Conformations of Peptides in Solution by Nuclear Magnetic Resonance Spectroscopy. Part 5.[1] Homoallylic Proton Spin Coupling in Linear Peptides

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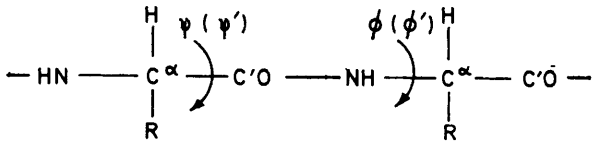
Five bond proton spin coupling, ^5J(HH), has been observed in some linear di- and tri-peptides with *trans* peptide bonds. Magnitudes of ^5J(HH) were analysed in terms of homoallylic coupling using *N*-methylacetamide and *NN*-dimethylacetamide as standard compounds for groups antiperiplanar across peptide bonds. Together with ^3J(HNCH) magnitudes the results for ^5J(HH) can be used to limit the range of conformations (ϕ, ψ) for peptides in solution. Attention has been focused on two peptide conformations studied by 100 MHz ^{1}H n.m.r. measurements of *N*-acetyl-L-alanyl-*N*-methylamide (C_7 structure) and *N*-acetyl-L-valyl-glycyl-*N*-methylamide (β-turn) in different solvents. The conformational properties are compared with previous studies using *X*-ray crystallography, theoretical calculations, and spectroscopy (n.m.r., i.r.).

THE conformational properties of linear and cyclic peptides in solution have been the subject of intensive study by various physical techniques.[2-8] It has been found that a limited number of conformations are observed which have been designated as α-helix, β-pleated sheet, 3_{10} helix, β-turn, *etc.* Such conformations are conveniently characterized by various combinations of the torsional angles, ϕ and ψ, corresponding to N–C$^\alpha$ and C$^\alpha$–C′ bond conformations, respectively, as shown in the peptide molecular fragment (I) where ϕ' and ψ' are the corresponding homoallylic angles.



(I)

N.m.r. spectroscopy has proved to be an excellent method for determining detailed conformational properties of peptides in solution. An estimate of the N–C$^\alpha$ bond conformation can be made from observed vicinal proton coupling (HNCH) using the Karplus–Bystrov relation between ^3J(HNCH) and the dihedral angle θ(HNCH).[3,9,10] One ^3J(HNCH) observation can be satisfied by four different single conformations for $0 < \theta < 360$ or by numerous conformational equilibria. Recently, potential energy calculations have increasingly been used to limit the number of possible conformations.[10-14] Despite the limitations imposed by variation of ^3J with such factors as bond lengths, bond angles, orientation and electronegativity of attached substituents, *etc.*,[15,16] ^3J(HNCH) is a most useful parameter for determining peptide conformations. With the advent of ^{13}C Fourier transform n.m.r. spectroscopy it is possible that observations of ^3J(HNC$^\alpha$C′), ^3J(HNC$^\alpha$C$^\beta$), and ^3J(C′NC$^\alpha$H$^\alpha$) may be used to determine N–C$^\alpha$ bond conformations. The latter coupling has an advantage over each of the other vicinal coupling constants as measurements can be made on peptide systems with fast NH exchange. It has already been shown[17-20] that vicinal ^{1}H–^{13}C coupling follows a Karplus-type angular dependence. It is expected that coupling between NH and β-^{13}C of peptide fragments follows an angular dependence that is 120° out of phase with that found for NH and α-CH vicinal proton coupling and so both observations could be used to limit the number of possible conformations.[21]

Methods for determining the conformational properties of the C$^\alpha$–C′ bond (ψ) are not so well developed. Recent work[22-24] on ^{15}N enriched amino-acids and peptides

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[24] M. Barfield and H. J. Gearhart, *Mol. Phys.*, 1974, **27**, 899.

has shown that $^3J(^{15}NC'C^\alpha H)$ is related to $\psi(C'C^\alpha)$. In principle it is also possible that $^3J(^{15}NC'C^\alpha C^\beta)$ may be used to determine C^α–C′ bond conformations. The observation of such coupling involves ^{15}N incorporation into peptide or protein and specialized instrumentation.

