

Experimental Calibration of a Karplus Relationship in Order to Study the Conformations of Peptides by Nuclear Magnetic Resonance¹

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ABSTRACT: An empirical calibration of a Karplus relationship between the J_{HH^α} vicinal coupling constant and the corresponding dihedral angle is achieved for the H-N-C $^\alpha$ -H $^\alpha$ sequence in amides and peptides. A first approach is made by referring to several heterocyclic rigid molecules such as 2-pyrrolidinone, 3-isoquinuclidone, and other ring structures. The results so obtained are subsequently confirmed by use of open-chain peptide model compounds previously studied, as regard to their conformation in solution, by infrared spectroscopy. The quite satisfactory agreement between these two sets of experimental data leads us to conclude that the measured J coupling constant is not much influenced by the cis or trans conformation of the amide linkage. In the same way, it seems that the "strain effect" (*i.e.*, the variation of J resulting from a distortion of the bond angles) for the H-N-C $^\alpha$ -H $^\alpha$ sequence is likely to be smaller than the ΔJ correction estimated through the formulas commonly used in the case of the H-C-C-H ethane-like sequence.

In most cases, the extensive application of nmr spectroscopy for elucidating the conformations of peptide molecules mainly relies on the measurement of the vicinal H-N-C $^\alpha$ -H $^\alpha$ coupling constant. From the theoretical work of Karplus,^{2a} it is usually assumed that this coupling constant J is related to the dihedral angle θ by (Figure 1).

$$J = A \cos 2\theta - B \cos \theta + C \quad (A, B, C > 0)$$

The relationship between θ and the rotational angle ϕ commonly used, according to the IUPAC-IUB recommendations^{2b} in peptide conformational studies, is

$$\theta = |\phi - 60^\circ| \quad (\text{for an L residue})$$

The nature and the hybridization state of the atoms involved in the coupling greatly influence the values of the coefficients A , B , and C ; it is therefore necessary to parametrize, through an empirical calibration for each series of homologous compounds, the most appropriate Karplus relation.

In the case of amides and peptides, such an attempt has been undertaken simultaneously by Bystrov, *et al.*,^{3,4} by Ramachandran, *et al.*,⁵ and by ourselves.^{6,7}

The calibration by Bystrov, *et al.*,³ relies mainly on measurements performed with two standard compounds, *i.e.*, *N*-methylformamide and *N*-acetyldialanyl methyl ester. Moreover, it takes account of an erroneous J coupling constant of 8.2 Hz previously reported on the basis of the nmr spectrum of 6-phenyldihydrouracil.⁸ This value has subsequently been rectified and corrected to 2.6 Hz.⁹

For the same purpose, Ramachandran, *et al.*,⁵ make use of eight flexible compounds. Because of the numerous conformational states available for such molecules, the resulting unequivocal plotting of J vs. θ obviously needs an averaged estimate based on some theoretical analysis, the reliability of which remains questionable.¹⁰ Furthermore, up to this date, theory is mainly concerned with molecules "*in vacuo*," free from any intermolecular constraints. This condition is not fulfilled in the experiments reported by these authors, the measurements being generally performed with samples taken in the pure liquid state or dissolved in dimethyl sulfoxide, a solvent which is well known to interact strongly with peptide groups.¹¹ In fact, the authors themselves acknowledge the poor reliability of their own calculations for aromatic and C $^\beta$ branched amino acids.

Finally, we will also mention a publication by Weinkam and Jorgensen¹² referring to a private communication by

Schwyzler proposing a Karplus relationship about which no precise information is available.

The use of these calibrations in order to obtain the θ values that we needed for our own investigations on peptide model molecules frequently led us to conclusions which were not consistent with other spectroscopic results. We were therefore led to collect our own measurements to obtain a more suitable Karplus relationship. We would like to report herewith our procedure and conclusions.

Calibration Procedure

The empirical calibration of a Karplus relationship can mainly be achieved two different ways. The first, chosen by Ramachandran,⁵ makes use of several flexible standard compounds; we have already discussed its reliability, pointing out that it needs assumptions concerning the favored conformations of these molecules.

The second approach consists in operating with model amides or lactams, the backbone of which presents a plane of symmetry and is rigid enough to prevent libration. These conditions are fulfilled by only a few strained heterocyclic species in which the amide linkage is in the cis conformation. The question may therefore be raised as to how the J coupling constant depends on the cis-trans isomerism and is eventually influenced by bond angle distortions in a strained molecule.

With regard to the first problem, the comparison of the experimental nmr data which are now available for various *cis*- and *trans*-*N*-alkylformamides^{13,14} and for *N*-methylacetamide^{14,15} shows that the J value is not very sensitive to the conformation of the amide link. The most recent theoretical treatments devoted to this question lead to the same conclusion.¹⁶⁻¹⁸

As for the second approach, it seems that reference to rigid cyclic model molecules could yield J values differing noticeably from those characterizing unstrained open-chain species.¹⁹ Theoretical formulas have already been proposed to account for such an effect in the case of the strained H-C-C-H ethane-like sequence and to correct accordingly the observed J_{HH} vicinal coupling constants.²⁰ However, the matter is still unsettled for the H-N-C-H sequence and, from our own experimental and theoretical work on this topic,²¹ it turns out that formulas commonly used for ethane-like structures do not hold for the H-C-N-H sequence (amides and related species) and would lead to rather overestimated corrections.²¹

We think therefore that the most reliable way to proceed

toward the expected Karplus relationship is a two-step approach. This relies first upon the use of rigid standard heterocyclic molecules in order to obtain an unequivocal J vs. θ correlation without any hypothetical averaging. It is then possible to ascertain the broader reliability of this calibration through further investigations with open-chain unstrained similar species. These last compounds have to be selected among those solutes the conformation of which has previously been elucidated by other physicochemical techniques such as, for example, infrared spectroscopy. Particular interest will therefore be attached to the rather rare open-chain model compounds having only a single conformation.

