High-resolution Features of the ^{13}C N.M.R. Spectra of Solid Amino Acids and Peptides

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Summary ^{13}C N.m.r. spectra of solid amino acids and peptides display resonance shifts and splittings not seen in solution; these solid state effects are illustrated with Tyr, Leu, and the mixed peptides Tyr-Leu and Leu-Tyr.

HIGH-resolution ^{13}C n.m.r. spectra of powders and solutions of organic molecules are expected to be essentially identical. By utilizing cross-polarization, proton-decoupling, and magic angle spinning, solid samples give ^{13}C n.m.r. spectra with resolution equivalent to and sensitivity greater than the same compounds in solution.[1] Individual carbon sites give single line resonances, the positions of which correspond to the isotropic chemical shift. These simple spectral correspondences have been seen for most, but not all, molecules in the solid state. Molecules with high chemical functionality have a number of ^{13}C n.m.r. features that differ between spectra of solids and solutions, with some of the most dramatic deviations in spectra occurring for amino acids, peptides, and proteins.[2] The most prominent of these features are (i) large changes in chemical shift for some aliphatic groups, (ii) splittings of ring carbon resonances from the influence of substituent groups, and (iii) splittings of resonances arising from carbons bonded to nitrogen. These effects are illustrated here with spectra from leucine, tyrosine, and their mixed dipeptides. The structure of the peptide Leu-Tyr is shown in Figure 1.

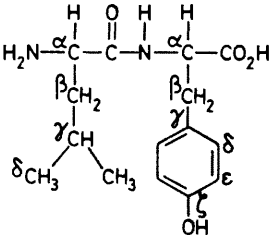

FIGURE 1. Structural formula of the dipeptide Leu-Tyr with the carbon atoms labelled.

Large changes in chemical shift of the aliphatic resonances of leucine are apparent in comparing spectra from aqueous solution with those from the solid state of the free amino acid. These shifts among the γ and δ carbons are as large as 5 p.p.m. and are sufficient to alter completely the appearance of spectra in the upfield (20—30 p.p.m.) region of Figure 2.† Such large phase change-induced shifts are surprising for methyl and methylene carbons which have relatively small shift anisotropies (20—35 p.p.m.),[3] and are unlikely to be involved in hydrogen bonding or in other highly directional chemical interactions.

