Direct Assignment of ^{13}C NMR Spectra of Rigid Solids by Two-Dimensional Magic-Angle-Spinning Separated-Local-Field Spectroscopy

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An alternative two-dimensional magic-angle-spinning separated-local-field (MASSLF) approach for directly assigning the ^{13}C spectra of rigid solids is proposed. This procedure does not employ homonuclear ^{1}H decoupling during the evolution period. The unique patterns of the dipolar spectra of CH, CH$_2$, CH$_3$, and C groups in the F_1 dimension are clearly distinguishable even under the influence of the homonuclear ^{1}H dipolar interaction. In the F_1 dimension, the CH group shows a Pake doublet pattern, the CH$_2$ group shows a "broad triplet-like" pattern, the CH$_3$ group shows a much narrower peak with high intensity due to its fast rotation at room temperature, and the C groups show a much narrower peak with low intensity (compared to the intensity of CH$_3$ groups) corresponding to the remote heteronuclear dipolar couplings. The CH$_3$ groups can be distinguished from the nontetrahedrally bound quaternary C groups by the significant intensity difference of the heteronuclear dipolar spectra in addition to the large chemical-shift difference. The ^{13}C heteronuclear dipolar spectra of CH and CH$_2$ groups suffer severe broadening due to remote couplings and spin exchange among protons. The MASSLF spectra are sensitive to the local environment of the carbons. The tetrahedrally bound quaternary carbons are not clearly distinguished from CH$_3$ groups in this approach, due to their similar chemical shifts, comparable intensities, and linewidths of the dipolar spectra. © 1997 Academic Press

INTRODUCTION

High-resolution solid-state ^{13}C NMR spectra with enhanced sensitivity can be obtained by combining cross polarization (*1*) and magic-angle spinning (*2, 3*) (CPMAS). This technique has proven to be extremely useful in studying the structure and physical properties of organic solids. However, the assignment of the ^{13}C spectra is not an easy task. In solution ^{13}C spectra, the methyl (CH$_3$), methylene (CH$_2$), methine (CH), and quaternary (C) carbons can be distinguished by APT, DEPT, and INDEPT techniques. Unfortunately, those techniques cannot be applied to solids due to the strong heteronuclear dipolar coupling between ^{13}C and ^{1}H and homonuclear dipolar coupling between the protons.

Comparison to the solution ^{13}C spectra can help the assignment of some peaks of solid-state ^{13}C spectra. In solution, due to the flexibility of the molecular structure and the isotropic tumbling of the molecules, some of the carbons become equivalent. However, in solids, those carbons may become nonequivalent due to the crystal symmetry and their different local environments. Thus, more peaks may appear and the chemical shifts may be different from those obtained in the solution ^{13}C NMR spectra. In general, solid-state ^{13}C spectra are more complicated than solution ^{13}C spectra.

Several techniques have been proposed so far to distinguish the CH$_3$, CH$_2$, CH, and C groups (*4–12*). The simplest and most used technique is dipolar dephasing in which a delay without high-power proton decoupling is inserted between the cross-polarization period and the acquisition period (*4*). By use of this technique, CH$_3$ and C can be distinguished from CH and CH$_2$. CH$_3$ can be distinguished from C (with the exception of tetrahedrally bonded carbons) by their large chemical-shift difference. However, this technique fails to distinguish CH from CH$_2$. Recently, a method for editing the ^{13}C spectra (*5*), based on chemical-shift anisotropy dephasing, was proposed but it also fails to distinguish CH from CH$_2$.

Techniques using one-dimensional and two-dimensional magic-angle-spinning separated-local-field spectroscopy (MASSLF) have been proposed to distinguish the CH$_3$, CH$_2$ and CH groups (*6, 7*). In these proposals, homonuclear proton decoupling either by multiple-pulse (*14*) or magic-angle decoupling (*8*) is used during the evolution period to suppress the homonuclear ^{1}H dipolar coupling, because such suppression of ^{1}H homonuclear decoupling is required to avoid a modification of the ^{13}C–^{1}H coupling by ^{1}H–^{1}H coupling. In the 1D MASSLF approach, the MAS spinning rate is set to 2000 Hz and the evolution time to 1/4 of the rotation period in order to obtain the best editing result; the phase of CH is inverted under such conditions (*6*). However, the spinning rate of 2000 Hz is not high enough to reduce the number and the intensity of the spinning sidebands which
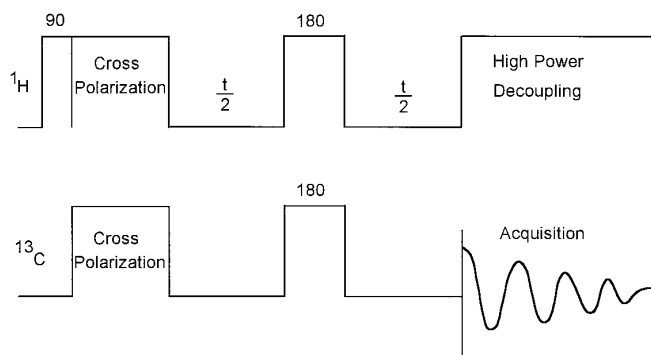
FIG. 1. Pulse sequence.