It should also be pointed out that the main purpose of such a calibration is its further utilization to obtain information about the conformations of more complex peptide species. In such molecules bond angles are likely to be distorted from their standard values, as already shown by recent X-ray investigations on low molecular weight peptides.²² It is therefore important to appreciate as exactly as possible to what extent the coupling constants are influenced by these deviations. In fact the effectiveness of nmr spectroscopy for elucidating the conformations of peptides and proteins mainly depends on the magnitude of this so-called strain effect.

(a) Standard Compounds. Let us note first, in close agreement with Bystrov, *et al.*,³ that the vicinal coupling constant in the $\text{NH}-\text{CH}_3$ system is equal to the coefficient C whatever assumption is made concerning its conformation, *i.e.*, either discrete state assumption

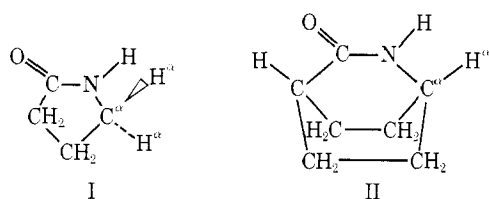
$$\langle J \rangle = 1/3 [J(\theta) + J(\theta + 120^\circ) + J(\theta - 120^\circ)] = C$$

or free rotation assumption

$$\langle J \rangle = \frac{1}{2\pi} \int_0^{2\pi} J(\theta) d\theta = C$$

Therefore, coefficient C may be estimated from vicinal coupling constants measured on N -methylamides.

For computing coefficients B and C we have selected as standard compounds 2-pyrrolidinone, I, and 3-isoquinuclidone (2-azabicyclo[2.2.2]-3-octanone), II. At this date the



actual geometry of I is not known. A consistent force field computation by Warshel, *et al.*,²³ predicts a nearly planar structure, *i.e.*, $\theta \approx 60^\circ$. The calculated geometrical parameters agree fairly well with those measured by X-ray diffraction on 5-substituted 2-pyrrolidinones,²⁴ although in these last derivatives the 2-pyrrolidinone ring is slightly warped because of the substitution. However, a slight distortion (about 10°) of I from planarity would not greatly influence the vicinal coupling constant. In fact, when the $\text{N}-\text{H}$ bond is contiguous to a methylene group as in I, one can show that the corresponding curve of $J_{\text{obsd}} = \Sigma I/2$ vs. θ passes through a minimum for $\theta = 60^\circ$.

The crystal structure of 3-isoquinuclidone, II, is now resolved.²⁵ In the solid state, the molecules of this compound are paired in centrosymmetric dimers by two linear hydrogen bonds $\text{N}-\text{H}\cdots\text{O}$. The associated amide functions lie in two slightly separated (0.27 \AA) parallel planes so that each

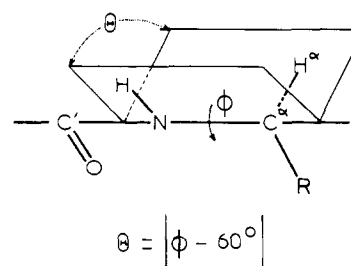


Figure 1. Relation between the dihedral angle θ for the vicinal $\text{NH}-\text{C}^\alpha\text{H}^\alpha$ coupling constant and the azimuthal angle ϕ (for L-amino acid residues).

Table I
Electronegativity Coefficient of Substituents Used in the Present Work

Substituent	Electronegativity coefficient
H	2.10 ^a
$\text{CH}_3, \text{CH}_2\text{R}, \text{CHRR}', \text{CRR}'\text{R}''$	2.50 ^a
OR	3.32 ^a
$\text{CO}-\text{OCH}_3$	2.58 ^b
$\text{CO}-\text{N}-(\text{C}_2\text{H}_5)_2$	2.70 ^b
$\text{CO}-\text{NH}-\text{CH}_3$	2.68 ^b

^a From ref 29. ^b Calculated by the formula from ref 30.

amide hydrogen atom is removed by 0.12 \AA out of the plane of its own amide group. This distortion, which is likely to be due to molecular packing forces in the crystal,²⁵ should disappear in solution.

Furthermore, inspection of the crystal structure shows that dimerization also induces an in-plane bending of the $\text{N}-\text{H}$ bond so that the $\text{H}-\text{N}-\text{C}^\alpha$ bond angle is increased to 125° in the paired molecules.²¹

By means of a proper choice of solvent and concentration, it is possible to obtain solutions of 3-isoquinuclidone, II, in which the solute is present either in a monomeric form or as dimeric aggregates and to observe by infrared spectroscopy the shift with dilution of the monomer-dimer equilibrium.²¹ Then, simultaneous measurements of the J_{HH^α} vicinal coupling constant allow an experimental estimation of the variation of J when the α angle is allowed to relax from 125° (in dimers) to its standard value (114°) in monomeric molecules. The corresponding ΔJ difference is found equal to 0.4 Hz .²¹ The magnitude of this experimental variation seems to indicate that the J_{HH^α} vicinal coupling constant for the $\text{H}-\text{N}-\text{C}^\alpha-\text{H}^\alpha$ sequence in amides is probably less sensitive to bond angle distortions than is generally assumed for the $\text{H}-\text{C}-\text{C}-\text{H}$ ethane-like system.²⁰

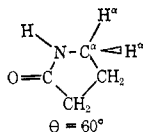
(b) Electronegativity Correction. There is some uncertainty concerning the substituent electronegativity influence on vicinal coupling constants. Several authors have stated that the correction should depend on the relative disposition of the coupled protons in ethane bonds.²⁶ The lack of precise information led us to neglect such an effect and we have merely referred to the formula of Banwell and Sheppard²⁷ which has also been previously used by Bystrov, *et al.*,^{3,4}

$$J_{\text{cor}} = J_{\text{obsd}} [1 - 0.1 \Sigma \Delta E_i]^{-1}$$

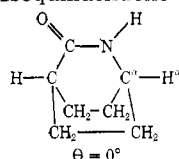
J_{obsd} and J_{cor} being respectively the observed and corrected vicinal coupling constants. Each of the ΔE_i terms is the difference between the substituent electronegativity and that of hydrogen in the Huggins scale.²⁸ In the following we have selected the electronegativity coefficients given by Laszlo and Schleyer;²⁹ when not available they were esti-

