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REGARDING H-C-C(O)-^{15}N COUPLING AS AN INDICATOR OF PEPTIDE TORSIONAL ANGLE

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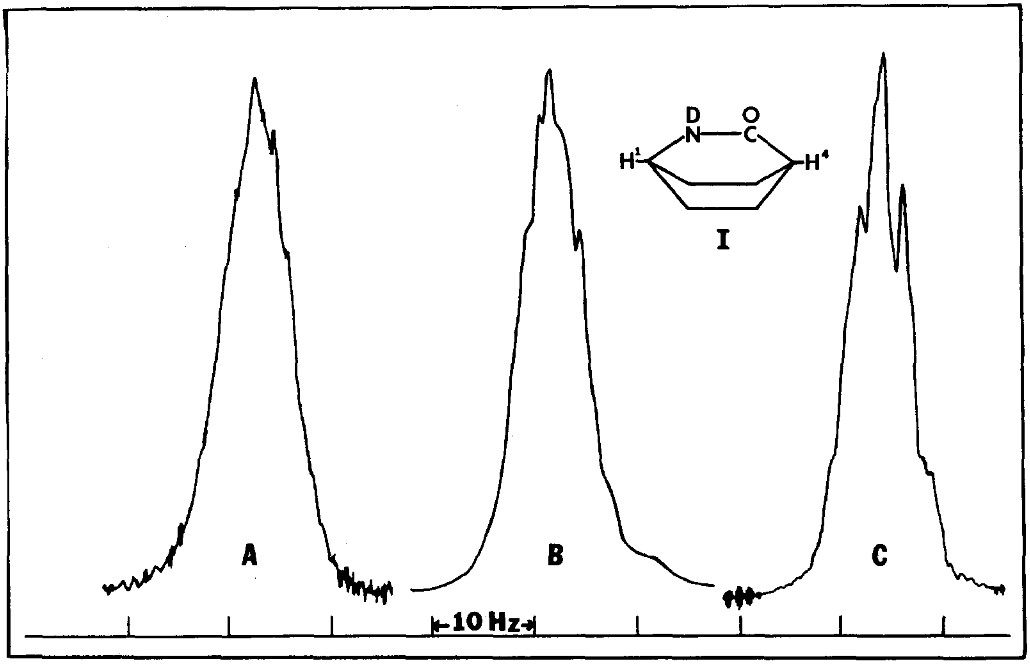
The use of vicinal H-C$^{\alpha}$-C'-^{15}N coupling constants as an indicator of the corresponding peptide dihedral angle, ψ, has been an attractive possibility to students of peptide conformation.[1,2] Although correlations have been proposed that predict readily observable magnitudes for $^{3}J_{HN}$ at some angles,[3,4] the couplings estimated from actual spectra have been small, less than 2 Hz. This has sometimes been attributed to conformational averaging of positive and negative values.

Because the largest (negative) coupling is expected for an H-C-C(O)-^{15}N angle of 180°, we prepared ^{15}N enriched 2-azabicyclo[2.2.2]octanone-3(I), in which the angle is maintained at that value.[5] Distinct splittings caused by the sought-for coupling are absent in its proton spectrum. We therefore compared the spectrum of the enriched material with that of the ^{14}N analog, in which coupling to nitrogen is virtually eliminated by rapid quadrupolar relaxation of the nitrogen nucleus. The comparison is shown in the Figure and yields an estimated coupling of ± 1.3 Hz (± 0.2 Hz).

In I the C^{1}-N-C^{3} and N-C^{3}-C^{4} bond angles (116° and 112°) are smaller than the corresponding angles in an unconstrained peptide bond (122° and 116° respectively),[5] but it is unlikely that 4° distortion at the carbonyl will have any large effect on the strength of the coupling, particularly when the coupled atoms are anti. We conclude that the H-C$^{\alpha}$-C'-^{15}N constant is likely to be generally too small for use in study of peptides.

The observed couplings (± 0.2 Hz) for I are compared in the Table with calculated values for I and with values we have determined for the strain-free dipeptide model acetylalanine N-methylamide, prepared from ^{15}N-enriched alanine. (The signs of the experimentally observed couplings were not determined.) Although the corresponding one and two-bond proton-nitrogen couplings are similar in the two substances, the one and two-bond carbon-nitrogen splittings do reflect the change in hybridization in the slightly strained bicyclic compound.



