Angular Dependence of Vicinal ^1H–^{14}N Spin Coupling[1]

Sir:

Dependence of the vicinal proton coupling constant, $J_{H,H(vic)}$, on the dihedral angle between two interacting protons has well been established and widely applied in organic chemistry.[3,4] Several workers recently provided evidence for the fact that $J_{H,^{13}C(vic)}$,[5] $J_{H,^{31}P(vic)}$,[6] $J_{H,^{19}F(vic)}$,[7] and $J_{H,^{199}Hg(vic)}$[8] depend also on dihedral angles between coupling nuclei. Further, it was revealed from pmr spectral analyses of vinylammonium salts that $J_{H,^{14}N(trans)}$ is larger than $J_{H,^{14}N(cis)}$.[2,9] Thus, we may reasonably expect the presence of angular dependence of $J_{H,^{14}N(vic)}$. However, the evidence has not so far been reported. The following factors may make the observation of H–^{14}N couplings very difficult: (1) the signal of a proton interacting with an ^{14}N nucleus is, in general, broadened by its quadrupolar relaxation except for the case of some ammonium ions or isonitriles where the electric-field gradient at their ^{14}N is highly symmetrical,[10] and (2) absolute values for $J_{H,^{14}N}$ are fairly small.[10]

We report here evidence for angular dependence of $J_{H,^{14}N(vic)}$ utilizing some rigid bicyclic compounds, dibenzobicyclo[2.2.2]octa-2,5-dien-7-yltrimethylammonium bromide (I)[11] and 2-*exo*- and 2-*endo*-hydroxybornan-3-*endo*-yltrimethylammonium bromides (II and III).[12] Signal assignments and determination of *J* values in their pmr spectra were carried out by using proton double- and triple-resonance techniques.[14] Figure 1 shows a portion of the pmr spectrum of I as an example. Table I lists the *J* values obtained together with the dihedral angles roughly estimated from molecular models. Evidently, dependence of $J_{H,^{14}N(vic)}$ on dihedral angles is present.

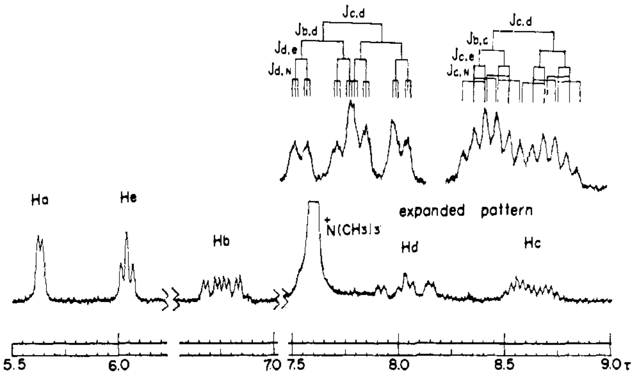
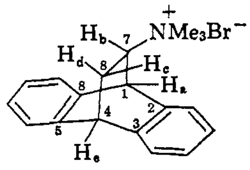
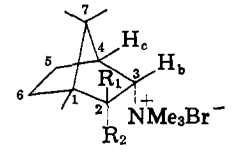


Figure 1. Pmr spectrum of I in CF_3COOD at 100 MHz (lower field parts are not shown).

The $J_{H,^{14}N(vic)}$ values at the dihedral angles 0 and 120° in II and III are smaller than the corresponding values in I. This fact can be ascribed to the effect of electronegativity of the hydroxyl group at C-2 in II and III and to the difference in bond angle concerned with the $-N^+CCH$ fragment. Since the sign of $J_{H,^{14}N(vic)}$ is believed to be positive,[2,9,15] the electronegativity effect of a hydroxyl group can be expected to decrease the *J* value by analogy with the relationship between $J_{H,H(vic)}$ and electronegativity of substituents.[16] The higher ring strain in II and III may considerably change bond angles and bond character, and therefore may decrease the *J* values.[3,17,18]



I



II, $R_1 = OH$; $R_2 = H_a$
III, $R_1 = H_a$; $R_2 = OH$

Table I. Vicinal Coupling Constants, *J*

Compd	Dihedral angle, deg	$J_{H,H(vic)}$, Hz	$J_{H,^{14}N(vic)}$, Hz
I	0	b,d = 10.0	N,c = 2.7
	60	a,b = 2.0 c,e = d,e = 2.7	N,a ≤ 0.3
	120	b,c = 5.8	N,d = 0.8
II	0		N,a = 1.4
	44	b,d = 3.5	
	79		N,c ≃ 0
	120	a,b = 5.3	
III	0	a,b = 9.8	
	44	b,c = 4.0	
	79		N,c ≃ 0
	120		N,a ≤ 0.3

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(1) Nmr Studies of Aliphatic Nitrogen-Containing Compounds. VIII. For part VII, see ref 2.

(2) M. Ohtsuru and K. Tori, *Chem. Commun.*, 750 (1966).

