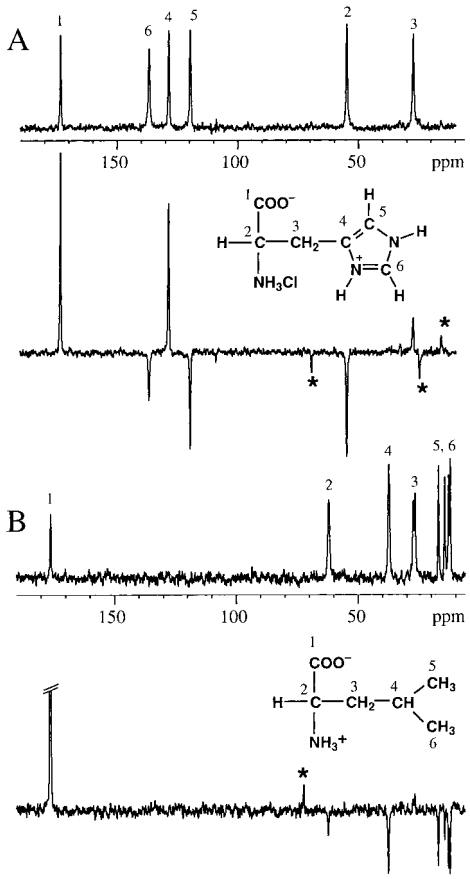

Figure 3. (A) One-dimensional cross-polarization spectrum and SS-APT spectrum of L-histidine monohydrochloride monohydrate. For CP, a contact time of 1 ms was used. The spectra were recorded with 200 scans and 2048 scans, respectively. τ was set to 4 ms for the editing experiment. (B) One-dimensional cross-polarization spectrum and SS-APT spectrum of L-isoleucine. The CP contact time was 700 μs. The spectra were recorded with 20 and 12000 scans, respectively. For the editing experiment, τ was set to 4.8 ms. In the case of L-isoleucine there are two crystallographically different molecules in the unit cell, giving rise to two distinct resonances for carbons 3, 5, and 6. The spinning sidebands are marked with an asterisk. The sign of these spinning sidebands varies for different values of τ (no rotor synchronization was used). A slightly shorter value of τ was chosen for L-histidine than for L-isoleucine, since L-histidine contains ring carbons having large coupling constants (peaks 5 and 6).

is clearly able to identify all the carbon multiplicities. Notably, both examples illustrate very well that the differentiation between the CH and CH_2 groups is completely unambiguous. In the L-histidine spectrum all the peaks are present with a good signal-to-noise ratio and comparable efficiency. In the L-isoleucine spectrum the two peaks corresponding to the CH_2 group in each of the two different molecules in the unit cell are weaker than the others, though still clearly visible. We assume that in this case the residual line width under FSLG decoupling is quite large, yielding a relatively strong attenuation of the signal by transverse relaxation. In a rigid compound, the carbon resonances corresponding to CH_2 groups are indeed the ones for which homonuclear decoupling is the most difficult to achieve, since they experience the strongest proton−proton couplings. However, as pointed out above, CH_2 groups will never become inverted and thus discrimination between them and CH or CH_3 groups remains possible. Thus, even in cases where the editing experiment does not lead to the observation of a positive peak, the CH_2 groups could still be identified by the absence of any negative peak (of course, this argument implies that the other multiplicities still give rise to residual peaks, but this has always been the case for all the samples we have studied so far). Finally, note that the sensitivity of the experiment should improve in the future as progress is made in the design of new homonuclear decoupling sequences under fast MAS.

Identification of multiplicities using the SS-APT technique is dependent on only a small range of J_{CH} values being present. Indeed, a complete edited spectrum can only be obtained if $1/(4J_{CH}) < \tau < 3/(4J_{CH})$), for all the J_{CH} values. In principle one-bond J_{CH} couplings can range from 100 to 280 Hz.[23] However, if one knows a priori, from molecular structure, that there is likely to be a large range of scalar couplings in the compound, recording the whole evolution curve (or at least a few points) of signal intensity as a function of τ, should lead to the unambiguous identification of the resonances. Despite this J_{CH} dependence, we have successfully used the SS-APT technique to identify the multiplicities of L-histidine which has J_{CH} values varying in a range of about 130 Hz to 220 Hz (values measured in solution). By taking a value of τ a little shorter (4 ms) than for L-isoleucine (4.8 ms), only one spectrum is necessary to assign all the resonances (Figure 3A). Liquid-state editing techniques, such as DEPT,[16] are less sensitive to variations in J_{CH}, but they require evolution of proton coherences (the APT sequence only involves carbon coherences). In solids this is likely to lead to the elimination of the signal due to the rapid dephasing of the proton coherences which are, using the current state of the art, still significantly larger than the carbon resonances under homonuclear decoupling.

