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REFINEMENT OF THE ANGULAR DEPENDENCE OF THE PEPTIDE VICINAL NH-C$^{\alpha}$H COUPLING CONSTANT

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Abstract—The refined dependence of the peptide NH-C$^{\alpha}$H vicinal coupling constant on the dihedral angle θ have been derived on the basis of the accumulated experimental data. The mean permissible values (in Hz) are approximated by

$$^{3}J_{NHCH} = 9{\cdot}4\cos^{2}\theta - 1{\cdot}1\cos\theta + 0{\cdot}4$$

An analogous relationship for the sum of two vicinal NH-C$^{\alpha}$H$_2$ coupling constants in the glycyl residue have been calculated from the above dependence. Measurements on N-methylacetamide in various solvents and in the presence of an alkali salt showed the vicinal constant NH-CH to vary by not more than ±3%. Some of the other proposed $^{3}J_{NHCH}(\theta)$ dependencies give too low values for the *cis*-oriented NH and C$^{\alpha}$H bonds. This may be due to the fact that in these correlations the data for compounds with *cis*-amide bonds have been used for $0° \leqslant \theta \leqslant 90°$ region of the dependence.

The earlier proposed dependence of the peptide NH-C$^{\alpha}$H vicinal coupling constant on the dihedral angle θ between the H-N-C$^{\alpha}$-H planes (Fig 1) derived from the NMR and IR data of dipeptides[1,2] has been successfully used in spatial structure determination of natural and synthetic peptides.[3-7]

However, the paucity of experimental data did not permit sufficient precision of the $^{3}J_{NHCH}(\theta)$ curve so that the θ values for a given $^{3}J_{NHCH}$ constant could vary within considerable limits (± 15°). Moreover, the wide range of permissible values led to overlap of the limits wherein, according to the $^{3}J_{NHCH}$ the NH-C$^{\alpha}$H protons could be either in *cis* ($\theta = 0°$) or *trans* ($\theta = 180°$) orientations.

With the accumulation of more data it has now become possible to overcome these shortcomings by refinement of the $^{3}J_{NHCH}(\theta)$ curve which is the subject matter of the present paper.

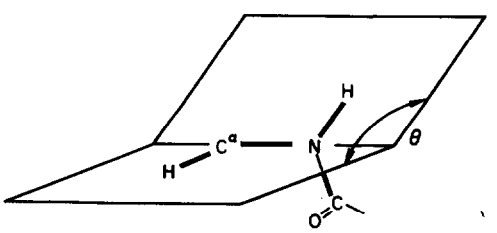


Fig 1. The dihedral angle θ of an NH-C$^{\alpha}$H bond.

†The deviation of the peptide bond from planarity is generally less than 10° even for comparatively strained cyclic compounds with *cis*-amide bonds.[12-14]

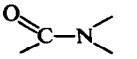
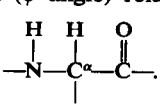
We assume here as before[1,2] that our correlation may be expressed in the form of Karplus-like equation.[8]

$$^{3}J = A\cos^{2}\theta - B\cos\theta + C\sin\theta, \qquad (1)$$

where A, B and $C \geqslant 0$. The constant is assumed to be of positive sign[9] and corrections for the substituent electronegativity are made according to

$$^{3}J = J_{obs}\left(1 - \alpha\sum_{i}\Delta E_{i}\right)^{-1}, \qquad (2)$$

where J_{obs} is the experimental value for the NH-C$^{\alpha}$H coupling, ΔE_i is the electronegativity difference between the C$^{\alpha}$ substituent and hydrogen. The proportionality factor α must have a negative sign, since $^{3}J_{NHCH}$ in substituted N-methylanilines decreases with increasing electronegativity of the substitutions.[10] The value $\alpha = -0{\cdot}1$ has been chosen in conformity with the experimental data for ethanes.[11]

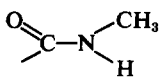
Since the peptide bond  is planar,† there are no reasons to expect any first-order orientation effects[15] from the substituents located at C$^{\alpha}$. On the other hand $^{3}J_{NHCH}$ constant might depend on the orientation of the carbonyl group of the next peptide bond (ψ angle) relatively to C$^{\alpha}$-H bond in the fragment  .