may overlap with other peaks. In the 2D MASSLF approach with homonuclear ^{1}H decoupling during the evolution period (7), the spectra in the F_1 dimension are the dipolar spectra of the carbons which are resolved in the F_2 dimension. The width of the MASSFL spectra in the F_1 dimension is significantly reduced due to the scaling effect of the homonuclear proton decoupling. The discrimination of CH from CH$_2$ groups is based on the comparison of spinning sideband patterns to the calculated theoretical patterns. The other editing techniques using cross polarization–depolarization (CPD) (9, 11), cross polarization–depolarization–repolarization (CPDR) (10, 11), and cross polarization combined with polarization inversion (CPPI) (12) require subtraction and addition of the spectra obtained under different conditions. The WIMSE (13) technique for ^{13}C spectral editing requires careful selection of experimental parameters and a well-controlled spinning rate, and the intensities of peaks must be scaled for editing.

In this paper, a relatively straightforward method based on the 2D MASSLF approach is proposed for discriminating the CH$_3$, CH$_2$, CH, and C groups directly from the 2D MASSLF spectra without any editing or theoretical calculation. The procedure does not employ homonuclear ^{1}H decoupling during the evolution period. This procedure and the pulse sequence will be discussed in detail in the next section.

THEORETICAL BACKGROUND AND THE PULSE SEQUENCE

The pulse sequence used in this procedure is shown in Fig. 1. After the cross-polarization period, there is an evolution period t without homonuclear proton decoupling. In the middle of the evolution period, there are 180° pulses on the ^{13}C channel and the ^{1}H channel, respectively, to refocus the chemical-shift dephasing and to preserve the dipolar evolution. After the evolution period, there is a normal acquisition period with high-power ^{1}H decoupling.

The Hamiltonians of the dipolar coupling interactions of isolated CH, CH$_2$, and CH$_3$ have been discussed elsewhere (15). Such couplings may be called direct couplings in order to distinguish those remote couplings (see below). Each group shows its unique powder pattern of the static dipolar spectrum. The dipolar spectrum of coupled two-spin $I = 1/2$ nuclei is a Pake doublet pattern shown in Fig. 2a (a C–H bond length of 1.09 Å and 15 kHz Gaussian broadening); the dipolar spectrum of coupled three-spin $I = 1/2$ nuclei is shown in Fig. 2b (a C–H bond length of 1.09 Å, an angle of 109.4° between the two CH, and 20 kHz Gaussian broadening). The lineshape of Fig. 2b may be called a ''broad triplet-like'' pattern for lack of a better name. Such a lineshape was obtained for the proton NMR of CH$_3$CCl$_3$ (16) and for the ^{13}C NMR of polyethylene (17). CH$_3$ shows a relatively narrow line due to its fast rotation at room temperature. For common organic compounds with high proton density, however, these groups are not isolated and there are remote heteronuclear dipolar couplings between ^{13}C and ^{1}H and remote homonuclear dipolar couplings between protons. The appearance of the dipolar spectra of ^{13}C will depend upon the remote couplings also.

According to the pulse sequence in Fig. 1, high-resolution CPMAS spectra in the F_2 dimension can be obtained at normal MAS spinning rates (3–6 kHz). Such spinning rates are much smaller than the dipolar interactions. The effect of the spinning on the dipolar spectra is not significant; therefore, the influence of fluctuation in the spinning rate on the MASSLF spectra is negligible. The appearance of the dipolar spectra will be influenced by homonuclear proton couplings. However, the unique feature of the dipolar spectrum of each
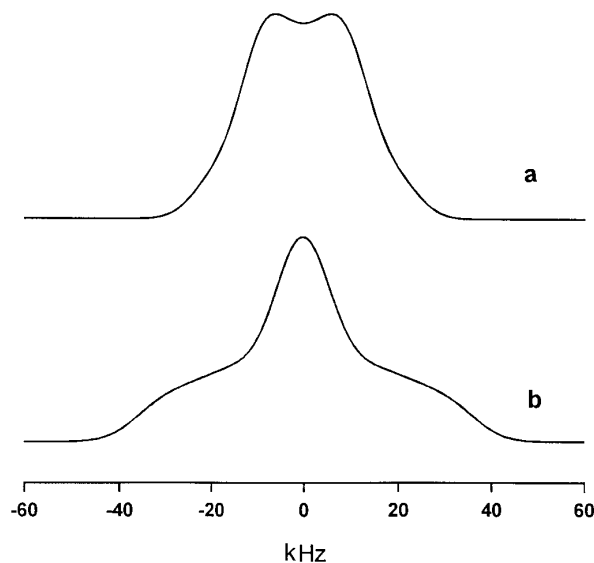