Table II
Vicinal Coupling Constant for the Selected Standard Compounds Used in the Calibration of the Karplus Relation
Fitting the NH-C^αH^α Bond in Peptide Compounds

Compd	Solvent	Vicinal NH-C ^α H ^α coupling constant		
		Obsd	Std	Cor ^a
<i>N</i> -Methylacetamide CH ₃ -CO-NH-CH ₃	Neat	4.7	4.7 ± 0.1	4.3 ± 0.1
	CCl ₄ ^a	4.6		
	CS ₂ ^a	4.8		
<i>N</i> -Methylpropionamide CH ₃ -CH ₂ -CO-NH-CH ₃	CS ₂ ^a	4.6		
<i>N</i> -Methylisobutyramide (CH ₃) ₂ CH-CO-NH-CH ₃	CS ₂ ^a	4.7		
<i>N</i> -Methylpivalamide (CH ₃) ₃ C-CO-NH-CH ₃	CS ₂ ^a	4.6		
2-Pyrrolidinone	Neat	0.7 ₅	0.75 ± 0.1	0.7 ± 0.1
	Dioxane ^a	0.8 ₀		
	(CH ₃) ₂ CO ^a	0.7 ₀		
	(CD ₃) ₂ CO ^a	0.7 ₅		
	CS ₂ ^a	0.6 ₅		
	CDCl ₃ ^a	0.7 ₅		
	CH ₃ -CN ^a	0.8 ₀		
3-Isoquinuclidone	(CD ₃) ₂ SO ^d	5.6	5.8 ± 0.2	5.7 ± 0.2
	CCl ₄ ^b	5.4		
	CCl ₄ ^c	5.7		
	CDCl ₃ ^a	5.8		
	CH ₃ -CN ^a	5.8		



3-Isoquinuclidone



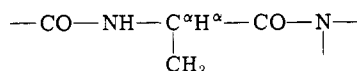
^a No apparent concentration effect. ^b Saturated solution: 0.1 mol l.⁻¹ ^c Dilute solution: 0.005 mol l.⁻¹ ^d From ref 5.
^e Electronegativity correction by reference to the dipeptide moiety -CO-NH-C^α(CH₃)H^α-CO-N<.

mated from the Dailey-Shoolery formula.³⁰

$$E_X = 1.78 + 0.684(\delta_{\text{CH}_3} - \delta_{\text{CH}_2})$$

E_X is the electronegativity of the substituent X; δ_{CH_3} and δ_{CH_2} are the chemical shifts characterizing the ethyl group in the molecule X-CH₂-CH₃.

In Table I are collected the electronegativity coefficients we have used in the present investigation. Corrections have been worked out by reference to a standard dipeptidic moiety



(c) **Results.** The vicinal J coupling constants of the selected standard compounds are reported in Table II. They have been measured under different experimental conditions.

The J value characterizing the various *N*-methylamides appears to be rather constant and insensitive to the nature of the solvent, with the exception of strongly acidic media (such as trifluoroacetic acid) which are known to protonate the amide group.^{5,14} So, the corrected vicinal coupling constant for *N*-methylamides was estimated to be 4.3 ± 0.1 Hz.

For 2-pyrrolidinone, the coupling constant is very low and the poor resolution of the N-H signal (triplet) makes the solvent effect hardly discernible. Resolution is better when one operates in acetonitrile or hexadeuterioacetone and then experiments yield a precise J value of 0.7₅ ± 0.1 Hz (0.7 ± 0.1 Hz after electronegativity correction).

We have already mentioned that the vicinal coupling constant for 3-isouquinuclidone, II, is solvent dependent, being lower in inert media than in dissociating media.²¹ The most significant J values are then obtained with solution in deuteriochloroform or acetonitrile in which dimerization is prevented by solvation. The corresponding corrected coupling constants are equal to 5.7 ± 0.2 Hz.

These experimental data enable us to compute the three coefficients A , B , C ³¹ (set I)

$$A = 4.3 \pm 0.2_5 \text{ Hz}$$

$$B = 2.9 \pm 0.3_5 \text{ Hz}$$

$$C = 4.3 \pm 0.1 \text{ Hz}$$

The Karplus relationship is then represented by the area included between the two solid lines drawn in Figure 2. The left ordinate scale refers to C^α branched residues (preceding set of coefficients A , B , and C) and the right one only to glycol units when the two H^α protons are magnetically nonequivalent. In such a case the absence of any substituent on the C^α carbon results in slightly different A' , B' , and C' values (set II)

$$A' = 4.45 \pm 0.2_5 \text{ Hz}$$

$$B' = 3.0 \pm 0.3_5 \text{ Hz}$$

$$C' = 4.45 \pm 0.1 \text{ Hz}$$

Finally, when the glycol residue contains two magnetically indistinguishable H^α hydrogen atoms, the vicinal coupling constant is an averaged one

$$J' = 1/2[J(\theta) + J(\theta + 120^\circ)]$$

Then, the Karplus relationship can directly be expressed as a function of the rotational angle Φ ^{2b}

$$J' = -\frac{A'}{2} \cos 2\Phi - \frac{B'}{2} \cos \Phi + C'$$

where A' , B' , and C' have the values listed in set II. The corresponding curve is drawn in Figure 3.

In Figure 2 are also depicted the relationships J vs. θ from Bystrov, *et al.*³ (dotted line), and from Ramachandran, *et al.*⁵ (broken line). Our own calibration curve shows an enhanced asymmetrical shape and reaches higher J values when the dihedral angle is close to 180°.