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(4) For example, see (a) H. Conroy, *Advan. Org. Chem.*, **2**, 311 (1960); (b) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day Book Co., Inc., San Francisco, Calif., 1964, Chapter 6; (c) A. A. Bothner-By, *Advan. Magnetic Resonance*, **1**, 195 (1965).

(5) (a) G. J. Karabatsos, C. E. Orzech, Jr., and N. Hsi, *J. Am. Chem. Soc.*, **88**, 1817 (1966); (b) G. J. Karabatsos, N. Hsi, and C. E. Orzech., Jr., *Tetrahedron Letters*, 4639 (1966).

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(7) (a) K. L. Williamson, Y. F. Li, F. H. Hall, and S. Swager, *J. Am. Chem. Soc.*, **88**, 5678 (1966); (b) G. Aranda, J. Jullien, and J. A. Martin, *Bull. Soc. Chim. France*, 2850 (1966), and references cited therein.

(8) M. M. Kreevoy and J. F. Schaefer, *J. Organometal. Chem.*, (Amsterdam), **6**, 589 (1966).

(9) J. M. Lehn and R. Seher, *Chem. Commun.*, 847 (1966).

(10) For example, see (a) Y. Kawazoe, M. Tsuda, and M. Ohnishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 214 (1967); (b) K. Tori, T. Iwata, K. Aono, M. Ohtsuru, and T. Nakagawa, *ibid.*, **15**, 329 (1967), and references cited therein.

(11) I (paste) was synthesized from 7-dimethylaminodibenzobicyclo-[2.2.2]octa-2,5-diene prepared according to S. Wawzonek and J. V. Hallum, *J. Org. Chem.*, **18**, 288 (1953), by treatment with CH_3Br.

(12) II (mp 305–306°) and III (mp ~270°, sublimation) were respectively prepared from 3-*endo*-methylaminoisoborneol[13] and 3-*endo*-methylaminoborneol[13] by N-formylation with formamide followed by reduction with $LiAlH_4$ and subsequent treatment with CH_3Br.

(13) Y. Hamashima, K. Tori, and A. Takamizawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 1052 (1965).

(14) The pmr spectra were taken with a Varian HA-100 spectrometer operating at 100 MHz by using D_2O and CF_3COOD solutions at elevated temperatures (60–100°). Spin-decoupling experiments were performed in the frequency-swept and DSS- or TMS-locked mode by using two Hewlett-Packard HP-200ABR audiooscillators and an HP-5212A electronic counter. The *J* values obtained in both solvents were essentially the same within experimental errors, ±0.1 Hz.

(15) (a) A. J. R. Bourn, D. G. Gillies, and E. W. Randall, "Nuclear Magnetic Resonance in Chemistry," B. Pesce, Ed., Academic Press Inc., New York, N. Y., 1965, p 277; (b) J. P. Maher, *J. Chem. Soc.*, *Sect A*, 1855 (1966).

(16) (a) K. L. Williamson, *J. Am. Chem. Soc.*, **85**, 516 (1963); (b) R. J. Abraham, L. Cavalli, and K. G. R. Pachler, *Mol. Phys.*, **11**, 471 (1966), and references cited therein.

(17) It is a known fact that $J_{H(endo),H(endo)}$ is smaller than $J_{H(exo),H(exo)}$ in the bicyclo[2.2.1]heptane ring system. See F. A. L. Anet, H. H. Lee, and J. L. Sudmeier, *J. Am. Chem. Soc.*, **89**, 4431 (1967), and references cited therein.

(18) Williamson, *et al.*,[7] also suggested that $J_{H,^{19}F(vic)}$ depends on bond angle.

More accumulated data on $J_{\mathrm{H},^{14}\mathrm{N}(vic)}$ values for a variety of dihedral angles may be necessary to derive the relationship between them. However, the $J_{\mathrm{H},^{14}\mathrm{N}(vic)}$ value of 2.2 Hz observed in ethyltrimethylammonium bromide and some other similar compounds can lead us to infer that the J value for 180° may be about 5–6 Hz[9,10] in view of the $J_{\mathrm{H},^{14}\mathrm{N}(vic)}$ value in I at the dihedral angle 60°.[19]

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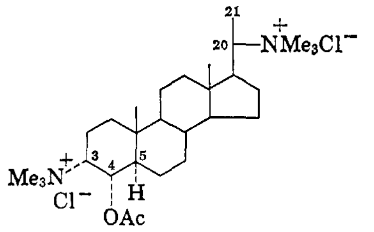
[19] In order to determine a $J_{\mathrm{H},^{14}\mathrm{N}(vic)}$ value at the dihedral angle of 180°, we synthesized 4α-acetoxy-3α,20α-di(dimethylamino)-5α-pregnane dimethochloride (IV) (mp 210–213.5° dec) from its free base[20]



IV

by the usual way. However, the pmr signal of $H_{4\beta}$ in IV, which is a quartet with spacings of 5.0, 6.0, and 5.0 Hz at τ 4.67 in D_2O, shows no splittings due to the ^{14}N at C-3α even at 110° but becomes very broad peaks, although the signal of the 21-methyl protons appears as a clear doublet of triplets at τ 8.48 (J = 7.0 and 2.2 Hz) at this temperature. Quadrupolar relaxation of the ^{14}N at C-3α may not be sufficiently slow at this temperature to show splittings by the ^{14}N atom.

