High Resolution Solid-state NMR Spectra of Leucine: a Re-examination

Mark D. Hollingsworth*[a] and Natsuko Cyr[b]

[a] *Chemistry Department, University of Alberta, Edmonton, Alberta T6G 2G2, Canada*
[b] *Alberta Research Council, Edmonton, Alberta T6H 5X2, Canada*

In the CP-MAS ^{13}C NMR spectrum of polycrystalline L-leucine, the splitting of the resonance from the β-carbon can be assigned to site differences in the $P2_1$ unit cell, and not to long-range residual dipolar coupling between ^{14}N and ^{13}C.

In the course of our solid-state NMR studies of functional group recognition in channel inclusion compound formation, we have needed to know the importance of residual dipolar coupling between ^{14}N and ^{13}C atoms separated from the nitrogen by one atom. Asymmetric splittings or broadened resonances that appear in ^{13}C cross-polarization-magic angle spinning (CP-MAS) NMR spectra[1] of solids containing ^{14}N are well known in the literature.[2—6] Owing to the nuclear quadrupole moment of ^{14}N, the axis of quantization of ^{14}N spins is shifted away from the applied field by electric field gradients within the molecule. Consequently, magic angle spinning cannot completely average the dipolar coupling between ^{14}N and carbon, and carbon resonances often appear as asymmetric doublets. Most often, these band splittings have been confined to carbon atoms directly bonded to ^{14}N, but there have been a few reports of long-range residual dipolar coupling,[4,7—9] especially at lower magnetic field strengths, where the quadrupolar interaction is significant compared to the Zeeman interaction.

We were intrigued by a report of such long-range coupling in L-leucine,[4] where splitting was observed not only for the α-carbon, but for the β-carbon as well. Both resonances were reported to be narrow singlets in the spectrum of polycrystalline [^{15}N]-leucine, as a subsequent review showed.[10] At 38 MHz, the resonance from the α-carbon of L-leucine exhibited a splitting of 1.4 ppm (53 Hz) while the β-carbon

resonance showed an even larger splitting of 1.8 ppm (68 Hz). Since the principal axis of the electric field gradient tensor at nitrogen should lie very close to the N–C_α bond,[11] and since the dipolar interaction varies with the inverse of the cube of the distance between ^{14}N and ^{13}C, we expected the ^{14}N–C_β coupling to be vanishingly small compared to that for the ^{14}N–C_α pair. A survey of the literature suggests that coupling between ^{14}N and C_β in L-leucine has been regarded as anomalous, but some authors have cited this result in papers in which residual dipolar coupling between distant atoms has been inferred.[8]

Our 50.3 MHz CP-MAS NMR spectra of carefully crystallized L-leucine [Figures 1(a) and 2(a)] exhibited the same general features as the previously reported spectra of crystalline L-leucine, including a single resonance for the methyl and methine carbons.† In our spectra, the doublet from the α-carbon was less well-defined than at 38 MHz,[4] as expected for the inverse field dependence of the residual dipolar coupling between ^{14}N and C_α.[14] However, the overall width of this broad band seemed to change only slightly when the field was increased. More significantly, the measured splitting in the β-carbon resonance (89 Hz) was *larger* than the splitting measured at 38 MHz, as expected for the normal scaling with magnetic field strength for two different sites. The carbonyl band was also doubled, just as in the full spectrum of crystalline L-leucine, as shown in ref. 15.

The 89 Hz splitting in the β-carbon resonance and the approximate 1 : 1 intensity ratio for this doublet (and for the carbonyl doublet) can be interpreted most readily in terms of the $P2_1$ space group of crystalline L-leucine,[16,17] in which two crystallographically inequivalent leucine molecules are related in pairs by a two-fold screw axis. The dihedral angles about C-1–C-2 are significantly different for the two different leucine molecules, so significant shielding differences are expected for C_β, which is in the vicinity of the magnetically anisotropic carboxyl group.[18] Similar crystallographic effects have been observed in the methine and methyl peaks in the CP-MAS spectrum of L-valine (space group $P2_1$), in which the dihedral angles about C_α–C_β are very different for the two molecules in the asymmetric unit.[15]

The spectrum of carefully crystallized [^{15}N]-L-leucine [Figures 1(b) and 2(b)] verifies our assignment of site differences for the two β-carbon resonances. Since ^{15}N does not have a quadrupole moment, magic angle spinning averages the dipolar interaction between ^{15}N and carbon atoms in the sample, and apart from the extremely small scalar coupling between ^{15}N and C_α, only site differences can give rise to splitting. In Figure 2(b), band doubling is again observed for the α-carbon in [^{15}N]-L-leucine, but the overall width is reduced by approximately 25%. Thus, at 50.3 MHz, both site differences and residual dipolar coupling contribute significantly to the linewidth of C_α in [^{14}N]-L-leucine.