Five-bond long-range proton spin coupling has been shown to exist between α-CH groups of adjacent amino-acids in peptide bonds.[25] From measurements on a series of cyclic dipeptides in [$^2H_6$]DMSO solution, it was found that the magnitude of the coupling depends on the N–C^α and C^α–C′ bond conformations according to relation (1) where A is a constant (A^s for groups in *syn*

$$^5J(HH) = nA \sin^2\phi' \times \sin^2\psi' \qquad (1)$$

conformations across peptide bonds) and n equals the number of equivalent coupling paths.[26] The angles ϕ' and ψ' corresponds to homoallylic torsional angles for N–C^α and C^α–C′ bonds, respectively, and they are related to the peptide torsional angles (ϕ,ψ) by relationships (2) and (3). Observations of $^5J(HH)$ of cyclic

$$\phi = 240 - \phi'(L) = 120 - \phi'(D) \qquad (2)$$

$$\psi = \psi'(L) - 240 = \psi'(D) - 120 \qquad (3)$$

dipeptides in [$^2H_6$]DMSO, $CDCl_3$, and D_2O solutions have been interpreted in terms of the conformations of these molecules in solution.[27]

In this work $^5J(HH)$ for groups in *anti* conformations across peptide bonds are discussed. There are few simple compounds with *trans* peptide bonds (α-CH groups antiperiplanar) and fixed conformations in solution which can be used to relate $^3J(HNCH)$ and $^5J(HH)$ in order to show that $^5J(HH)$ for such systems conforms to homoallylic coupling[26] according to equation (1). Hence, it is assumed that $^5J(HH)$ observed for *trans* peptide bonds depends on ϕ' and ψ' according to equation (1) with $n = 1$ and with A^a a constant for α-CH groups antiperiplanar across the peptide bond. The calibration of A^a from measurements on mono- and dimethylacetamide in different solvents is discussed. Homoallylic coupling has been observed in peptides whose conformations have previously been determined, *i.e.*, *N*-acetyl-L-valylglycyl-*N*-methylamide (β-turn, type II) and *N*-acetyl-L-alanyl-*N*-methylamide (C_7 structure). The results are compared with the conformational models derived from *X*-ray analysis, n.m.r. and i.r. measurements, and theoretical calculations.

EXPERIMENTAL

N-Methyl- (NMA) and *NN*-dimethyl-acetamide (DMA) purchased from B.D.H. were purified by fractional distillation and stored over activated molecular sieve (3A). *N*-Acetyl-L-Ala-NMe was purchased from Fox Chemical Company, U.S.A.; the purity was checked by t.l.c. and n.m.r. *N*-Acetyl-L-Val-Gly-NMe was synthesized and described elsewhere.[28] Deuteriated solvents were purchased from Fluorochem; $CDCl_3$ (99.8%), [$^2H_6$]DMSO (99.9%), CD_3OD (99.9%), and D_2O (99.9%).

[25] D. B. Davies and M. A. Khaled, *J.C.S. Perkin II*, 1973, 1651.

100 MHz ^1H N.m.r. spectra of *ca.* 0.1M solutions of the amides were measured using a JEOL PS100 n.m.r. spectrometer operating in the internal lock mode. Sodium [2,2,3,3-$^2H_4$]-3-trimethylsilylpropionate (TSP) (D_2O) and tetramethylsilane (TMS) (DMSO, CD_3OD, and $CDCl_3$) were used as internal lock. Magnitudes of $^5J(HH)$ for NMA and DMA in D_2O (0.5 Hz) were determined from line separations of resolved quartets observed at 54 Hz sweep width (1.5 Hz cm^{-1}). Smaller long-range coupling constants (<0.5 Hz) were determined from at least five measurements of the line widths of coupled and decoupled signals observed at 27 Hz sweep width (0.75 Hz cm^{-1}). Chemical shifts and spin-coupling constants of NMA and DMA in the different solvents are summarized in Table 1. The complete n.m.r.