Assuming the Pauling scale of electronegativities,[16] Eq. (2) for the peptide fragments becomes

$$^3J_{NHCH} = 1{\cdot}09\,J_{obs}. \quad (3)$$

In what follows only constants corrected according to Eq. (3) are used.

For any torsional potential of internal rotation about the N-CH_3 bond in the



fragment the averaged coupling constant is

$$^3J_{NHCH_3} = \sum_i x_j \langle J(\theta_j)\rangle, \quad (4)$$

where

$$\langle J(\theta_j)\rangle = 1/3[J(\theta_j)+J(120°+\theta_j)+J(120°-\theta_j)]. \quad (5)$$

Angles θ_j (Fig 2) correspond to the energy minima of the rotational states; x_j is the population of the j-level and $\sum_i x_j = 1$. Inserting Eq. (1) into Eq. (4) we have

$$\langle J(\theta_j)\rangle = (A+C)/2$$

and taking into account Eq. (5)

$$^3J_{NHCH_3} = (A+C)/2$$

Thus the averaged constant (4·9 Hz for N-methylformamide[9,17] and 4·8 Hz for N-methylacetamide[18]) regardless of the torsional potential, and also in the case of free rotation (Ref 1) equals the half-sum of the coefficients A and C.

In addition new maximum $^3J_{NHCH}$ values for the regions $0° \leqslant \theta \leqslant 90°$ and $90° \leqslant \theta \leqslant 180°$ were selected from the data on peptides with trans-amide bonds. For the first region the value of 8·0 Hz was selected, being the constant of the corresponding NH-C$^\alpha$H fragment of the D-Val residue of valinomycin in the "A" type conformation predominant in non-polar media.* For the region $90° \leqslant \theta \leqslant 180°$ the maximum coupling constant of 10·2 Hz was selected as that exhibited by the corresponding fragment of the L-Val and L-Leu residues of gramicidin S.[5]

We thus have the following boundary conditions for the coefficients of Eq. (1):

$$A+C = 9{\cdot}8\ \text{Hz}, A-B \geqslant 8{\cdot}0\ \text{Hz}, A+B \geqslant 10{\cdot}2\ \text{Hz}.$$

With these constraints, linear programming[20] gave the angular dependence of the $^3J_{NHCH}$ coupling constant presented in Fig 3.

The refined dependence lies within the region of the one proposed earlier,[1,2] but confines permissible angles for a given experimental coupling constants to narrower limits and more definitely discriminates between the *cis* and *trans* orientation of the NH-C$^\alpha$H protons. The mean permissible values,

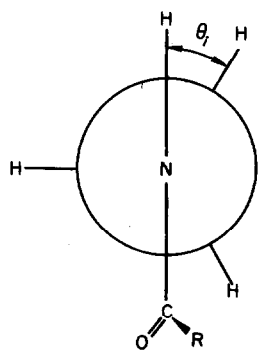


Fig 2. Newman projection of the N-CH_3 bond for N-methylamides.