FIG. 2. Theoretical patterns of ^{13}C dipolar spectra. (a) CH: the C–H bond length is 1.09 Å; 15 kHz Gaussian broadening is used. (b) CH$_2$: the C–H bond length is 1.09 Å and the angle between the two CH bonds is 109.4°; 20 kHz Gaussian broadening is used.

group will be preserved in the F_1 dimension if such influence is not too high to smear the feature. Therefore, CH, CH$_2$, CH$_3$, and C groups can be distinguished by their unique patterns in the spectra in the F_1 dimension. The remote couplings and the proton–proton spin exchange are major sources of line broadening.

EXPERIMENTAL

Two commercial samples (*trans*-4-methyl-1-cyclohexanecarboxylic acid and L-tyrosine ethyl ester) were used without further treatment. The 2D MASSLF spectra were obtained with a Bruker MSL-300 NMR spectrometer operating at 75.5 MHz for ^{13}C. A Bruker CP/MAS broadband probe was used. The samples were packed into a 7 mm zirconium oxide rotor. The 90° pulse for ^{13}C was 5 μs.

RESULTS AND DISCUSSION

Figure 3 shows the 2D MASSLF spectrum of L-tyrosine ethyl ester obtained with a spinning rate of 4.3 kHz, with a contact time of 50 μs, and with a recycle time of 0.5 s. The number of increments of t is 64 with each increment being 8 μs. For such a 2D spectrum, the linewidths of the CH and
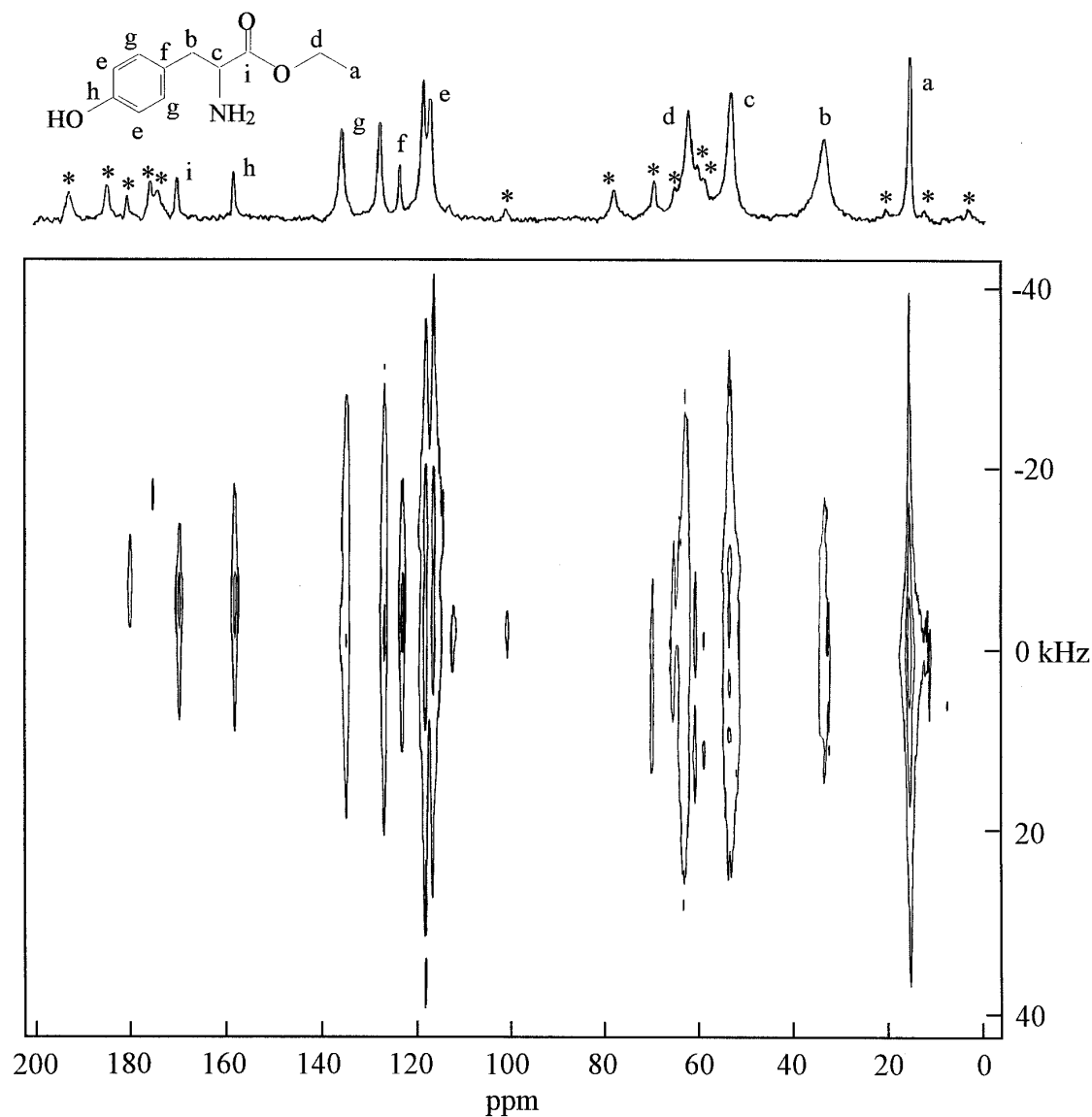