Discussion

We will now discuss the reliability of our results, taking

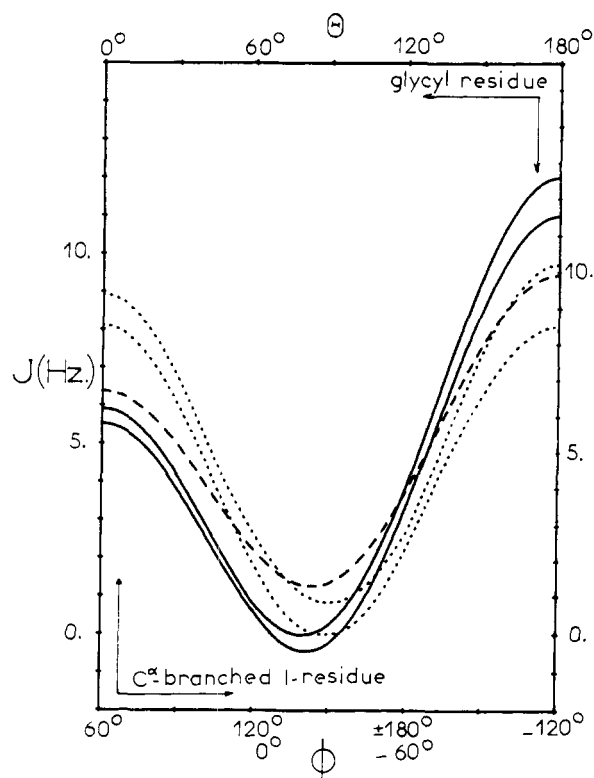


Figure 2. Variation of the vicinal $\text{NH-C}\alpha\text{H}\alpha$ coupling constant *vs.* the dihedral angles Θ and Φ . The left and lower scales refer to observed values for $\text{C}\alpha$ substituted residues (coefficients *A*, *B*, and *C*; set I); the right and upper scales refer to observed values for glycylic residues with nonequivalent $\text{H}\alpha$ protons (coefficients *A'*, *B'*, and *C'*; set II): (—) this work; (···) from V. F. Bystrov, *et al.*³ (---) from G. N. Ramachandran, *et al.*⁵

into account the most recent theoretical work in this field, several additional nmr data which are available in the literature, and a few experimental *J* values we have measured with open-chain peptide model compounds.

(a) Comparison with Theoretical Results. Recently, three theoretical investigations have been devoted to the evaluation of the vicinal coupling constant for the $\text{H-N-C}\alpha\text{-H}\alpha$ sequence in *N*-methylamides.¹⁶⁻¹⁸ Each of these studies results in two Karplus-like relationships: for a *cis* and for a *trans* amide conformation. Figure 4 reproduces the corresponding theoretical curves after correction for the electronegativity of the substituents in order to allow a direct comparison with the previous experimental traces.

First, it appears that theory corroborates the experimental conclusion according to which the coupling constant is not very sensitive to the *cis* or *trans* arrangement of the amide moiety (excepting perhaps the conformation where Θ is close to 0 or 180°). Moreover, although theoretical *J* values seem to be systematically larger in the INDO approximation, there is, with regards to the asymmetry of the curves, a rather better agreement with our empirical calibration than with the experimental curves proposed by Bystrov, *et al.*³ and by Ramachandran, *et al.*⁵

(b) Comparison with Nmr Data from Literature. A second way to support our calibration consists of collecting any nmr result available in the literature dealing with heterocyclic molecules with a *cis* amide group.^{19,32-34} In Table III we have reported these experimental *J* values and we have corrected them, as usual, for the electronegativity of the substituents. Then, referring to our own calibration, we have deduced a dihedral angle Θ which is compared with the Θ value measured by X-ray analysis or more roughly estimated by use of a Dreiding model.

The fairly good agreement obtained in the case of the

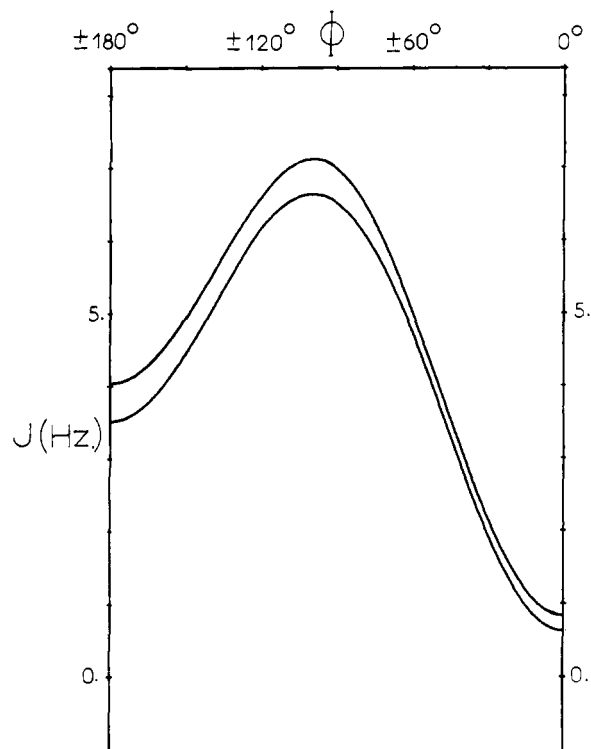


Figure 3. Variation of the observed averaged vicinal $\text{NH-C}\alpha\text{H}_2\alpha$ coupling constant *vs.* the dihedral angle Φ for glycylic residues with two equivalent $\text{H}\alpha$ protons (coefficients *A'*, *B'*, and *C'*; set II).