TABLE 1

Chemical shifts and spin coupling constants [a,b]

Parameter	Solvent: D_2O	CD_3OD	[$^2H_6$]-DMSO	$CDCl_3$
N-Methylacetamide				
δ(CH_3CO)	1.98	1.91	1.77	1.97
δ(NCH_3)	2.71	2.69	2.54	2.78
^5J(HH) *anti*	0.5 [c]	0.22 (±0.06)	0.20 (±0.05)	0.23 (±0.05)
^3J(HNCH)			4.5	4.5
NN-Dimethylacetamide				
δ(CH_3CO)	2.09	2.07	1.95	2.07
δ(NCH_3)	2.91	2.91	2.78	2.94
δ(NCH_3)	3.06	3.05	2.95	3.01
^5J(HH) *syn*	0.13 (±0.04)	0.12 (±0.04)	0.08 (±0.05)	0.06 (±0.06)
^5J(HH) *anti*	0.52 (±0.02) 0.5 [c]	0.24 (±0.05)	0.21 (±0.05)	0.23 (±0.05)
N-Acetyl-L-Ala-*N*-methylamide [d]				
^3J(HNCH, Ala)				7.5
^3J(HNCH$_3$)				4.5
^5J(CH_3CO, α-CH)	0.05 (±0.02)			0.10 ±0.02)
^5J(α-CH,NCH_3)	0.10 (±0.02)			<0.03
N-Acetyl-L-Val-Gly-*N*-methylamide [d]				
^3J(HNCH, Val)			6	
^3J(HNCH, Gly)			7.4	
^3J(HNCH$_3$)			4.5	
^5J(CH_3CO, α-CH)	0.15		0.1 (±0.02)	
^5J(Val α-CH, CH_2 Gly)	0		0	
^5J(CH_3, NCH_3)	0.7 (±0.02)		0.5 (±0.02)	

[a] Chemical shifts in p.p.m. from internal TSP (D_2O) or TMS (CD_3OD, [$^2H_6$]DMSO, and $CDCl_3$). [b] Spin-coupling constants measured from one-third of difference in linewidth of coupled and decoupled signals (measured under off resonance and double resonance conditions, respectively). [c] Measured from peak separations of resolved multiplet, J (±0.05) Hz. [d] Complete n.m.r. parameters to be published.[28]

parameters of *N*-acetyl-L-Ala-NMe and *N*-acetyl-L-Val-Gly-NMe will be published elsewhere [28] but the relevant magnitudes of $^3J(HNCH)$ and $^5J(HH)$ are included in Table 1.

DISCUSSION

Previous measurements have shown that $^5J(HH)$ is observed not only in cyclic dipeptides [26,27] but also in linear dipeptides and *N*-substituted amino-acids and

[26] D. B. Davies and M. A. Khaled, *J.C.S. Perkin II*, 1976, 187.
[27] D. B. Davies and M. A. Khaled, *J.C.S. Perkin II*, 1976, 1238.
[28] M. A. Khaled, K. Okamoto, and D. W. Urry, in preparation.

peptides.[25] In order to allow for considerable flexibility about N–C$^\alpha$ and C$^\alpha$–C′ bonds of linear peptides, ^5J(HH) in equation (1) is generalized to (4) where the summation

$$^5J(\mathrm{HH}) = A^a \sum_i^k p_i \sin^2\phi_i' \times \sum_i^m p_i' \sin^2\psi_i' \quad (4)$$

includes all k conformations with angles ϕ_i' and all m conformations with angles ψ_i' weighted according to the relative populations, p_i and p_i' for each conformer. For free rotation about N–C$^\alpha$ and C$^\alpha$–C bonds $p_i = 1/k$ and $p_i' = 1/m$. In practice, one parameter, ^5J(HH), cannot be used to determine four other variables (ϕ_i', p_i, ψ_i', and p_i') though there are a number of cases where equation (4) can be used to obtain information otherwise not available. One case relies on the fact [26] that any group with free rotation between classical staggered conformers makes a contribution to ^5J(HH) of a factor 0.5. Another case for simplification of equation (4) occurs for systems where ^3J(HNCH) is observed as this coupling is related to the peptide torsional angle ϕ through the vicinal Karplus [9] dependence of ^3J with dihedral angle θ(HNCH) according to equation (5).

