

Conformational Dependence of Vicinal $^{13}\text{C}'\text{NC}^\alpha\text{H}$ Coupling Constants in Peptides: A Dirac Vector Model Investigation*

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Synopsis

Theoretical calculations of the heteronuclear vicinal coupling constant $^3J(^{13}\text{C}'\text{NC}^\alpha\text{H})$ in peptides have been carried out using the Dirac vector model. The results showed an angular dependence for this coupling constant, which can be expressed in the form $^3J(^{13}\text{C}'\text{NC}^\alpha\text{H}) = A \cos^2 \theta + B \cos \theta + C$, where A , B , and C are constants and θ is related to the torsional angle ϕ of the peptide backbone. The results of the present calculations are in very good agreement with those obtained using finite perturbation theory at the INDO level of approximation.

INTRODUCTION

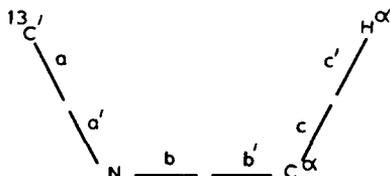
Considerable progress has been made in studies of the conformations of peptides and proteins using nmr spectroscopy.^{1,2} A Karplus-like³ angular relationship connecting the vicinal $\text{HNC}^\alpha\text{H}$ coupling constant $J_{\text{HNC}^\alpha\text{H}}$, with the peptide backbone dihedral angle ϕ has been found to be extremely useful in the conformational analysis of a large number of peptides in solution.⁴⁻⁸ However, the estimation of ϕ for peptides in solution based on $^3J_{\text{HNC}^\alpha\text{H}}$ sometimes leads to ambiguity since the same value of coupling constant can correspond to more than one relative orientation of the coupled protons. To make unambiguous assignments, it was suggested by Solkan and Bystrov⁹ that the vicinal heteronuclear $^{13}\text{C}'\text{NC}^\alpha\text{H}$ coupling constants could be used. The calculations performed using the finite perturbation theory at the INDO (intermediate neglect of differential overlap) level of approximation^{10,11} have shown an orientational dependence of $^3J(^{13}\text{C}'\text{NC}^\alpha\text{H})$ with the peptide backbone torsional angle ϕ . In this communication, we present the results of our theoretical investigation of vicinal $^{13}\text{C}'\text{NC}^\alpha\text{H}$ coupling constant $^3J(^{13}\text{C}'\text{H}^\alpha)$ using Dirac vector model formalism.^{12,13}

It has been found in previous studies¹⁴⁻¹⁶ that useful results can be obtained by evaluating the total coupling constants in π -electron systems as a sum of σ - and π -contributions separately. We have made use of this approximation, since the peptide C—N bond has a partial double-bond character. For the evaluation of the π -contribution, a recent formulation¹⁷ was used.

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THEORETICAL CONSIDERATIONS AND METHOD OF CALCULATION

A six-electron fragment was used for the evaluation of σ and π contributions each. The fragments considered can be schematically represented as follows:



a , b' , etc. represent the orbitals involved in the formation of the localized bonds. The a — a' bond between C' and N can be a σ - or a π -bond as the case may be.

The Fermi contact term for the σ contribution is given by¹⁸

$${}^3J_{\sigma}(^{13}C'H^{\alpha}) = \left[\frac{-2}{3h\Delta E} \right] \left[\frac{16\pi\beta\hbar}{3} \right]^2 \gamma_H\gamma_C\phi_H^2(0)\phi_{\sigma C}^2(0) \times \langle \psi_0 | S_{1s_H} \cdot S_{\sigma C} | \psi_0 \rangle \quad (1)$$

where $\phi_H^2(0)$ and $\phi_C^2(0)$ are the electron densities in the hydrogen $1s$ and carbon σ -orbitals at the respective nuclei, γ_H and γ_C are the magnetogyric ratios of proton and ^{13}C nuclei, and ΔE is the "average singlet-to-triplet excitation energy." The wave function ψ_0 in Eq. (1) refers to the singlet ground state. In the Dirac vector formulation of nuclear spin coupling constants, one uses a "spin description" of the localized bonds. The operator in the matrix element of Eq. (1) then has the property of mixing the excited singlet states with the singlet groundstate, which is a product of antisymmetrized two electron spin states, each one of them representing one localized bond.¹⁹ A perturbation calculation consisting of intraatomic and interbond exchanges as perturbation has been made.