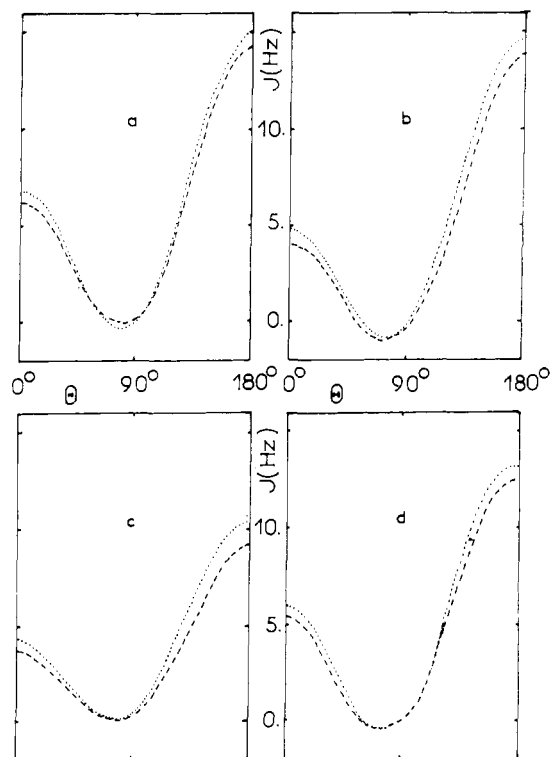
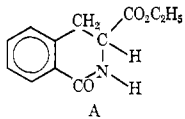
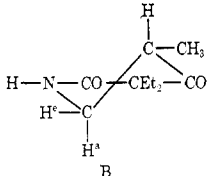
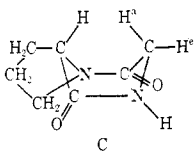
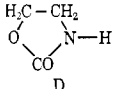
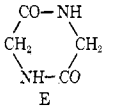


Figure 4. Theoretical variation of the vicinal coupling constant corrected for electronegativity by reference to the dipeptide moiety $-\text{CO-NH-C}(\text{CH}_3)\text{H-CO-N}<$ for comparison with empirical curves: (---) *cis* amide bond; (···) *trans* amide bond. (a) From Barfield and Gearhart¹⁶ in the INDO approximation. (b) From Giessner-Prettre¹⁷ in the INDO approximation. (c) From Giessner-Prettre¹⁷ in the PCILO approximation. (d) From Solkan and Bystrov¹⁸ in the INDO approximation.

first three compounds allows a conclusive checking of our empirical relationship when Θ is near 20 and 100°.

Furthermore, the observation according to which none of

Table III
Vicinal NH-CH Coupling Constant for Several Heterocycle Compounds Containing a Cis Amide Function^a

Compd		J_{obsd} , Hz	J_{cor} , Hz ^b	Θ , deg	Θ , deg Dreiding
D-3-(carboethoxy-3,4-dihydroisocarbostyryle) ^{32a} (A)					
 A	H ^a	1.7	1.7	108	100
	H ^e	4.2	4.2	27	20
3,3-Diethyl-5-methyl-2,4-piperazinedione ^{32b} (B)					
 B	H ^a	<1.0	<1.0	18	100
	H ^e	5.3	5.0		20
cyclo-(Glycyl-L-prolyl) ³³ (C)					
 C	H ^a	1.5	1.4 _s	106	100
	H ^e	4.5	4.3	26	20
2-Oxazolidinone ¹⁶ (D)					
 D		0.9	0.8 _s	60	60 ^{c 35}
cyclo-(Diglycyl) ^{33, 34} (E)					
 E		2.2	2.1	30 90	60 ^{c 28}

^a Comparison between the dihedral angle Θ calculated from our calibration and estimated from Dreiding models or crystal molecular structure. ^b Electronegativity correction by reference to the dipeptide moiety $-\text{CO}-\text{NH}-\text{CH}(\text{CH}_3)-\text{CO}-\text{N} <$. ^c From crystal molecular structure.

the three J values corresponding to $\Theta = 20^\circ$ exceeds 5 Hz corroborates the maximum of 5.7 Hz we have ascribed to $\Theta = 0^\circ$ by reference to our own measurement performed with 3-isouquinolidone.

It is also worthwhile to point out the experimental J value of 0.85 Hz reported in the case of 2-oxazolidinone¹⁹ which is very close to our result obtained with 2-pyrrolidinone (0.7 ± 0.1 Hz). The crystal structure of 2-oxazolidinone is now known and it shows a nearly planar molecule.³⁵ All these observations are quite consistent with the low value we ascribe to the coupling constant when Θ is near 60° .

In the lower part of Table III we mention cyclo(diglycyl) or 2,5-diketopiperazine, which also has a planar structure ($\Theta = 60^\circ$) as shown by X-ray examination of crystalline samples.³⁶ Referring to our calibration we would therefore expect a low J value (about 0.7 Hz) which is not consistent with the reported experimental one (2.1 Hz). Such a discrepancy leads us to consider that the cyclo(diglycyl) molecule is not planar in solution but adopts, on the contrary, two rapidly interconverting boat conformations where the Θ dihedral angles are respectively equal to 30 and 90° . Similar conformations have actually been identified in the solid state by X-ray crystallography in the case of other 3,6-substituted 2,5-diketopiperazines such as cyclo-(glycyl-L-tyrosyl),³⁷ cyclo-(di-L-alanyl),³⁸ and cyclo-(L-prolyl-L-leucyl).³⁹ If this is the case, the observed vicinal coupling constant (2.1 Hz) is an averaged value. According to the relationship we have established for glycine derivatives with two equiv-

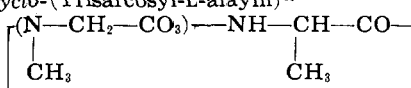
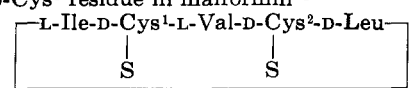
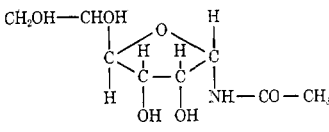
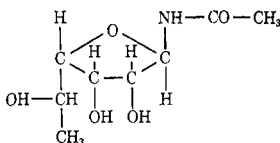
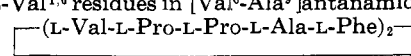
alent H^a protons (Figure 3), it turns out that the average Φ angle is close to -30° and this result is quite consistent with our interpretation.

In Table IV emphasis is put on several compounds, containing a trans amide function, which are characterized by corrected J values higher than 10 Hz⁴⁰⁻⁴³ and which therefore support the asymmetrical shape of our calibration curve. This is the case for the D-cysteinyl residue indexed 2 in malformin⁴¹ and the L-valyl residues indexed 1 and 6 in the (Val⁶-Ala⁹) antanamide analog.⁴³ Such high values are not predicted by previous empirical Karplus relationships³⁻⁵ whereas they correspond to Θ angles close to 180° in our own calibration. The highest coupling constant is observed with *N*-acetyl-L-rhamnofuranosylamine and concerns the acetamido group at the C₁ carbon atom.⁴² The corrected J value (11.7 Hz) reaches the estimated J (180°) limit (11.5 ± 0.5 Hz).