FIG. 3. 2D MASSLF ^{13}C spectrum of L-tyrosine ethyl ester; the CPMAS spectrum and the structure are at the top. Asterisks indicate spinning sidebands.
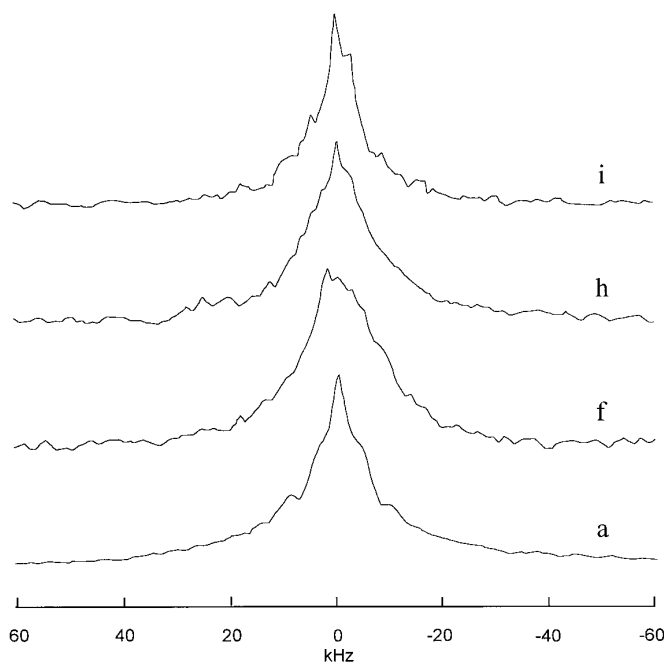
FIG. 4. MASSLF ^{13}C spectra in the F_1 dimension from Fig. 3. The spectra are not plotted to the same intensity scale.

The MASSLF spectra in the F_1 dimension suffer severe broadening (7–20 kHz). The major broadening may be due to remote coupling and proton spin exchange. Because the dephasing of the CH and CH_2 groups is very fast, only the FIDs of the first few increments of t contain the information of CH and CH_2. Therefore, the 180° pulse may not completely refocus chemical-shift dephasing of CH and CH_2. The dipolar spectra suffer broadening from the chemical-shift anisotropy. However, the distinguishable patterns for different groups are still seen. In Fig. 5, the MASSLF spectra of all CH groups show the Pake doublet pattern (Fig. 5c, 5e's, and 5g's) and of all the CH_2 groups (Figs. 5b and 5d) show a ''broad triplet-like'' pattern. The space between the two singularities of the doublet is about 12 kHz, which is much smaller than the value of a normal heteronuclear dipolar spectrum of CH (23 kHz if the average bond length is about 1.09 Å). Such distortion is due to the influence of homonuclear proton couplings and remote $^{13}C-{}^1H$ couplings.
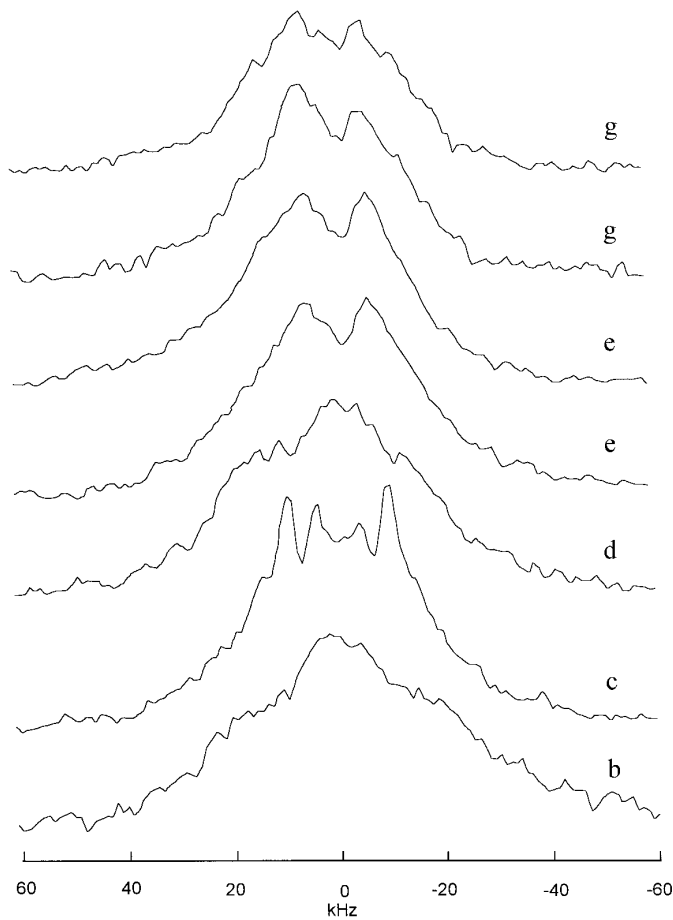
It is interesting to note that the MASSLF spectrum of