When there is a π -orbital based at one of the coupled nuclei, the procedures for evaluation of the contribution arising via that π -orbital have to be modified because of the nodal character of this orbital at the nucleus. The π contribution can arise only from the polarization of the core electrons, leading to a finite density at the nucleus. The contact term analogous to Eq. (1) for the π contribution in the present case can then be written as¹⁷

$${}^3J_{\pi}(^{13}C'H^{\alpha}) = \left[\frac{-2}{3h\Delta E} \right] \left[\frac{16\pi\beta\hbar}{3} \right]^2 \left(\frac{6K_{\sigma\pi}}{E_{\sigma\pi}} \right) \gamma_H\gamma_C \times \phi_{1s_H}^2(0)\phi_{\pi C}^2(0) \langle \psi_0 | S_{1s_H} \cdot S_{\pi C} | \psi_0 \rangle \quad (2)$$

where $K_{\sigma\pi}$ is the carbon one-center exchange integral, $\langle 1s(1) 2p(2) | r_{12}^{-1} |$

$2p(1)2s(2)$, $E_{\sigma\pi}$ is the $1s-2p$ excitation energy for carbon, and $\phi_{\pi C}^2(0)$ is the electron density on the carbon π -orbital at the nucleus. The other terms in Eq. (2) have their usual meaning.

For $\phi_{\pi C}^2(0)$ in Eq. (2) the product of $1s$ and $2s$, we used the Hartree-Fock functions of carbon, $\phi_{1s2s}(0)$. The numerical values used for $\phi_{1s2s}(0)$, $K_{\sigma\pi}$, and $E_{\sigma\pi}$ were the same as the ones used earlier by Karplus and Fraenkel²⁰ in the study of ^{13}C hyperfine splittings in π radicals. The $\pi-\pi^*$ transition frequency²¹ of $61,000\text{ cm}^{-1}$ is 6.54 eV . The ΔE term in Eq. (2) was taken as half the sum of this value, and the normal singlet-to-triplet excitation energy of a C—H bond is 9 eV .

The Dirac vector model calculations were performed exactly in the same way as described in previous studies.^{19,22-24} The necessary valence bond exchange integrals, other than those for which the numerical values are available from previous calculations, were evaluated using the "approximate two-electron Hamiltonian" of Karplus.³ All the numerical evaluations were carried out on an IBM 360/44 computer at the Computer Centre, Indian Institute of Science, Bangalore.

RESULTS AND DISCUSSION

The results of the present calculation of $^3J(^{13}\text{C}'\text{H}^\alpha)$ as a function of θ , where θ is the dihedral angle between $\text{C}'-\text{N}$ and $\text{C}-\text{H}$ bonds, are listed in Table I, along with the σ and π contributions. The σ contributions to the total coupling has an angular dependence of the form $A \cos^2 \theta + B \cos \theta + C$, where A , B , and C are constants. Hence the angular dependence of the σ contribution is not symmetric. This is shown in curve a of Fig. 1. The angular variation of the π contribution is symmetric (curve b of Fig. 1) and is of the form $A' \cos^2 \theta + B'$. The π contribution has a maximum magnitude (and a negative sign) at $\theta = 90^\circ$, but the magnitude decreases as θ changes from 90° and becomes positive at $\theta = 0^\circ$ or 180° .

The dihedral angle θ is related to the torsional angle ϕ ²⁵ for peptides, as $\phi = \theta - 120^\circ$. The ϕ angle dependence of $^3J(^{13}\text{C}'\text{H}^\alpha)$ values calculated herein is shown in Fig. 2. The results of Solkan and Bystrov,⁹ using the finite perturbation theory (FPT) at the INDO level of approximation, as

TABLE I
Angular Variation of $^3J(^{13}\text{C}'\text{H}^\alpha)$ and its σ and π Contributions

θ^a (deg)	σ (Hz)	π (Hz)	Total $^3J\text{C}'\text{H}$ (Hz)
0	4.64	0.13	4.77
30	3.41	-0.47	2.94
60	1.07	-1.73	-0.66
90	0.06	-2.40	-2.34
120	1.48	-1.73	-0.25
150	4.16	-0.47	3.69
180	5.52	0.13	5.65

^a θ is the dihedral angle between the $\text{C}'-\text{N}$ and $\text{C}-\text{H}$ bonds.