This result enables us to ascertain that 2-pyrrolidinone is a reliable standard compound for parametrizing the $J(\Theta)$ function. In fact, if we agree with the Karplus formulation, it turns out that $J(180^\circ) - 2J(60^\circ) = 3C = 12.9$ Hz. So, $J(180^\circ) = 11.7$ Hz implies that $J(60^\circ) = 0.6$ Hz, a result which is in satisfactory agreement with the vicinal coupling constant we have measured with 2-pyrrolidinone ($J = 0.7 \pm 0.1$ Hz).

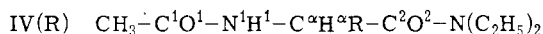
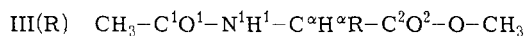
(c) **Comparison with Ir and Nmr Data Obtained with Open-Chain Peptide Model Compounds.** Two sets of simple peptide model compounds III(R) and IV(R) have been investigated by using ir and nmr spectroscopies. Ex-

Table IV
Several High Experimental $J_{\text{HH}\alpha}$ Values which Are Not Accounted for by the Empirical J vs θ Relationships from References 3–5

Compd	J_{obsd} Hz	J_{cor} Hz ^a
cyclo-(Trisarcosyl-L-alanyl) ⁴⁰ 	10	10
3-Acetylamino-2,2,4,4-tetramethyl-pentane ^b $\text{CH}_3\text{-CO-NH-CH}[\text{CH}(\text{CH}_3)_2]_2$	10.2	10
D-Cys ² residue in malformin ⁴¹ 	10.7	10.7
N-Acetyl-α-D-glycofuranosylamine ⁴² (F) 	10	10.6
N-Acetyl-L-rhamnofuranosylamine ⁴² (G) 	11	11.7
L-Val ^{1,6} residues in [Val ⁶ -Ala ⁹]antanimide ⁴³ 	10.7	10.7

^a Electronegativity correction by reference to the dipeptide moiety $-\text{CO-NH-C}(\text{CH}_3)\text{H-CO-N}<$. ^b This work (solvent: CS_2 and saturated iodine).

periments were performed with dilute solutions in an inert solvent (6×10^{-3} mol l.⁻¹ in CCl_4) in order to prevent self-association of the solute.



The J values we have measured with these species are collected in Table V.

The conformational features of the model derivatives III(R) are now well known from our previous ir investigations.⁴⁴ The N-H stretching absorption spectra of these compounds (Figure 5) show that apart from the glycine derivative III(H) nearly all the solute molecules are in the so-called C_5 conformation which is stabilized by the $\text{H}^1\text{-O}^2$ interaction (Figure 6a).

The nmr data in Table V indicate that the C_5 conformation is progressively distorted out of planarity as the R substituent becomes more and more bulky. Such a warping is probably due to the asymmetrical repulsion between the O^1 oxygen atom and the side substituent (Figure 6a). It is also worth noticing that the Φ rotational angle ($\Phi = -161^\circ$) so estimated for the alanine derivative III(Me) is very close to the value ($\Phi = -160^\circ$) obtained from the X-ray examination of a very similar compound (*N*-chloroacetyl-*dl*-alanine) which is found to adopt the C_5 conformation in the solid state.⁴⁵

Moreover, ir spectroscopy shows that the deviation from the planar stretched structure ($\Phi = -180^\circ$) is in no case important enough to exclude the $\text{H}^1\text{-O}^2$ interaction even

Table V
Vicinal $\text{NH-C}^\alpha\text{H}^\alpha$ Coupling Constant (J) for Compounds III(R) and IV(R)^a

Compd	J_{obsd} , Hz	J_{cor} , Hz ^b	θ , deg	$-\Phi$, deg
III(R)				
$\text{CH}_3\text{-CO-NH-C}^\alpha\text{H}^\alpha\text{R-CO-O-CH}_3$				
R = H ^c	4.8	4.6		
CH_3	7.2	7.1	139	161
$\text{CH}_2\text{-CH}_3$	7.6	7.5	141	159
$\text{CH}_2\text{-CH}(\text{CH}_3)_2$	8.0	7.9	144	156
$\text{CH}(\text{CH}_3)_2$	8.5	8.4	147	153
$\text{C}(\text{CH}_3)_3$	9.3	9.2	152	148
IV(R)				
$\text{CH}_3\text{-CO-NH-C}^\alpha\text{H}^\alpha\text{R-CO-N(C}_2\text{H}_5)_2$				
R = H	3.8 ₅	3.7	120	180
CH_3 ^d	8.3	8.3	146	154
$\text{CH}_2\text{-CH}_3$ ^d	8.7	8.7	148	152
$\text{CH}_2\text{-CH}(\text{CH}_3)_2$ ^d	8.9	8.9	150	150
$\text{CH}(\text{CH}_3)_2$ ^d	9.5	9.5	154	146
$\text{C}(\text{CH}_3)_3$	9.7	9.7	155	145

^a Progressive distortion of the C_5 conformation with the bulkiness of the side-chain R (planar structure: $\Phi = -180^\circ$). (Solvent, CCl_4 ; concentration, 6×10^{-3} mol l.⁻¹). ^b Electronegativity correction worked out by reference to the dipeptide moiety $-\text{CO-NH-C}^\alpha\text{H}^\alpha(\text{CH}_3)\text{-CO-N}<$. ^c Rapid exchange of two conformations with different Φ angles. ^d Rapid exchange of two conformations with very similar Φ angles.