For L-histidine, the efficiency of the SS-APT experiment, calculated under the experimental conditions of Figure 3A, is quite good. Note especially that for the CH peaks numbered 2 and 5 (having line widths of around 80 Hz in the normal CP spectrum), an efficiency of about 30% was calculated. As pointed out above, the efficiency of the editing experiment is dependent on the value of τ with respect to the J_{CH} couplings, but it is also strongly dependent on the effective line width Δ due to relaxation (dephasing) during the period 2τ. It is important to note that this *effective* line width may be much narrower than the one observed directly when applying FSLG decoupling during the acquisition period (as in Figure 2b), since the 180° pulse eliminates broadening arising from any chemical shift distribution and from the bulk susceptibility. This probably explains why such high efficiencies can be observed on compounds having line widths of almost 100 Hz in the 1D CPMAS spectrum. This reinforces the point that the couplings do not by any means need to be fully resolved for the experiment to work. Also note that a J-resolved two-dimensional spectrum, for which the delay τ is replaced by a τ_1 evolution period,[29] would provide more highly resolved multiplet structures than in Figure 2b, and should be useful to measure J_{CH} couplings in ordinary organic molecules in the solid state.

Because of the dependence of the experimental efficiency on J_{CH} and on line width, it is not possible to obtain individual subspectra for each carbon multiplicity using this method. However, if the resonances are fully resolved, a complete identification of all carbon groups can be achieved. The quarternary resonances can be easily distinguished from the CH_2 resonances since the intensity of the quaternary resonances never goes through zero as a function of τ, and since they decay only very slowly with τ, unlike the CH_2 resonances, resulting in intense positive peaks for the quaternary carbons for all values

(29) Miura, H.; Terao, T.; Saika, A. *J. Magn. Reson.* **1986**, *68*, 593−596.

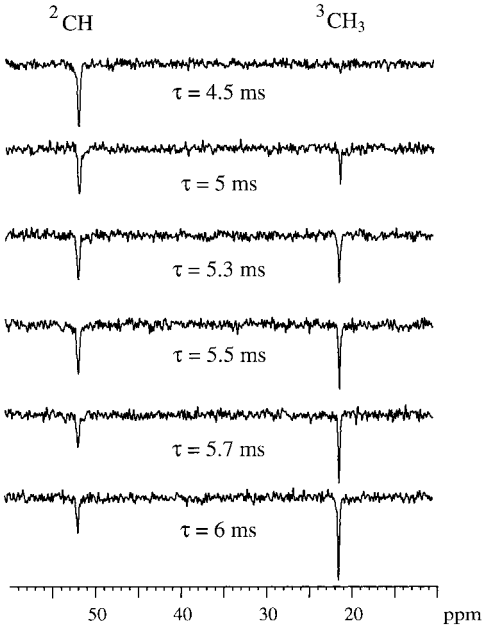

Figure 4. Edited spectra of L-alanine recorded for various values of the delay τ. The other experimental conditions are the same as in Figure 2. Only the region of the CH and CH_3 resonances is shown; the carbonyl resonance always remains positive. The CH and CH_3 groups can be distinguished by recording spectra with various values of τ.

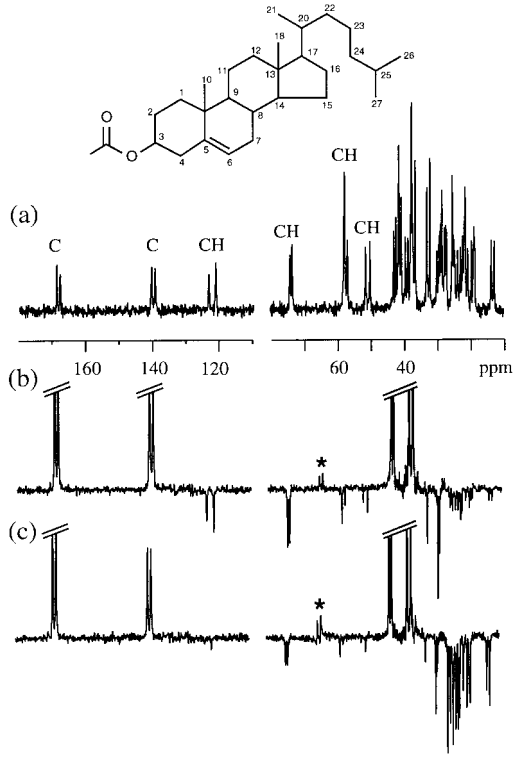

Figure 5. (a) One-dimensional cross-polarization spectrum of cholesteryl acetate (128 scans). The contact time for CP was 1.5 ms. The other experimental parameters are described in the text. (b) SS-APT spectrum of cholesteryl acetate recorded with $\tau = 4.5$ ms (2048 scans) in a total acquisition time of 35 min. (c) SS-APT spectrum of cholesteryl acetate recorded with $\tau = 6$ ms (2048 scans). Spinning sidebands are marked with an asterisk.