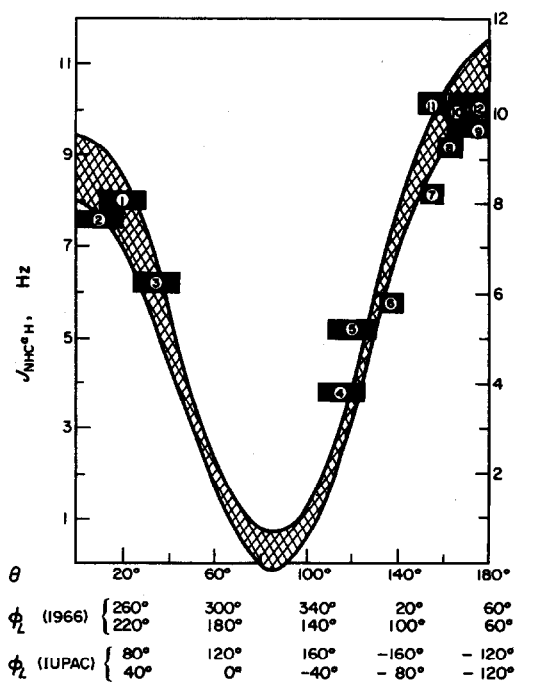


Fig 3. Refined dependence of the spin-spin peptide vicinal NH-C$^\alpha$H coupling constant on the dihedral angle θ. Experimental data: **1** and **3**, D- and L-Val residues of valinomycin in the "bracelet" conformation,[6] **2** and **10**, L- and D-Val of valinomycin in the "propeller" conformation;[6] **4**, D-Phe of gramicidine S[5]; **5**, L- and D-Val of valinomycin K^+-complex;[3,6] **6–8**, L-$Orn_{1,2,3}$ of alumichrome A[4] (X-ray diffraction data for ferrichrome A, see ref 18); **9**, L-Orn of gramicidine S[5]. **11** and **12**, L-Leu and L-Val of gramicidin S[5].

*Earlier[1,2] the maximum value of $^3J_{NHCH} = 8{\cdot}9$ Hz from the spectrum of 6-phenyldihydrouracil[19] was used for the $0° \leqslant \theta \leqslant 90°$ region. Since this heterocyclic molecule has *cis*-amide bonds we believed it more feasible to leave it out when deriving a dependence for peptides with a *trans*-amide bonds.

shown on Fig 3 by hatched area, are approximated by

$$^{3}J_{NHCH} = 9{\cdot}8\cos^2\theta - 1{\cdot}1\cos\theta + 0{\cdot}4\sin^2\theta,$$

which on rearrangement gives

$$^{3}J_{NHCH} = 9{\cdot}4\cos^2\theta - 1{\cdot}1\cos\theta + 0{\cdot}4.$$

Figure 3 also presents the most reliable experimental values for θ values obtained by the composite physicochemical method of conformational analysis in solution[3,5,6] and determined by X-ray analysis.[21,22] The extreme values for $^{3}J_{NHCH}$ known up to now (2·7 Hz for evolidine;[23] $< 2{\cdot}6$ Hz for the Na^+-antamanide complex[24] and the maximal value of 11·7 Hz for one of the forms of Val,[6] Ala[9]-antamanide[25]) are in good accord with the general range of values for this newly derived dependence (Fig 3).

The good agreement with the experimental data gives grounds for the belief that the proposed angular dependence will find an ever increasing use in conformational studies of peptides by ^{1}H-NMR. Dihedral angles θ can be converted into the conventional angles ϕ[26,27] as shown in Table 1.

For peptide systems with a fixed spatial structure four ϕ angles would in general correspond to a given experimental value of $^{3}J_{NHCH}$ (Table 1). This is due to symmetry of the $^{3}J_{NHCH}(\theta)$ dependence with respect to $\theta \sim 90°$. Only for $^{3}J_{NHCH} > 9{\cdot}4$ Hz do but two ϕ angles remain and these are in the comparatively narrow range $\pm(15\text{–}30°)$. In order to overcome this ambiguity it is necessary to obtain supplementary data from other physicochemical methods and/or theoretical conformational calculations (see for instance[3–6,23–25]).

In conformationally flexible systems the observed NH-C$^{\alpha}$H coupling constants reflect the averaged rotational distributions,[7,28] so that, as a rule the preferred conformation can be determined only if the ratios of the equilibrium forms are known. However, a value of $^{3}J_{NHCH} \geqslant 10$ Hz should always indicate preference for transoid orientation of the NH-C$^{\alpha}$H protons, whereas a constant less than 3 Hz means a preferably gauche orientation.

