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Vicinal ^{13}C–^{15}N Coupling Constants in Peptides

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Summary Vicinal ^{13}C–^{15}N coupling constants for three ^{15}N-labelled peptide models having C^{β}–C^{α}–$C^{15}N$ dihedral angles constrained to 110 or 160° are smaller than 1 Hz, which minimizes their potential utility in peptide conformation studies.

Lichter *et al.*[1] have reported ^{13}C–^{15}N coupling constants for some saturated primary amides labelled with ^{15}N, in which rotation about the C^{α}–C' bond is not obviously restricted. They were unable to observe three-bond couplings between ^{15}N and $^{13}C^{\beta}$, and suggested that this might in part be the result of rotational averaging. The magnitude and dihedral angle dependence of the C^{β}–N three-bond coupling would be of interest as a possible indication of the dihedral angle ψ in peptide systems[2,3] and is worth additional study. We report here related results, obtained from peptide systems with restricted ψ rotation, which confirm that the ^{15}N–C'–C^{α}–C^{β} coupling is in fact very small.

Two diketopiperazines, cyclo-(Gly-Pro) and cyclo-(Gly-Phe), both 95% substituted with ^{15}N in the glycine residue, were examined. The dipeptide ring of cyclo-(Gly-Pro) is constrained to a boat form in which the proline β carbon is quasi-equatorial. The ^{15}N–C'–C^{α}–C^{β} angle may be estimated as *ca.* 155° by analogy with the structure of cyclo-(L-Pro-L-Leu) established by *X*-ray crystallography.[4] The diketopiperazine ring of cyclo-(Gly-Phe) is also a boat, but with the phenylalanine β-carbon quasi-axial.[5] The N-C'-C^{α}-C^{β} angle is probably close to that determined in *X*-ray studies of cyclo-(Gly-L-Tyr),[6] *ca.* 110°.

^{13}C n.m.r. spectra of these substances were measured with a Varian XL-100 instrument, equipped with a VFT-100 Fourier transform accessory, with noise decoupling. In neither of the diketopiperazines was splitting of the C^{β} line observed. An upper limit to the magnitude of the N–C^{β} coupling may be estimated from the linewidth data. For cyclo-(Gly-Pro) in water, the observed width of the C^{β} line was 1·2 Hz and the width of the C^{γ} line was 1·4 Hz. The width of the line from dioxan added to the sample was 1·0 Hz. For cyclo-(Gly-Phe) in methanol containing 40% hexafluoroacetone,1·5D_2O the width of the C^{β} line was 2 Hz and that of added internal dioxan was 1·2 Hz.

We also examined, with similar results, *N*-acetylprolinamide bearing primary amide ^{15}N. For that species of this substance with a trans Ac–Pro amide bond the conformation about C^{α}–C' in non-polar solvents is probably determined by an intramolecular hydrogen bond between primary amide N–H and acetyl C=O.[7] The ^{15}N–C'–C^{α}–C^{β} angle is thus held in a range near 160°; on the other hand, it is not so constrained in water.[7] For AcPro $^{15}NH_2$ we observed the following widths at half-height: in $CDCl_3$ (0·5% CF_3CO_2H), C^{β} 1·5, C^{γ} 1·2, C^{Me} 1·2 Hz; in H_2O, C^{β} 0·9 C^{γ} 0·8, C^{Me} 1·0 Hz.

In these measurements the actual resolution attainable was limited to 0·5 Hz by the acquisition time, so that it appears that at a dihedral angle of 160° the magnitude of

$J(^{15}NCC^{13}C)$ is $<$ *ca.* 0·5 Hz, and that at 110° it is no greater than *ca.* 1·0 Hz. It is not likely, therefore, that ^{15}N–^{13}C couplings will be of service in experimental determinations of the peptide conformational angle ψ.

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