CH_2 groups in the F_1 dimension are much broader than those of the CH_3 and C groups. For such a short contact time and recycle time, the total intensities of all groups will be reduced. Such reduction of intensities can be compensated by increasing the number of transitions without increasing the total length of the experimental time. Because of different cross-polarization rates, the intensities of the CH and CH_2 groups will be relatively enhanced and the intensities of the C and CH_3 groups will be relatively suppressed under a short contact time. The unique features of the CH and CH_2 groups will be seen more clearly. Figure 4 and Fig. 5 show the MASSLF spectra of the different carbons. The CPMAS spectrum and the structure are shown at the top of Fig. 3. The MASSLF spectra in Fig. 4 and Fig. 5 are not plotted to the same intensity scale. In solution, two carbons e are equivalent and two carbons g are equivalent; in the solid state, however, these equivalent carbons become nonequivalent. It is difficult to distinguish which g peak corresponds to which g carbon; therefore, the two peaks from the different g's are both labeled as g; the same is true for the two peaks of the two carbon e's. The efficiency of the separation of the local field along the F_1 dimension depends on how well the different carbons are resolved in the F_2 dimension. In Fig. 3, different carbons are well resolved in the F_2 dimension, and the projection on the F_2 dimension gives a CPMAS spectrum of the sample. The dipolar spectra of different carbons in the F_1 dimension are well separated due to the separation of the carbons in the F_2 dimension.

FIG. 5. MASSLF ^{13}C spectra in the F_1 dimension from Fig. 3. The spectra are not plotted to the same intensity scale.

carbon c shows a spinning sideband pattern. However, the MASSLF spectra of the CH groups in the phenyl ring do not. This indicates that the carbons in the phenyl ring probably experience stronger remote couplings than carbon c in this sample. The influence of $^{14}N-{}^{13}C$ dipolar coupling (~ 680 Hz if the average bond length of C–N is about 1.47 Å) is negligible under this line broadening (at least 7.5 kHz or higher) and the MAS condition. If it is significant, it should be seen in the F_2 dimension.

Figure 4a shows the residual ^{13}C dipolar spectrum of the CH_3 group which is located at the high-field side in the F_2 dimension. The spectral width is about 13 kHz, which is much smaller than that of the CH and CH_2 groups. This is because the fast rotation of the CH_3 group partially averages