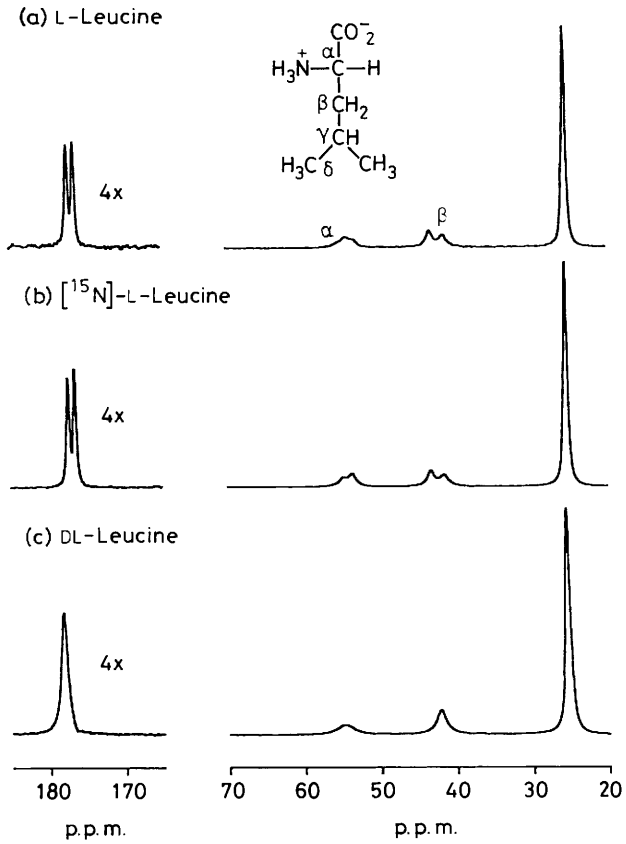

Figure 1. 50.3 MHz CP-MAS NMR spectrum of polycrystalline samples in regions of significant absorption, excluding spinning side-bands. For (a) L-leucine δ 176.3, 177.2 (C-1), 54.0 (br., C_α), 41.3, 43.1 (C_β), 25.3 (C_γ), 25.3 (C_δ). (b) [^{15}N]-L-Leucine δ 176.3, 177.2 (C-1), 53.4, 54.6 (C_α), 41.3, 43.1 (C_β), 25.3 (C_γ), 25.3 (C_δ). (c) DL-Leucine δ 178.1 (C-1), 55.0 (C_α), 42.1 (C_β), 25.5 (C_γ), 25.5 (C_δ). Spectra were collected with sample spinning rates of 4.75, 4.4, and 4.0 kHz, respectively.

In trying to reconcile our results with the previous observations of single bands for α-, β-, and carbonyl carbon atoms in carefully crystallized [^{15}N]-leucine,[10] we explored the possibility of polymorphism in L-leucine, but in our experiments, slow crystallization always gave the expected crystal form. Crystals that grew on the surface of the aqueous solution gave virtually the same CP-MAS NMR spectra as those growing on the bottom of the flask. A much more plausible explanation is that the earlier workers had inadvertently used [^{15}N]-DL-leucine instead of the L enantiomer, since both forms were available from the manufacturer (KOR Isotopes Inc.) in 1980. Unfortunately, the isomeric form was not specified in the paper in which the spectrum of [^{15}N]-leucine was displayed.[10]

In our spectrum of carefully crystallized DL-leucine [Figures 1(c) and 2(c)], the C_α resonance is broadened by residual dipolar coupling, but the C_β and carbonyl resonances occur as simple singlets, as expected for the $P\bar{1}$ space group, in which