$$^3J = D\cos^2\theta + E = D + E - D\sin^2\theta \quad (5)$$

For rotation about the N–C$^\alpha$ bond observed ^3J is the time-averaged value of J_i of each conformer weighted for the relative proportions for each conformer, p_i, as shown in equation (6). It is found for peptide N–C$^\alpha$

$$^3J_{(\mathrm{obs})} = \Sigma_i\, p_i\, ^3J_i = D + E - D\,\Sigma_i\, p_i \sin^2\theta_i \quad (6)$$

bonds that $\sin^2\theta = \sin^2\phi'$ as $\phi' = (180 \pm \theta)$ for L- and D-amino acids. Hence, the ϕ' contribution to ^5J(HH) can be calculated from observed ^3J(HNCH) values according to equation (7) so that ^5J depends on

$$\Sigma p_i \sin^2\phi'_i = (D + E - {}^3J)/D \quad (7)$$

the ψ' term only. In principle observations of ^5J(HH) can be used to determine conformations of the peptide C$^\alpha$–C′ bond though, in practice, it is found that the small values of ^5J(HH) so far observed can only be used to limit the possible range of conformations. In this work the combination of ^3J(HNCH) and ^5J(HH) observations are used to investigate the postulated conformations (β-turn, C_7 structure) of linear dipeptides in solution.

(i) *Calibration of* A^a.—It is necessary to determine A^a in order that equations (1) or (4) can be used in conformational analyses of peptides. From the quartet splitting patterns of expanded signals ^5J(HH) for NMA in D_2O was found to be 0.5 Hz; the value is the same as that observed between groups *anti* to each other in DMA in D_2O (Table 1). It is expected that both C–CH_3 (corresponding to ψ') and N–CH_3 (ϕ') bonds exhibit free rotation [^3J(HNCH) of 4.5 Hz for *N*-methylacetamide in different solvents (Table 1) is in accord with previous work [29-33] on NMA (4.6—5.0 Hz) and *N*-methylformamide (4.9 ± 0.1 Hz [29-33])] and A^a is calculated to be 2.0 Hz. It should be noted that the value of A^a (2.0 Hz) > A^s (1.40 Hz) in D_2O is predicted from INDO-MO theory.[34] A^a was determined in a similar manner from measurements on NMA and DMA in different solvents. The results which are summarized in Table 2 indicate

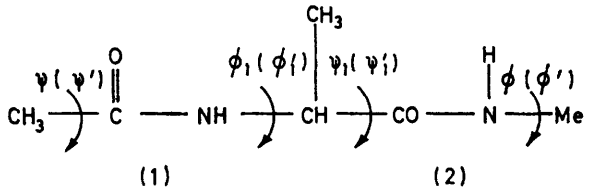
TABLE 2

Values of homoallylic coupling parameter (A) for *cis* and *trans* peptides in different solvents

	cis Peptide [a]		*trans* Peptide [b]			
	^5J(HH)/Hz		^5J(HH)/Hz			
Solvent	*c*-Gly-L-Pro [c]	A^a/Hz	NMA	DMA	Mean [d]	A^a/Hz
D_2O	2.6	1.38 (±0.04)	0.5	0.5	0.5 (±0.05)	2.0 (±0.2)
DMSO	1.65	0.88 (±0.03)	0.20	0.21	0.21 (±0.03)	0.8 (±0.1)
CD_3OD	2.1	1.1 (±0.03)	0.22	0.24	0.23 (±0.03)	0.9 (±0.1)
$CDCl_3$	1.7	0.9 (±0.03)	0.23	0.23	0.23 (±0.03)	0.9 (±0.1)

[a] Determined from observed ^5J(HH) for *c*-Gly-L-Pro assuming $n = 2$ and $\phi' = 280$ in the relation $^5J = nA\sin^4\phi'$.[26] [b] Determined from average of observed ^5J(HH) for *N*-methylacetamide and *NN*-dimethylacetamide assuming $n = 1$ and $\Sigma_i p_i \sin^2\phi_i' = \Sigma_i p_i' \sin^2\psi_i' = 0.5$ in equation (4). A^a values likely to be underestimated for all but D_2O solutions. [c] Error in J(±0.05) Hz. [d] Average error determined from measurements in Table 1.

that A^a is approximately constant (*ca.* 0.9 Hz) in CD_3OD, [$^2H_6$]DMSO, and $CDCl_3$ solutions and the value is significantly smaller than that observed in D_2O solutions (2.0 Hz). Magnitudes of A^s determined by quite a different procedure from cyclic dipeptides also vary with solvent.[26] For peptides in all solvents except D_2O it can be seen from the results in Table 2 that A^a = *ca.* A^s within the experimental accuracy of the present measurements.