the heteronuclear dipolar interaction between the ^{13}C and the protons. In addition, the homonuclear dipolar interaction between protons is significantly reduced. The spectrum is modulated by the sample spinning, which indicates that the remote coupling is small because of its fast rotation. The peaks of three quaternary carbons are located at the low-field side in the F_2 dimension. Figures 4f, 4h, and 4i show the dipolar spectra of three quaternary carbons. Because there are no protons bound to such carbons, the dipolar spectra are the remote coupling spectra of the quaternary carbons coupled with neighboring protons. Comparing the MASSLF spectra (Figs. 4f, 4h, 4i) of three quaternary carbons, the widths of the spectra in Figs. 4f, 4h, and 4i are about 16, 11, and 7.5 kHz, respectively. Such differences
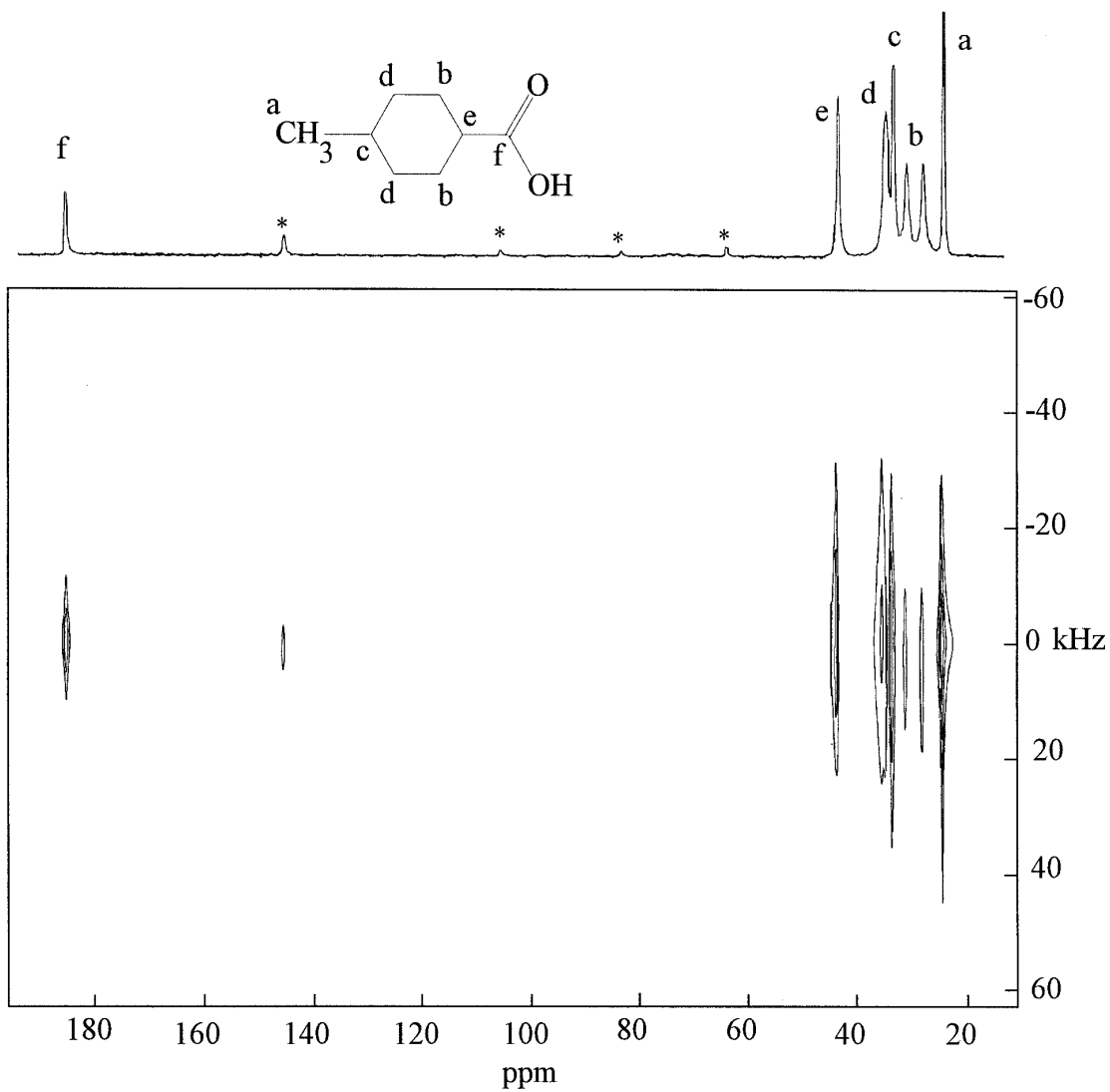

FIG. 6. 2D MASSLF ^{13}C spectrum of *trans*-4-methyl-1-cyclohexanecarboxylic acid; the CPMAS spectrum and the structure are at the top. Asterisks indicate spinning sidebands.

may reflect the different local environments of each quaternary carbon. Carbon f is bound to two CH groups and one CH_2 group. Carbon h is bound to two CH groups and one OH group, and carbon i is bound to one CH group and two O groups. Therefore, the total first remote coupling (coupled with nearest neighboring protons) of carbon f is stronger than that of carbon h, and the total first remote coupling of carbon i is less than that of carbon h.