Resonance of H^4 of 2-azabicyclo[2.2.2]octanone-3 at 250 MHz, 0.1 M solution in D_2O. Spectrum A is of 95% 15-N enriched material and spectrum C is of the normal compound. The spectra were obtained by rapid scan correlation spectroscopy, at the same instrumental line width, about 0.5 Hz for chloroform. A resolution enhancement of 0.5 Hz was applied to emphasize the structure of the lines. Trace B is synthesized by summing two traces of spectrum C shifted relative to each other by 1.30 Hz. Shifts of 0.52, 1.04, 1.56 or 2.08 Hz produced traces distinctly different from spectrum A.

Table. Couplings to ^{15}N in Amides

Coupling (Hz)	I (exp)	I (calc)	$Ac^{15}N$-Ala-NHMe
$^{3}J_{HC^{\alpha}C'(O)N}$	1.3[a]	-5.9	1.2[b]
$^{3}J_{HC^{\beta}C^{\alpha}N}$	-	-	2.9[b]
$^{2}J_{HCN}$	1.5[a]	1.8	1.1[a]
$^{1}J_{HN}$	92.8[b]	-83.1	93.4[b]
$^{2}J_{C^{\alpha}C'(O)N}$	4.2[b]	-4.1	7.2[b]
$^{1}J_{C^{\alpha}N}$	7.0[b]	-31.2	11.0[b]
$^{1}J_{C'N}$	13.2[b]	-21.1	14.5[b]
$^{4}J_{HC^{\alpha}C'(O)NH}$	2.1[b]	2.3	

(a) Estimated as in Figure above. Sign undetermined.
(b) Direct observation of splitting. Sign undetermined.
(c) C^{α} refers to C^4 of I, C' to C^3.

To investigate the possibility that the small magnitude observed for the vicinal H-C^{α}-C'-^{15}N coupling in I was due to some electronic feature implicit in the actual molecule and not in the model compounds of reference 3, molecular orbital calculations of the Fermi contact contributions to the coupling constants in I were based on the finite perturbation theory (FPT) formulation with INDO (intermediate neglect of differential overlap) wavefunctions,[6,7] with a molecular geometry based on the X-ray structural data.[5] The calculated coupling constants are included in the table; the calculated value of - 5.9 Hz for the vicinal H-C^{α}-C'-^{15}N coupling in I is somewhat smaller in magnitude than the calculated value of - 6.5 Hz for N-methylacetamide in the cis amide arrangement in which the C^1-N-C^3 and N-C^3-C^4 angles were taken to be 126° and 118°, respectively.[3] Another calculation was performed for I using an idealized geometry based on the boat form of cyclohexane, but with C^1-N-C^3 and N-C^3-C^4angles of 120° and 109°, respectively. In this case the calculated value of the H-C^{α}-C'-^{15}N coupling constant was - 6.3 Hz. Thus, the calculated values are relatively insensitive to the molecular geometry, but are more than four times the magnitude of the experimental value! This appears to be a major anomaly for calculations of vicinal coupling constants in which at least one of the coupled nuclei is a proton. For certain coupling constants, which do not involve protons, i.e., directly bonded C-N coupling,[8] it is often possible to implicate non-Fermi contact mechanisms. However, in the case of vicinal H-C^{α}-C'-^{15}N coupling the inadequacy may be related to the one noted in detailed comparisons of the calculated INDO-FPT results and experimental data for long range H-H coupling constants;[9] because of the neglect of two-center exchange integrals in the INDO approximation,[10] certain types of spin polarization mechanisms,which would transmit the nuclear spin-spin coupling information, are absent.

Relevant calculated data for comparison with the other experimental data are also included in the Table. Except for the vicinal H-C^{α}-C'-^{15}N coupling constant value, others involving a proton are in good conformity with the experimental values. Furthermore, vicinal H-H coupling constants between H^4 and the adjacent methylene protons were calculated to be 2.0 Hz and 3.0 Hz. These values give a triplet of triplets with outer components separated by 10 Hz in conformity with spectrum C.

Enriched I was prepared by pyrolysis of the corresponding amino acid,[11]obtained by us via Hoffmann rearrangement of 4-carboxycyclohexane carboxamide. The largely cis amido acid was prepared by treatment of cis cyclohexane-1,4-dicarboxylic acid anhydride[12] with an equivalent of 95% enriched ammonium chloride plus an equivalent of triethylamine.

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