(ii) N-*Acetyl*-L-*Ala*-N-*methylamide*.—The molecular formula of *N*-acetyl-L-Ala-NMe consists of two amide or peptide bonds labelled (1) and (2) with corresponding peptide (ϕ, ψ) and homoallylic torsion angles (ϕ', ψ').

X-Ray crystallographic studies of *N*-acetyl-L-Ala-NMe indicate that successive molecules form intermolecular hydrogen bonds which generate an anti-parallel chain β-pleated sheet structure twisted to form a left-handed helical unit.[35] The peptide torsional angles (ϕ_1, ψ_1) of the two molecules per unit cell which are listed in Table

[29] M. Liler, *J.C.S. Perkin II*, 1972, 720.
[30] M. T. Cung, M. Marraud, and J. Néel, *Macromolecules*, 1974, **7**, 606.
[31] J. S. Powles and J. H. Strange, *Discuss. Faraday Soc.*, 1963, **34**, 30.
[32] E. W. Randall and J. D. Baldeschweiler, *J. Mol. Spectroscopy*, 1962, **8**, 365.
[33] A. T. R. Brown and E. W. Randall, *Mol. Phys.*, 1964, **8**, 567.
[34] M. Barfield and S. Sternhell, *J. Amer. Chem. Soc.*, 1972, **94**, 1905.
[35] Y. Harada and Y. Iikaka, *Acta Cryst.*, 1974, **B30**, 1452.

3 show that both molecules have essentially the same conformation. Spectroscopic studies (n.m.r.[28,36] and i.r.[37-41]) of *N*-acetyl-L-Ala-NMe in non-polar solvents ($CDCl_3$, CCl_4, and tetrachloroethylene[42]) indicate that an intramolecular hydrogen bond is formed between the acetyl carbonyl group and the amide amino-group to form a seven-membered ring. For other than glycine residues there are two possible C_7 structures in which the amino-acid side chain exhibits approximate axial differentiated by comparing observed and predicted ^5J(HH) for both bond (1) (C–CH_3, α-CH; ψ',ϕ') and bond (2) (α-CH, N–CH_3; ψ_1',ϕ'). Magnitudes of ^5J(HH) were calculated from equation (4) assuming free rotation about C–CH_3 and N–CH_3 bonds and the results for *N*-acetyl-L-Ala-NMe in $CDCl_3$ (A^a 0.9 Hz) and D_2O (A^a 2.0 Hz) are summarized in Table 3. For each conformational model [bond (1), ϕ_1 and, hence, θ(HNCH)] the magnitude of ^3J(HNCH, Ala) was calculated using