The proton–proton spin exchange is another source of line broadening, because the protons have finite lifetimes in their Zeeman states due to proton–proton spin exchange. Therefore, the more protons that are coupled to the carbon, the more broadening the carbon will suffer. Clearly, the MASSLF spectra are very sensitive to the local environment. The second and higher remote couplings will be much smaller due to the $1/r^3$ dependence, where r is the distance between the coupled nuclei. The broadening by these couplings will be significantly reduced by the fast MAS spinning. The linewidth of the dipolar spectra of those quaternary carbons gives the magnitude (7.5–16 kHz) of the line broadening caused by remote dipolar couplings and proton spin exchange, which are responsible for the severe broadening in all spectra of Fig. 5. Comparison of the dipolar spectra of the CH_3 and C groups shows that the intensity of the CH_3 spectrum is much higher than that of the C spectrum (the MASSLF spectra in Fig. 4 and Fig. 5 are not plotted to the same intensity scale). One reason for this is that the intensity of the spinning sidebands of the COO group is much higher than that of the CH_3 group. Another possible reason is that there is no ${}^1H$ directly bound to quaternary carbons; the cross-polarization transfer is by remote heteronuclear couplings, which lead to a lower rate of cross polarization. Therefore, the CH_3 group can be distinguished from the C group by the significant difference in the intensities of their ${}^{13}C$ heteronuclear dipolar spectra, in addition to a significant difference in their chemical shifts.

Figure 6 shows the 2D MASSLF spectrum of *trans*-4-methyl-1-cyclohexanecarboxylic acid obtained with a spinning rate of 3.0 kHz, with a contact time of 2.5 ms, and with a recycle time of 2.5 s. Figure 7 shows the MASSLF spectra of the different carbons. The structure and the CPMAS spectrum are at the top of Fig. 6. The MASSLF spectra in Fig. 7 are not plotted to the same intensity scale. In solution, the two carbons b are equivalent and the two carbons d are equivalent. In the solid state, the two carbons b become nonequivalent, and two peaks appear, and so they are both labeled with the letter b. The different carbons are resolved in the F_2 dimension; therefore, the local field of each carbon is separated along the F_1 dimension.

One can see in Fig. 7 that the MASSLF spectra of all CH and CH_2 groups suffer severe line broadening. However, the patterns of the dipolar spectra for different groups are still distinguishable in Fig. 7. A doublet pattern is seen for all
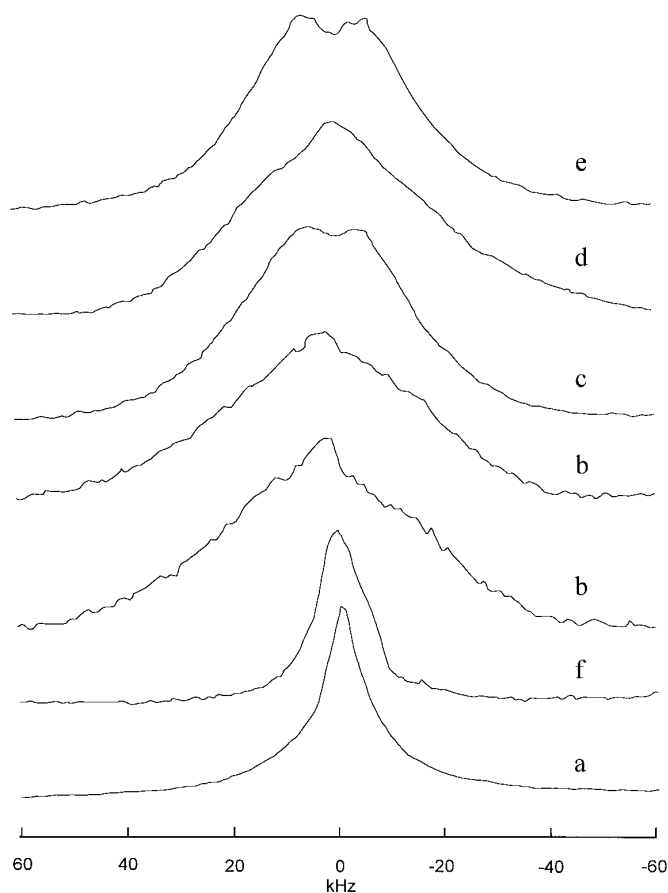

FIG. 7. MASSLF ${}^{13}C$ spectra in the F_1 dimension from Fig. 6. The spectra are not plotted to the same intensity scale.