[20] M. Tomita, S. Uyeo, and T. Kikuchi, *Chem. Pharm. Bull.* (Tokyo), **15**, 193 (1967).

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The Optical Rotation of Ribonuclease[1]

Sir:

One of the most characteristic features of the optical rotatory spectrum of the α helix is the trough at 233 mμ. It is an important and interesting fact that in model polypeptide systems this trough diminishes in magnitude without appreciable change in position as the helical content is diminished until at very low helicity it drifts up into the small anomaly of the random polypeptide chain at 239 mμ (Figure 1). Curve C, for example, has a trough at 233 mμ even though it represents only 6–7% helix as estimated by the trough amplitude method.[2] The absorption band responsible for this trough has been located at 222 mμ by studies of circular dichroism[3] and has been assigned to the n,π* transition of the peptide group. This is an unusual position for this transition. Model studies place it at about 228 mμ in solvents which cannot donate hydrogen bonds[4] and at about 212–214 mμ in solvents such as water and methyl alcohol.[5] This is the well-known blue shift of n,π* transitions in highly polar solvents. The constancy of position of the trough of the α helix in a wide variety of solvents and in many proteins suggests that the helix itself provides a relatively constant environment for the n orbitals of the peptide group which is distinct from that of water or nonpolar media. Inspection of space-filling models of helices reveals that the nonbonding n orbitals are nestled in the helix in such a way that direct interaction with solvent is prevented. The hydrogen bonds of the α helix itself are in the nodal planes of the n and π* orbitals and consequently will affect the n,π* energies only as a secondary effect.

The trough of beef pancreatic ribonuclease is at 228 mμ.[6] Since this is a deviation from model helix behavior which may be of deep structural significance, we have performed a number of experiments to demonstrate that this is a general feature of the ORD spectrum of ribonuclease and not dependent on special conditions of solution or the protein preparation. These experiments spanned the following conditions: eight enzyme preparations,[7] pH 2–8, in a variety of salts: KCl, Tris-HCl, and $(NH_4)_2SO_4$ at concentrations of 0.05–3.0 M. Measurements were made on a Cary 60 spectropolarimeter. In almost all cases the trough was at λ_t 228–229 mμ. Exceptions were two old preparations, Sigma R6B-053 and Pentex 10669, which had troughs at 230 and 231 mμ, respectively. Evidently long storage or impurities can lead to higher values for λ_t. The mean residue rotation at the trough, averaged over 21 experiments, was −4100° with a standard deviation of 300°. These values are corrected for refractive index using the values for water. Curves from a given preparation were quite reproducible and did not change much with the variation of pH and ionic strength. The standard deviation given above represents essentially the variation among protein preparations.

Circular dichroism was also investigated using a Jasco dichrograph (Figure 2), and the data were analyzed by a curve-fitting procedure derived from the program of Carver, Shechter, and Blout.[8] The data are analyzable in terms of two negative Cotton effects. The upper one at 217 mμ has a molar rotatory strength of −5.6 Debye-magnetons and is responsible for the trough at 228 mμ.[9] The calculated Gaussian band width is 12 mμ, which is a reasonable value for the protein chromophores which may be involved as established in model studies. Consequently, this Cotton effect is evidence either for a single type of transition or the superposition of a number of transitions at the same wavelength. On the other hand, the lower band has an apparent width of only 6 mμ. This indicates that it is a composite of a number of

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[1] This research was supported by PHS Grant CA 4216 of the National Cancer Institute.

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[3] G. Holzworth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

[4] E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, **71**, 3914 (1967).

[5] J. A. Schellman and E. B. Nielsen in "Conformation of Biopolymers," Vol. 1, G. N. Ramachandran, Ed., Academic Press Inc., New York, N. Y., 1967, p 109.

[6] R. E. Cathou, G. G. Hamnes, and P. R. Schimmel, *Biochemistry*, **4**, 2687 (1965).

[7] Pentex, Lots 2626, 10669, 26Z8; Worthington, RNase A, Lot 6504; Mann, Lot F 2789; Sigma, Lots R61B-053, R101B67, R22B70.

[8] J. P. Carver, E. Shechter, and E. R. Blout, *J. Am. Chem. Soc.*, **88**, 2550 (1966).

[9] In calculating the rotatary strength Δε values were corrected for refractive index by means of the formula $\Delta\epsilon' = (3/(3^2 + 2)^2 n\Delta\epsilon$, where Δε is the observed molar dichroism and n is the refractive index of water.