TABLE 3

Comparison of observed and predicted ^5J(HH) for *N*-acetyl-L-Ala-NMe

	Peptide bond (1)							Peptide bond (2)		
				^3J (HNCH)/		$^5J_{calc}$/Hz		$^5J_{calc}$/Hz		
	ϕ_1 (°)	ψ_1 (°)	θ (°)	Hz [a]	ϕ'_1 (°)	$CDCl_3$ [b]	D_2O [c]	ψ'_1	$CDCl_3$ [b]	D_2O [c]
1. Crystal structure [d]										
Molecule 1	−84.3	159	144.3	7.5	324.3	0.15	0.34	39	0.18	0.40
Molecule 2	−87.6	154.8	147.6	8.0	327.6	0.13	0.29	34.8	0.15	0.32
2. Solution conformations										
(a) C(7) axial										
Bystrov *et al.*[e]	60	−60	0	8.7	180	0	0	180	0	0
Renugopalakrishnan *et al.*[f]	70	−65	10	8.4	170	0.01	0.03	175	0	0
Néel *et al.*[g]	45	−50	15	8.1	195	0.03	0.07	190	0.01	0.03
Pullman *et al.*[h]	90	−30	30	6.5	150	0.11	0.25	210	0.11	0.25
Bláha *et al.*[i] (B)	60	−30	0	8.7	180	0		210	0.11	
(b) C(7) equatorial										
Mizushima *et al.*[j]	−60	60	120	3.3	300	0.34	0.75	300	0.34	0.75
Néel *et al.*[g]	−75	50	135	5.9	315	0.22	0.50	290	0.40	0.88
Pullman *et al.*[h]	−90	60	150	8.4	330	0.11	0.25	300	0.34	0.75
Bláha *et al.*[i] (A)	−90	75	150	8.4	330	0.11		315	0.22	
(c) Extended conformations										
Bystrov *et al.*[e]	−60	−60	120	3.3	300	0.34	0.75	180	0	0
Renugopalakrishnan *et al.*[f]	−120	−60	180	10.9	0	0	0	180	0	0
Pullman *et al.*[h] (C_5)	−180	180	120	3.3	60	0.34	0.75	60	0.34	0.75
Néel *et al.*[g] (C_5)	−160	170	140	6.8	40	0.19	0.41	50	0.26	0.59
Bláha *et al.*[i] (C)	−150	150	150	8.4	30	0.11		30	0.11	
(d) N.m.r. observations [k]				7.5		0.10	0.05		<0.03	0.10
				(±0.1)		(±0.02)	(±0.02)			(±0.02)
Previous work [g]				7.8						

[a] Calculated from Karplus–Bystrov relation with A = 9.4, B = −1.1, C = 0.4 Hz.[3] [b] $A^a(CDCl_3)$ 0.9 Hz. [c] $A^a(D_2O)$ 2.0 Hz. [d] Ref. 35; antiparallel chain β-pleated sheet structure with the sheet twisted to form a left-handed helical unit. [e] Ref. 36. [f] Ref. 44. [g] Ref. 41. [h] Ref. 43. [i] Ref. 42. [j] Ref. 37. [k] Present work.

(C_7^{ax}) or equatorial (C_7^{eq}) relationships to the seven-membered ring (shown in Figure 13 of ref. 36). Extended conformations have also been suggested (C_5 structure[37-41]) particularly for molecules of the *N*-acetyl-L-Ala-NMe type in both polar[36] and non-polar[42] solvents. These conformations have been characterized by n.m.r.[36] and i.r. studies[37-42] and by theoretical calculations;[43-45] the corresponding ϕ,ψ parameters are summarized in Table 3. The results show that a range of ϕ,ψ angles have been postulated for each conformation.

In principle the different conformations can be the Karplus–Bystrov relation[3] and the results in Table 3 compared with the observed value, 7.5 (±0.1) Hz.

The predicted magnitudes of ^5J(HH) vary for the different conformational models [*e.g.*, bond (1), $CDCl_3$ solutions; C_7^{ax} 0—0.11, C_7^{eq} 0.11—0.34, and extended conformations 0—0.34 Hz] though the similar C_7^{ax} structures proposed by Bystrov *et al.*[36] and Renugopalakrishnan *et al.*[44] predict similar ^5J(HH) values for both bonds (1) and (2) of *N*-acetyl-L-Ala-NMe (*ca.* 0 Hz). The observed ^5J(HH) values differ with solvent such that

[36] V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Y. A. Ovchinnikov, *Tetrahedron*, 1969, **25**, 493.
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[42] J. Smolíkova, A. Vítek, and K. Bláha, *Coll. Czech. Chem. Comm.*, 1971, **36**, 2474.
[43] B. Pullman and B. Maigret in ref. 41, p. 13, and references therein.
[44] V. Renugopalakrishnan, S. Nir, and R. Rein, in 'Environmental Effects on Molecular Structure and Properties,' Reidel, Dordecht, 1976, pp. 109—133.
[45] G. N. Ramachandran in ref. 41, p. 1.

the magnitudes for $CDCl_3$ [(1), 0.1; (2), <0.03 Hz] are in the reverse order for D_2O solutions [(1), 0.05; (2), 0.1 Hz]; the values do not conform to those predicted for any conformational model when both bonds (1) and (2) are considered.