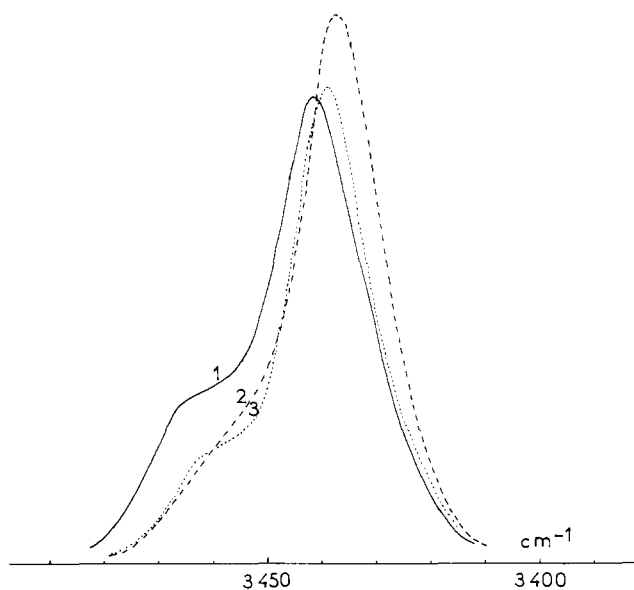


Figure 5. N-H stretching absorption spectra of compounds III(R) in dilute CCl_4 solution (concentration, 5×10^{-3} mol l.⁻¹): (1) III(H); (2) III(Me); (3) III(*t*-Bu).

when the side substituent is a tertiary butyl group as in III(*t*-Bu) (Figure 5). We consequently deduce that the rotation around the $\text{N}^1\text{-C}^\alpha$ bond can hardly exceed 30° (i.e., $\Phi = -150^\circ$) in order to account for the preservation of a weak $\text{H}^1\text{-O}^2$ interaction. Accordingly, the J value for the derivative III(*t*-Bu) reaches 9.2 Hz corresponding to $\Phi = -148^\circ$ in our calibration. On the contrary, reference to the empirical curves of Bystrov, *et al.*,^{3,4} and of Ramachandran, *et al.*,⁵ would result in a Φ value close to -120° (rotation of 60° around the $\text{N}^1\text{-C}^\alpha$ bond). Such a large distortion would obviously relax the $\text{H}^1\text{-O}^2$ intramolecular interaction, in opposition to our ir experimental observations. We think therefore that these authors have underestimated the values of the J coupling constant for dihedral θ angles near 180° .

As for model compounds IV(R), ir spectra generally ex-

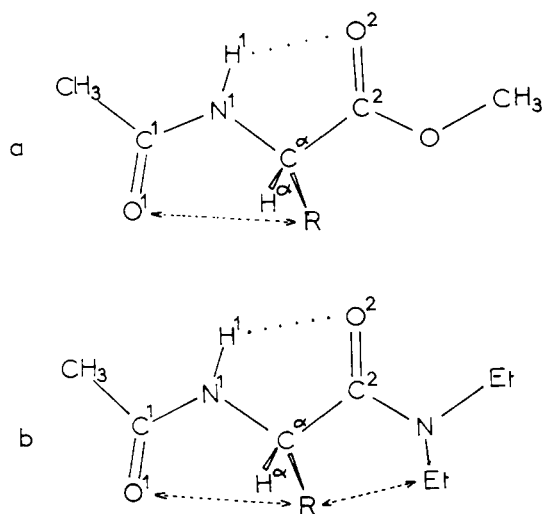


Figure 6. C_5 conformation of compounds III(R) and IV(R). Intramolecular repulsions distort the C_5 conformation out of planarity: (a) III(R); (b) IV(R).

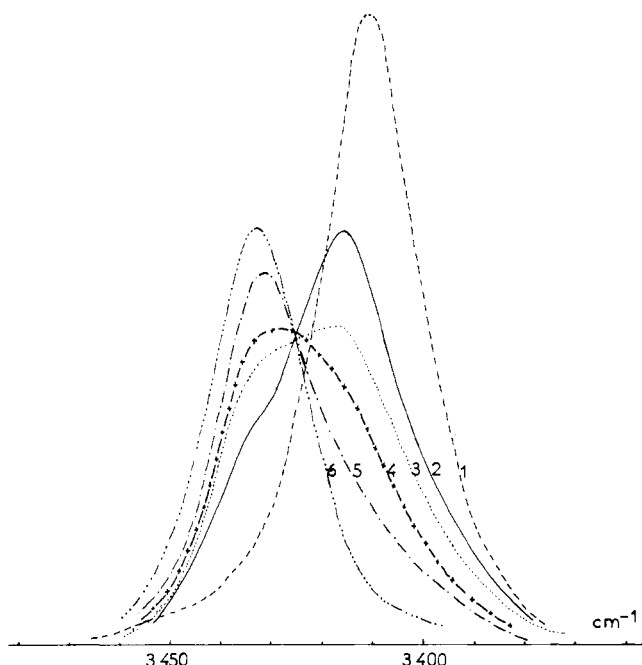


Figure 7. N-H stretching absorption spectra of compounds IV(R) in dilute CCl_4 solution (concentration, 5×10^{-3} mol l. $^{-1}$): (1) IV(H); (2) IV(Me); (3) IV(Et); (4) IV(*i*-Bu); (5) IV(*i*-Pr); (6) IV(*t*-Bu).

hibit, in the same 3350–3450 cm^{-1} frequency range, two overlapping absorption bands at 3415 and 3435 cm^{-1} (Figure 7). The different curves pass through an isosbestic-like crossing and consequently we believe there is an equilibrium, depending on the bulkiness of the R substituent, between the C_5 conformation ($\nu_{\text{N-H}} = 3415 \text{ cm}^{-1}$) and some open structure ($\nu_{\text{N-H}} = 3435 \text{ cm}^{-1}$), the latter being the only stable one in the case of the pseudoleucine derivative IV(*t*-Bu). In our opinion, the easier relaxation of the C_5 conformation, in comparison with the behavior of the III(R) analogous compounds, is due to an additional sterical repulsion between the side-substituent R and the N-terminal ethyl group (Figure 6b). Both opened and C_5 conformations are therefore characterized by nearly equal Φ rotational angles (Table V).