Vicinal NH-C$^{\alpha}$H$_2$ coupling in glycyl residues should be considered separately as the spectrum of these protons is either of the ABX or AA'X type. In this case the line separation of the NH signal (quartet or triplet) as a rule does not directly give the $^{3}J_{NHCH}$ coupling constant. Only the separation between the outer components is strictly equal to the sum of J_{AX} and J_{BX}.[29] Assuming the projection angle between the two N-C$^{\alpha}$-H glycyl planes to be 120°, one may then use the above $^{3}J_{NHCH}$ dependence to calculate an analogous relationship for the sum of the glycyl residue constants:

$$\Sigma(^{3}J_{NHCH_2}) = J(\theta) + J(120° \pm \theta).$$

The result obtained is shown in the Fig 4 as a function of the conventional angles ϕ.

The mean permissible values are approximated by the following function of the IUPAC-IUB conventional angle[27]

$$\Sigma(^{3}J_{NHCH_2}) = 10{\cdot}7\cos^2\phi - 1{\cdot}5\cos\phi + 15{\cdot}9.$$

The maxima on the curve ($\sim$ 16 Hz) correspond to the rotamer with the N-H bond eclipsed by the C$^{\alpha}$-H bond, whereas the minima ($\sim$ 3 and 6·5 Hz) correspond to rotamers with the C$^{\alpha}$-C'(O) bond eclipsed by the N-H or the N-C'(O) bond. The experimental over-all glycyl NH-C$^{\alpha}$H$_2$ coupling constants ranging from 13·5 Hz* for glycylalanyl cyclopeptides[30] to 7·7 Hz for alumichrome[4] fall within the extrema of the derived glycyl coupling curve (Fig 4).

From general considerations one could have expected the NH-C$^{\alpha}$H coupling constants to depend also on intra- and inter-molecular interactions (such as hydrogen bonding NH...X, ion-dipole interactions C=O$\cdots$M$^+$ etc). However measurements on N-methylacetamide in various

*Electronegativity corrections given according to $\Sigma(^{3}J_{NHCH2}) = 1{\cdot}04\,\Sigma J_{obs}$.

Table 1. Relation between the dihedral angles θ and the conventional angles ϕ for L- and D- amino acid residues in peptides

	θ	0°	20°	40°	60°	80°	100°	120°	140°	160°	180°
According to the nomenclature (1966)[26]	ϕ (L-)	240°	260° 220°	280° 200°	300° 180°	320° 160°	340° 140°	360°, 0° 120°	20° 100°	40° 80°	60°
	ϕ (D-)	120°	140° 100°	160° 80°	180° 60°	200° 40°	220° 20°	240° 0°, 360°	260° 340°	280° 320°	300°
According to the IUPAC nomenclature[27]	ϕ (L-)	60°	80° 40°	100° 20°	120° 0°	140° −20°	160° −40°	±180° −60°	−160° −80°	−140° −100°	−120°
	ϕ (D-)	−60°	−40° −80°	−20° −100°	0° −120°	20° −140°	40° −160°	60° ±180°	80° 160°	100° 140°	120°

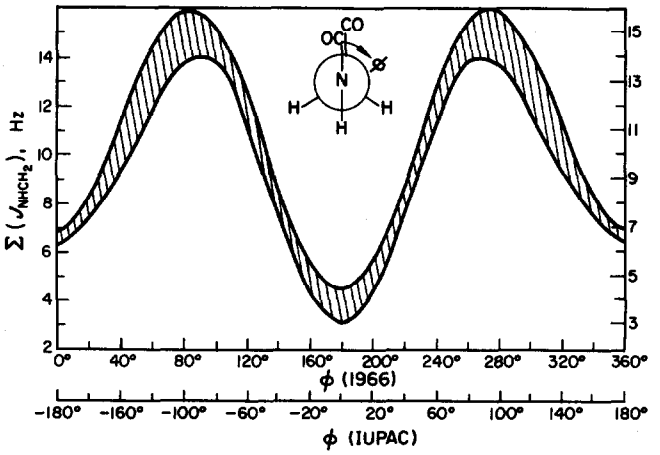


Fig 4. Over-all vicinal coupling constant in the glycyl NH-C$^{\alpha}$H$_2$ fragment plotted against rotational states.