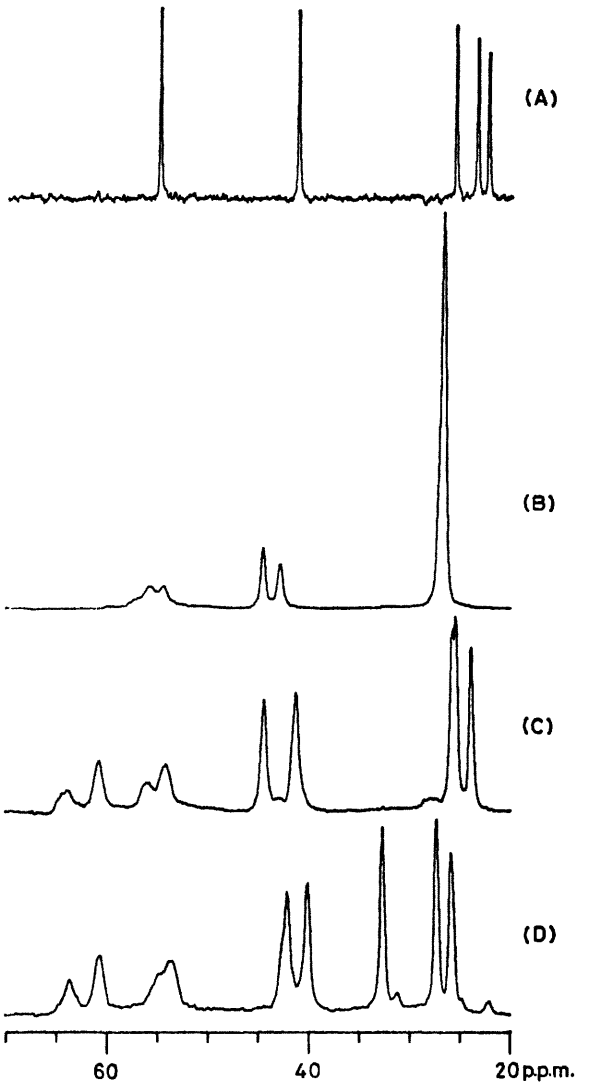

FIGURE 2. Aliphatic region of ^{13}C spectra (δ/p.p.m., external Me_4Si). (A) Leu in solution at pH 6·0 (α, 54·8; β, 41·0; γ, 25·5; δ, 23·4, 22·2), (B) Leu powder (α, 55·6, 54·2; β, 44·4, 42·6; γ, 26·5; δ, 26·5), (C) Leu-Tyr powder (α, 63·6, 60·5, 55·8, 53·8; β, 44·1, 41·0; γ, 25·5; δ, 25·1, 23·5), (D) Tyr-Leu powder (α, 63·5, 60·4, 54·6, and 53·2; β, 41·8, 39·9; γ, 32·1; δ, 27·1, 25·6).

Substituent groups on aromatic rings generally exist in a single asymmetric conformation in the solid state resulting in adjacent carbons having different environments. This

† The powder spectra which have 5 Hz line broadening added were obtained at 38 MHz on a homebuilt double-resonance spectrometer with magic angle sample spinning (3·4 kHz); each spectrum is the result of 300 single 1 ms cross-polarizations recycled every 3 s and data acquired for 100 ms with 2·4 mT proton decoupling. The solution spectrum which has 2·2 Hz line broadening added was taken on a Nicolet NT-150 spectrometer in a 20 mm tube with 256 90° pulses recycled every 10 s with data acquired for 450 ms. The amino acids and peptides were obtained from Sigma Biochemicals as the L isomers. The powder samples were prepared by crystallization from boiling water at neutral pH.

is seen in solid state n.m.r. spectroscopy as a line doubling or splitting. This effect has been used previously for conformational analysis of polymers and small molecules.[4]