In the particular case of the glycine derivative IV(H) the corresponding single maximum curve (Figure 7) shows that

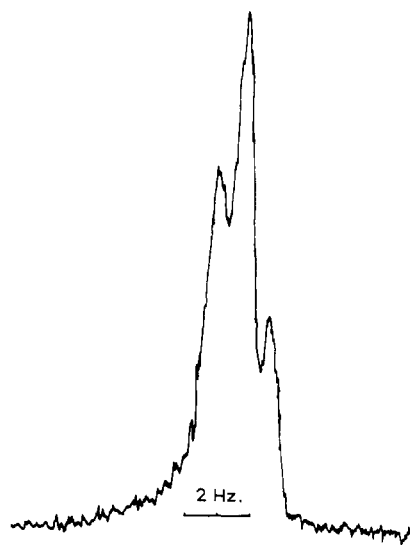


Figure 8. N-H signal of 2-pyrrolidinone from hexadeuterioacetone solution (concentration 4%) with ^{14}N heteronuclear irradiation.

nearly every molecule is in the C_5 conformation. When the temperature is raised to 70°, the open form is hardly discernible as a very weak absorption band located around 3460 cm^{-1} .¹¹ Thus, the glycine derivative IV(H) is one of those rare single-conformation open-chain trans amides we can use to support our previous calibration. Referring to our curve (Figure 2), the corresponding J value ($J_{\text{cor}} = 3.7$ Hz, Table V) is related to a dihedral angle $\theta = 120^\circ$ (i.e., $\Phi = -180^\circ$) and is consistent with a nearly planar C_5 conformation. Such a result also agrees with the symmetry of the model molecule and with the magnitude of the $\Delta\nu_{\text{N-H}}$ frequency shift (45 cm^{-1}), which indicates a rather strong $\text{H}^1\text{--O}^2$ interaction.

Since the Karplus formulation implies that $J(0^\circ)$ and $J(120^\circ)$ are related by $J(0^\circ) + 2J(120^\circ) = 3C = 12.9$ Hz, it turns out that $J(0^\circ)$ should be close to 5.5 Hz and this conclusion corroborates the J value we have measured with 3-isosquinolidone (5.7 ± 0.2 Hz).

Conclusion

An empirical calibration of a Karplus relationship between the $J_{\text{HH}\alpha}$ vicinal coupling constant and the corresponding dihedral angle has been achieved for the $\text{H--N--C}^\alpha\text{--H}^\alpha$ sequence in amides, peptides, and similar species. A unique formula is proposed which approximately accommodates experimental results obtained with rigid heterocyclic lactams and with open-chain trans amides as well. This extensive agreement leads us to conclude that the $J_{\text{HH}\alpha}$ coupling constant is not very sensitive to the cis or trans arrangement of the amide link. Furthermore, it seems to be less influenced by bond angle distortions resulting from internal strain in ring structures than the corresponding J_{HH} coupling constant for the ethane-like H--C--C--H sequence.

The most striking feature of the resulting $J_{\text{HH}\alpha}$ vs. θ curve is its enhanced asymmetry arising from a relatively high value of the coefficient B (compared to previous calibrations from other authors). As an example of the significance of this point, it can be concluded from our curve that an experimental J constant higher than 6 Hz is inconsistent with a cis disposition of the N-H and C-H adjacent bonds.

Experimental Section

Nmr spectra were measured at room temperature on a J.N.M.-P.S. 100 spectrometer equipped with a J.N.M.-S.B.-2 S/N booster. Heteronuclear irradiation of the quadrupolar ^{14}N nucleus was accomplished with a J.N.M.-S.D.-HC heterospin decoupler and a

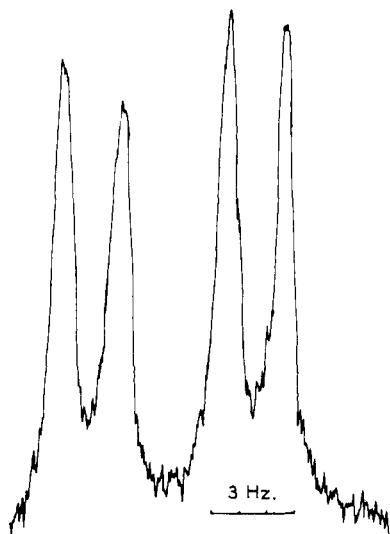


Figure 9. Accumulated N-H signal of 3-isoquinuclidone from dilute acetonitrile solution (concentration, 2×10^{-2} mol l.⁻¹) with ¹⁴N heteronuclear irradiation.

J.N.M.-O.A.-1 FR oscillator adaptor (7 MHz). Irradiation frequency for ¹⁴N nucleus is 7,224,024 Hz.

Vicinal NH-C^αH^α coupling constants were measured on hetero-decoupled N-H signals (accumulated for dilute solutions; sweep width = 0.75 Hz/cm) using an internal lock control on the TMS signal in a field/frequency sweep mode.

Measurement of the vicinal coupling constant for 2-pyrrolidinone (I) was performed by the JEOL Co. The N-H signal, decoupled by ¹⁴N irradiation, was obtained using a 4% hexadeuterioacetone solution and is reproduced in Figure 8. Measurements on a dilute acetonitrile solution (concentration 2×10^{-2} mol l.⁻¹) gave quite similar results.

All other compounds were studied in our laboratory.

Figure 9 represents the accumulated N-H signal (S/N factor = 16) for 3-isoquinuclidone (II) in acetonitrile solution (concentration 2×10^{-2} mol l.⁻¹) with ¹⁴N decoupling. We account for the splitting of the N-H signal (quartet) by the rather high value (ca. 2 Hz) of the ⁴J coupling in the >CH-CO-NH- moiety.²¹

As for the open-chain peptide compounds III(R) and IV(R), vicinal NH-C^αH^α coupling constants were measured using dilute CCl₄ solutions (concentration 6×10^{-3} mol l.⁻¹) under conditions such that any self-association cannot be detected by ir spectroscopy. Except for glycine derivatives III(H) and IV(H), vicinal coupling constants are not significantly dependent on concentration and this feature indicates that self-association of these solutes does not noticeably perturb the rotational state of the N-C^α bond.

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