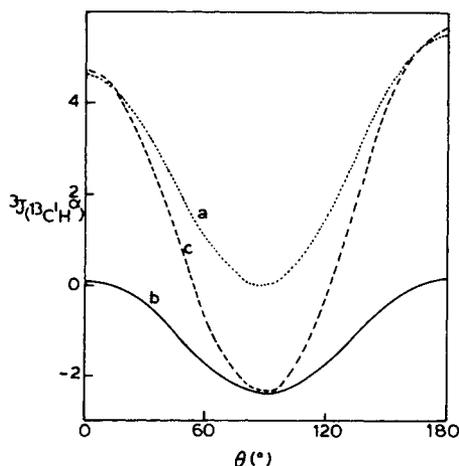


Fig. 1. Angular dependence of vicinal $^{13}\text{C}'\text{NC}^{\alpha}\text{H}^{\alpha}$ coupling constant in peptides: a, σ contribution; b, π contribution; c, total coupling constant.

well as on the ones obtained by Bystrov et al.²⁶ from the available experimental values, are also shown in Fig. 2. The results of the present calculation are in better agreement, in certain ranges of ϕ (namely, 0° to -180°), with the experimental values deduced by Bystrov et al.²⁶ than with those of the calculations by Solkan and Bystrov.⁹

The angular dependence of $^3J(^{13}\text{C}'\text{H}^{\alpha})$, as found from the present calculation, is expressible in form

$$^3J(^{13}\text{C}'\text{H}) = A \cos^2 \theta + B \cos \theta + C$$

A , B , and C were estimated to be $+7.55$, -0.44 , and -2.34 Hz, respectively. The corresponding values by Bystrov et al.²⁶ are 9.0 , -4.4 , and -0.8 Hz, respectively. In this connection, we note the following. First, only three

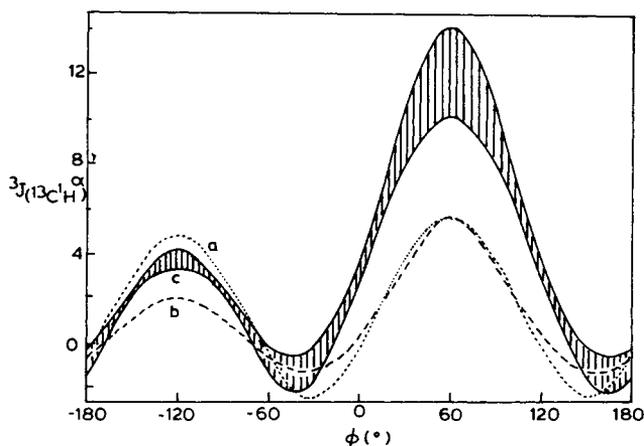


Fig. 2. ϕ angle dependence of $^3J_{\text{C}'\text{NC}^{\alpha}\text{H}^{\alpha}}$: a, present calculation; b, from calculation of Solkan et al. (Ref. 9); c (shaded area), from experimental values of Bystrov et al. (Ref. 25).

experimental coupling constants were available for deducing the experimental curve of Bystrov et al.²⁶ Second, the experimental value quoted for $\theta = 90^\circ$ by Bystrov et al.²⁶ has been taken from the nmr studies of the conformation of *N*-acetyl-L-tryptophan in its complex with α -chymotrypsin by Rodgers and Roberts.²⁷ The latter authors have obtained this value by plotting the $^3J(^{13}\text{C}'\text{H}^\alpha)$ against the fraction of bound substrate and extrapolating to the fully bound situation. The fully bound case ($\theta = 90^\circ$) was reported by this procedure to have a value of -0.78 ± 0.8 Hz. Naturally there is quite a bit of spread in the experimental curve of Bystrov et al.²⁶ Since the core polarization contributing to the π -electron coupling could be dependent on the environment, the π -contribution may not necessarily be the same for both free and bound substrates, even if θ has the same value in both cases. Thus one has to be extremely careful in making stereochemical assignments from $^3J(^{13}\text{C}'\text{H}^\alpha)$. It is also quite important to make studies of $^3J(^{13}\text{C}'\text{H}^\alpha)$ as a function of pH in different solvents for systems for which the conformations are known.

CONCLUSION

The present calculations indicate that the vicinal $^{13}\text{CNC}^\alpha\text{H}$ coupling constants in peptides show an angular dependence which can be utilized in the studies of the conformation of the peptide backbone in solution. The findings of the present study are in reasonably good agreement with those from FPT-INDO calculations by Solkan et al.⁹ It shows that one can indeed obtain reasonable estimates of heteronuclear coupling constants by evaluating the π -contribution explicitly in terms of the polarization of core electrons if one of the coupled nuclei is an unsaturated center. This prompts us to propose that a similar treatment can be employed for other heteronuclear coupling situations such as the ψ angle dependence of vicinal $^{15}\text{NC}'\text{C}^\alpha\text{H}$ coupling constants.

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