of τ (Figure 2c and 3). Discrimination between CH and CH_3, which have the same parity, should also be possible since the curves of signal intensity as a function of τ are significantly different for these two groups, the CH_3 signals pass through zero with an inflection point (Figure 1, b and c). Thus, we can

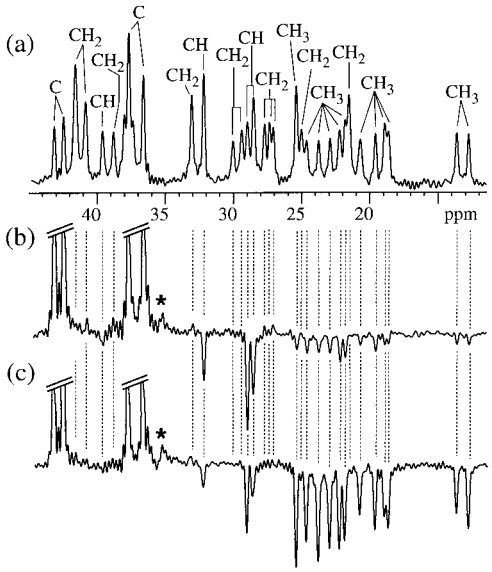

Figure 6. Expansions of the spectra shown in Figure 5 between 12 and 45 ppm. The assignment indicated on the top of the figure was deduced as described in the text: the CH and CH_3 groups can be distinguished by the fact that the CH resonances give intense negative peaks for τ equal to 4.5 ms, which diminish in intensity for τ equal to 6 ms, whereas the opposite effect is observed for CH_3 resonances. The CH_2 groups give peaks which are weakly positive (like those around 27 ppm) or even null (like those around 30 ppm), whereas the quaternary carbons give intense positive resonances.

expect to see mainly the CH resonances in the spectrum for a τ value just after the signal cancellation, whereas the CH_3 resonances will become stronger for longer values of τ. This difference should be strengthened by the fact that the CH groups will dephase more rapidly and thus, for longer values of τ, will be more strongly attenuated than CH_3 groups. This reasoning depends on the values of J_{CH} being similar. Notably, identification could be compromised in cases where $J_{CH}(CH) < J_{CH}(CH_3)$. However, in normal organic compounds, it is found that $J_{CH}(CH)$ is usually roughly the same as or larger than $J_{CH}(CH_3)$. This behavior is confirmed experimentally on the model sample of L-alanine as shown in Figure 4. For τ equal to 4.5 ms we obtain an SS-APT spectrum which contains only the α resonance. As τ increases the methyl resonance appears, and increases in intensity while the α resonance decreases.

To summarize, in cases where all the couplings are similar, for τ values close to the zero-crossing point, the intense negative peaks correspond to CH resonances. For longer values of τ, the intense negative peaks correspond to CH_3 resonances, and the CH peaks are strongly attenuated. The intense positive peaks correspond to quaternary carbons while the CH_2 resonances give small positive peaks (or in some cases disappear completely). We have applied this protocol to characterize the CP-MAS spectrum of cholesteryl acetate. The results are illustrated in Figures 5 and 6. Like L-isoleucine, cholesteryl acetate crystallizes with two molecules per unit cell. Using the protocol described above, we have been able to assign all the resolved resonances to C, CH, CH_2, or CH_3 groups. The proposed assignment, shown in Figures 5 and 6 is in complete agreement with previous studies on this compound,[10] indicating that the method is reliable.

5. Conclusions

In conclusion, we have shown that heteronuclear scalar couplings provide a useful interaction for natural abundance

carbon-13 spectral editing in ordinary organic solids having line widths of several tens of hertz. (Note that the technique does not work in the presence of homonuclear C−C scalar couplings, i.e., in multiply carbon-13 labeled systems.) The technique is robust and relatively easy to implement. Since the technique does not depend on dipolar couplings, it provides an unambiguous differentiation between CH and CH_2 groups in solid-state NMR of powders, even in the presence of motional averaging effects. We have used the technique to identify multiplicities in some amino acids, which are notoriously difficult samples using older methods, and in cholesteryl acetate, a rigid molecule having 29 carbon atoms. The technique should become widespread for spectral characterization in solids.

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