CH groups (Fig. 7c and Fig. 7e) and a ''broad triplet-like'' pattern is seen for all CH_2 groups (Fig. 7b and Fig. 7d). The doublet pattern of the carbon e is more evident than that of carbon c. One possible reason for this could be the difference in their remote couplings. Carbon c is connected to a CH_3 group in addition to the two CH_2 groups, whereas carbon e is connected to a COOH group in addition to the two CH_2 groups. The total remote coupling to carbon c is stronger than that of carbon e. Another possible reason for this could be that peak c(CH) is partially overlapped with peak d(CH_2) in the F_2 dimension. Such overlapping may result in incomplete separation of the local field in the F_1 dimension. The MASSLF spectrum of CH_3 in the F_1 dimension has a Lorentzian lineshape with a linewidth of 10 kHz. The linewidth of the MASSLF spectrum of the COO group is about 8 kHz. The intensity of the dipolar spectrum for f is much lower than that of CH_3 because of higher intensity of the spinning sidebands of COO.

It should be pointed out that the tetrahedrally bound carbons are not clearly distinguished from the CH_3 groups in this approach because of the comparable linewidth and inten-

sities of their dipolar spectra and their similar chemical shifts. Other techniques may be used. The difference between the tetrahedrally bound carbons and other quaternary carbons could be that the tetrahedrally bound carbons may, in general, have more protons in the nearest neighboring sites, in addition to the significant difference in their chemical shifts. This may result in a higher cross-polarization rate which is comparable to that of the CH_3 groups.

Compared to other editing approaches, one can see from these two examples that this approach is relatively simple and straightforward without any calculation and editing. The magnitude of the line broadening is the major concern in this approach. The magnitude of the line broadening observed here probably represents the situation for most organic solids because the two compounds have high proton densities which should be comparable to most other organic solids. If the line broadening for a compound is much higher than that observed here, other editing approaches should be used.

CONCLUSION

The results of this experiment demonstrate that the CH, CH_2, CH_3, and nontetrahedrally bonded quaternary C resonances in high-density ^1H organic solids can be directly distinguished by ^{13}C 2D MASSLF spectroscopy without homonuclear proton decoupling during the evolution period. For the MASSLF spectra in the F_1 dimension, CH's show a Pake doublet pattern, CH_2's show a ''broad triplet-like'' pattern, and CH_3's show much narrower dipolar spectra than those of CH and CH_2 because of fast methyl rotation. The dipolar spectrum of a quaternary C is dictated by the remote couplings with the neighboring protons. Nontetrahedrally

bound C can be distinguished from CH_3 by the significant difference in intensities of the heteronuclear dipolar spectra, in addition to the large chemical-shift difference. The MASSLF spectra are influenced and broadened by homonuclear proton dipolar interactions, remote couplings, and proton spin-exchange effects. The MASSLF spectra are sensitive to the local environment of the carbons.

REFERENCES

1. A. Pines, M. G. Gibby, and J. S. Waugh, *J. Chem. Phys.* **59,** 569 (1973).
2. E. R. Andrew, A. Bradburg, and R. G. Eades, *Nature* **182,** 1659 (1958).
3. I. J. Lowe, *Phys. Rev. Lett.* **2,** 285 (1959).
4. S. J. Opella and M. H. Frey, *J. Am. Chem. Soc.* **109,** 5854 (1979).
5. J. Peng and L. Frydman, *J. Magn. Reson. A* **113,** 247 (1995).
6. N. K. Sethi, *J. Magn. Reson.* **94,** 352 (1991).
7. G. G. Webb and K. W. Zilm, *J. Am. Chem. Soc.* **111,** 2455 (1989).
8. T. Terao, H. Miura, and A. Saika, *J. Chem. Phys.* **75,** 1573 (1981).
9. M. T. Melchior, 22nd Experimental NMR Conference, Poster B-29, Asilomar California, 1981.
10. J. S. Hartman and J. A. Ripmeester, *Chem. Phys. Lett.* **168,** 219 (1990).
11. R. Sangill, N. Rastrup-Anderson, H. Bildson, H. J. Jakobsen, and N. C. Neilsen, *J. Magn. Reson. A* **107,** 67 (1994).
12. X. Wu and K. W. Zilm, *J. Magn. Reson. A* **102,** 205 (1993).
13. D. P. Burum and A. Bielecki, *J. Magn. Reson.* **95,** 184 (1991).
14. B. C. Gerstein and C. R. Dybowski, ''Transient Techniques in NMR of Solids,'' p. 164, Academic Press, Orlando, Florida, 1985.
15. E. F. Ryaczewski, B. L. Neff, and J. S. Waugh, *J. Chem. Phys.* **67,** 1231 (1977).
16. H. S. Gutowsky, G. B. Kistiakowsky, G. E. Pake, and E. M. Purcell, *J. Chem. Phys.* **17,** 972 (1949).
17. T. Nakai, J. Ashida, and T. Terao, *J. Chem. Phys.* **88,** 6049 (1988).