Table 2. $^3J_{NHCH_3}$ coupling constants for N-methylacetamide (30°, JEOLCO JNM-4H-100 Spectrometer)

Solvent	Concentration in moles/l	$^3J_{NHCH_3}$ in HZ
CCl_4	0·48	4·80 ± 0·05
	0·05	4·94 ± 0·07
	0·02	4·94 ± 0·07
	0·01	4·8 ± 0·1
$(CD_3)_2CO$	0·50	4·75 ± 0·05
	0·03	4·68 ± 0·07
	0·01	4·7 ± 0·1
$(CD_3)_2SO$	0·46	4·72 ± 0·05
CH_3OH	0·48	4·66 ± 0·05
H_2O	0·56	4·81 ± 0·05
	0·04	4·89 ± 0·07
	0·02	4·87 ± 0·07
0·21 moles/l KBr in H_2O	0·02	4·85 ± 0·07

solvents and in the presence of a ten fold excess of an alkali salt (Table 2) showed the vicinal constant to vary by not more than ± 3%. A similar result is known for the $^3J_{HOCH}$ constant of methanol[31] which varies by ± 4% depending upon the nature of the solvent.

Recently several other sets of coefficients for Eq. (1) have been proposed[28,32–34] (Table 3). The coefficients proposed by Schwyzer[34] practically coincide with those for a curve passing through the average values of our band dependence for the free rotation model.[2] The theoretically calculated dependence of Karplus and Barfield[32] does not reflect the actual difference between the *cis*- and *trans*-oriented NH-C$^{\alpha}$H protons. The empirical dependencies of Thong *et al.*[33] and of Ramachandran *et al.*[28] give too low $^3J_{NHCH}$ values for the *cis*-oriented NH and C$^{\alpha}$H bonds ($\theta = 0°$). This may be due to the fact that these authors used 6-membered heterocyclic compounds with a *cis*-amide bond for deriving the $0° \leqslant \theta \leqslant 90°$ region of the curve. Quite possibly the strain occurring in these compounds, particularly in isoquinuclidone,[28] leads to

Table 3. Coefficients proposed for Eq. (1)

Reference	Coefficients in Hz			$^3J_{NHCH}$ in Hz	
	A	B	C	*Cis* ($\theta = 0°$)	*Trans* ($\theta = 180°$)
This paper*	9·4	−1·1	0·4	8·0–9·4	10·2–11·6
Bystrov *et al.*[1]*	8·9	−0·9	0·9	8·0–9·8	8·0–11·6
Bystrov *et al.*[2]*	9·6	−0·3	0·4	8·9–9·8	8·9–10·7
Barfield and Karplus[32]	12·0	0·0	0·2	12·0	12·0
Thong *et al.*[33]†	9·3	−3·5	0·3	5·8	12·8
Schwyzer[34]	9·68	−0·42	0·12	9·26	10·10
Ramachandran *et al.*[28]†	8·6	−1·7	1·5	6·9	10·3

*The coefficients correspond to a curve in the middle of the allowed region.
†Electronegativity corrections made according to Eq. (3).

somewhat smaller $^{3}J_{NHCH}$ constants than they would have been for the *trans*-amide bonds. Another possibility is that the NH-C$^{\alpha}$H coupling constants at least for this region are in general less for the *cis*-amide bond than for the *trans*-amide bond.

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