† Crystals of L-leucine (General Intermediates of Canada) and DL-leucine (Eastman Organic Chemicals) were formed at their isoelectric points by slow evaporation of H_2O from saturated solutions at 5 °C. Crystals of [^{15}N]-L-leucine (≥ 99 atom % ^{15}N, Isotec Inc.) were prepared by very slow cooling of a similar solution from 94 to 5 °C. Polarimetry gave the expected optical rotation for each sample. Fast atom bombardment mass spectrometry of L-leucine and [^{15}N]-L-leucine was used to verify the virtually complete incorporation of ^{15}N in the latter. In each case, the large crystals were checked with polarized light microscopy before grinding for powder *X*-ray diffractograms (Philips model 12045B/3 powder diffractometer), which were used to verify the correctness of crystal form. CP-MAS NMR spectra were obtained at 50.3 MHz on a Bruker CXP-200 spectrometer utilizing a probe from Doty Scientific Inc. (7 mm id sapphire rotors spinning at 4.0—4.75 kHz). Each spectrum was the result of either 3000 (DL-leucine, [^{15}N]-L-leucine) or 2044 (L-leucine) scans (6 μs 90° pulses) with cross-polarization contact time of 2 ms, acquisition time of 102 ms (4096 data points) during proton decoupling, and a 5 s recycle delay. Each 4 K block was zero-filled to 16 K and transformed with 5 Hz line broadening. For unlabelled samples we used an internal chemical shift standard, tetrakis(trimethylsilyl)silane (δ_C = 3.50),[12] which was synthesized by the literature procedure.[13] Chemical shifts of the carboxyl in [^{15}N]-L-leucine were referenced to those of L-leucine. Solid-state NMR spectra were simulated with the program GLINFIT, by Alex D. Bain of McMaster University.

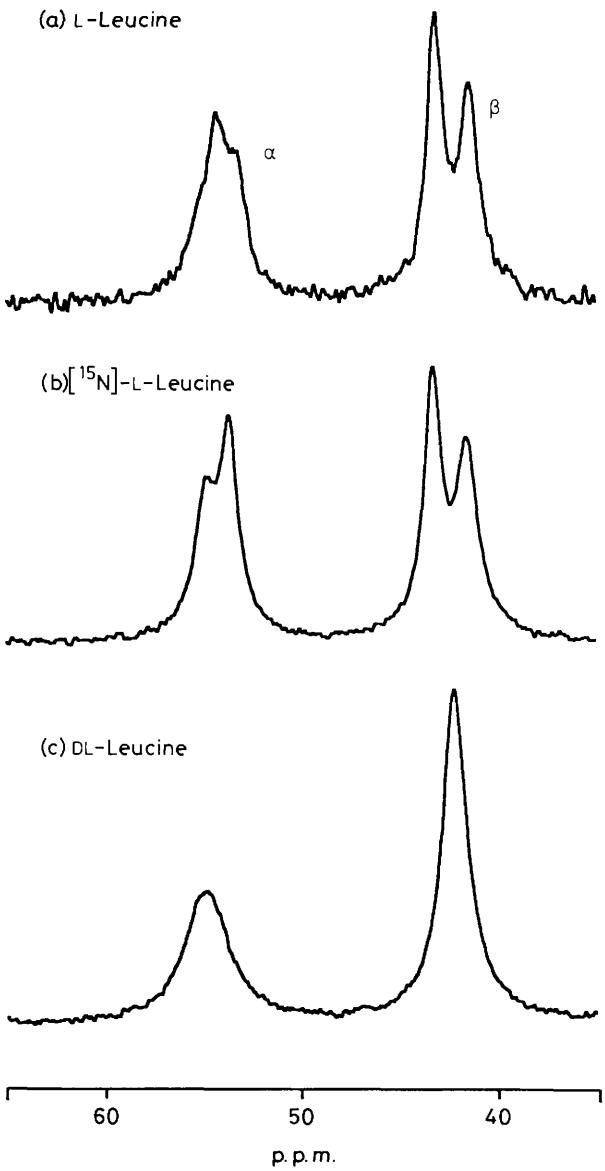

Figure 2. 50.3 MHz CP-MAS NMR spectra of polycrystalline samples of (a) L-leucine, (b) [^{15}N]-L-leucine, and (c) DL-leucine, expanded to show resonances from C_α and C_β.

the two enantiomers share a centre of symmetry.[19] For [^{15}N]-DL-leucine, a similar spectrum is expected, but without the broadening in the resonance for C_α. Downfield shifts in the carboxy resonance in DL-leucine and in the spectrum shown in ref. 10 support our explanation for the singlets observed in the previously recorded spectra of [^{15}N]-leucine.‡

The present results indicate that although such long-range effects have been documented[20] caution must be exercised in assignments of band doubling to long-range residual dipolar coupling, especially in cases where crystallographic inequivalence is significant.

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‡ *Note added in proof:* Prof. S. J. Opella has confirmed that the spectrum shown in ref. 10 was that of [^{15}N]-DL-leucine.