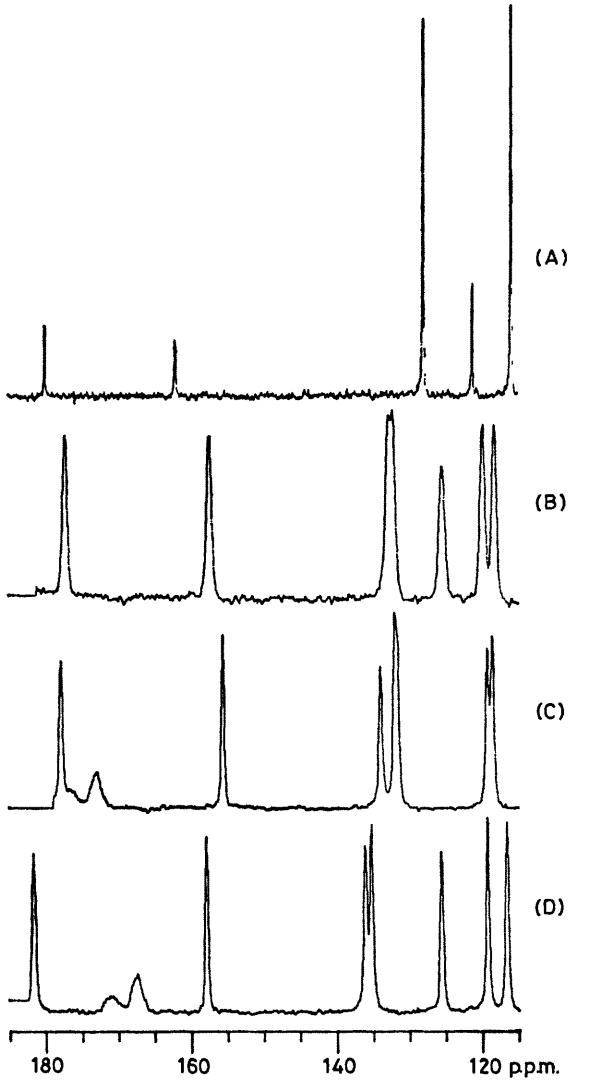

FIGURE 3. Aromatic and carbonyl region of ^{13}C spectra. (A) Tyr in solution at pH 12·6 (carboxy, 181·0; ζ, 163·1; δ, 129·0; γ, 122·3; ε, 116·9), (B) Tyr powder (carboxy, 177·0; ζ, 157·3; δ, 132·6, 132·0; γ, 125·4; ε, 119·7, 118·1), (C) Leu-Tyr powder (carboxy, 177·7; carbonyl, 176·6, 172·8; ζ, 155·4; δ, 133·7, 131·5; γ, 131·8; ε, 119·1, 118·4, (D) Tyr-Leu powder (carboxy, 181·5; carbonyl, 171·1, 167·3; ζ, 157·8; δ, 135·9, 135·1; γ, 125·5; ε, 119·1, 116·5). Powder spectra are from the same data used for Figure 2. The zeroed data points at the extreme downfield end of the spectra mark the positions of spinning sidebands from the Delrin rotor.

In Figure 3A the ^{13}C n.m.r. spectrum of tyrosine in solution has all chemically unique carbons as single lines, but in Figure 3B for polycrystalline tyrosine the ε carbons, *ortho* to the hydroxy group, are split by *ca.* 2 p.p.m. and there is a slight splitting of the δ carbons, *meta* to the hydroxy group. The hydroxy group-induced splitting of tyrosine ring carbons varies substantially in magnitude among the two peptides and solid tyrosine shown. The resonance shifts of the aromatic carbons between Tyr crystallized from water at neutral pH and Tyr in solution at high pH are due to the titration of the phenol group and not to the change in phase.

A third major influence on spectral appearance in solid state ^{13}C n.m.r. spectroscopy is the presence of nitrogen in the molecule. Most resonances from carbons bonded to nitrogen are split into broadened asymmetric doublets and some resonances from more distant carbons are also affected; the spectra of Figure 2 show that the extent of splitting is variable. This effect results from the ^{14}N nuclear quadrupole moment interfering with the ability of magic angle spinning to average out carbon–nitrogen dipolar interactions.[5] Leucine alone shows a broad doublet around 55 p.p.m. from its α carbon as well as a split β carbon resonance near 43 p.p.m.; both of these carbons give sharp single lines in [^{15}N]Leu. The peptides Leu-Tyr and Tyr-Leu have both α carbon resonances split, but to varying extents. In Figures 3C and 3D the downfield terminal carboxy resonance is a narrow line, while the more upfield peptide carbonyl resonance is a broadened doublet. The peptide bond nitrogen of Tyr-Leu splits its α carbon (1·4 p.p.m. in Figure 2D) much less than its carbonyl carbon (3·8 p.p.m. in Figure 3D).

The results from a simple peptide system show that caution is indicated when comparing ^{13}C n.m.r. spectra for solids and solutions. These spectral features have potential for providing useful structural information, although they greatly complicate the interpretation of ^{13}C n.m.r. spectra of biopolymers where chemically interesting inter- and intra-molecular interactions must be distinguished from solid state effects. Even though the changes in isotropic chemical shift and the substituent-induced splittings may be amenable only to empirical correlations, they certainly can be an effective monitor of symmetry and motional averaging. There is a good chance that molecular geometry can be deduced from the asymmetric splittings resulting from ^{13}C–^{14}N coupling. In addition, since the presence of a characteristically split resonance is usually indicative of a covalently bonded nitrogen, resonance assignments can be based on this effect.

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