It was suggested by Bystrov and his co-workers [36] on the basis of ^3J(HNCH) magnitudes of alanyl dipeptides that an equilibrium exists between the C_7^{ax} (ϕ 60, ψ −60°) and an extended conformation (−60, −60°) with a predominance of the hydrogen-bonded C_7^{ax} form in non-polar solvents ($CDCl_3$; 80—90%) decreasing with increasing polarity of solvent [$(CD_3)_2SO$ 70—80; H_2O, 40—60%]. The observed ^3J(HNCH) and ^5J(HH) values for both bonds (1) and (2) of *N*-acetyl-L-Ala-NMe in $CDCl_3$ solutions are consistent with a preference (*ca.* 80%) of the C_7^{ax} form. On the other hand, the Bystrov model [36] is at complete variance with the results for D_2O solutions; the predicted ^5J[(1)] *ca.* 0.37 and ^5J[(2)] *ca.* 0 Hz are in the reverse order of those observed ^5J[(1)] *ca.* 0.05 and ^5J[(2)] *ca.* 0.1 Hz. A similar conformational model was suggested by Bláha and his co-workers [42] on the basis of i.r. measurements of *N*-acetyl-L-Ala-NMe in tetrachloroethylene solutions at 363 K. The equilibrium between the C_7^{ax} (ϕ 60, ψ −30°; conformation B[42]), C_7^{eq} (−90, 75°; A) and an extended conformation (−150, 150°; C) has the relative proportions of (A + B) *ca.* 36 ± 2% and C *ca.* 60 ± 5%. It was also suggested from n.m.r. measurements [42] that conformation B is more stable than A. Such a conformational equilibrium [assuming all B and no A and assuming $A^a(C_2H_2Cl_4)$ *ca.* A^a ($CDCl_3$) *ca.* 0.9 Hz] predicts magnitudes of ^5J[(1)] or 0.07 and ^5J[(2)] of 0.11 Hz. The results agree with the observed values for bond (1) (0.1 Hz) but not for bond (2) (<0.03 Hz); also ^3J(HNCH) for the equilibrium (8.4—8.7 Hz) is considerably higher than that observed (7.5—7.8 Hz). However, as *N*-acetyl-L-Ala-NMe was previously observed [42] in $C_2H_2Cl_4$ at 363 K, it is not expected that the present observations ($CDCl_3$, 295 K) conform to the same equilibrium. Within the scope of the present measurements the conformational model suggested by Bystrov and his co-workers [36] holds for *N*-acetyl-L-Ala-NMe in non-polar solvents ($CDCl_3$) but not for polar solvents (D_2O).

The observed ^5J[(1)] and ^5J[(2)] values for *N*-acetyl-L-Ala-NMe in aqueous solutions can be interpreted in terms of a unique conformation using the results of potential energy calculations to limit the four possible values calculated for both ϕ and ψ. For example, the observed ^5J[(2)] of 0.1 Hz yields four possible ψ' values corresponding to of 138, −78, −42, and 102° of which ψ *ca.* −42° lies closest to a potential energy minimum region.[43,*] Similarly ^5J[(1)] of 0.05 Hz yields four possible ϕ' and ϕ (−133, 73, 47, −107°) values of which ϕ *ca.* 73° lies closest to a potential energy minimum region. Hence, one possible conformation compatible with both ^5J values and the results of potential energy calculations is an approximate C_7^{ax} conformation with ϕ *ca.* 73 and ψ *ca.* −42°. However, such a conformation would predict ^3J(HNCH, Ala) *ca.* 8.2 Hz which is significantly greater than those values observed for alanyl dipeptides in aqueous solution [43] (corrected J 6.4—7.8 Hz). It is likely that *N*-acetyl-L-Ala-NMe in aqueous solution has considerable flexibility such that the observed ^5J(HH) and ^3J(HNCH) values result from a time-average of a number of different conformations (probably mainly extended) which are populated to different extents.

* Calculated ϕ, ψ plots in ref. 43 are presented in terms of the nomenclature by Edsall *et. al.*[46] whereas the present results are denoted in the IUPAC-IUB standard nomenclature.[47]

TABLE 4

Comparison of β-turn crystal conformations

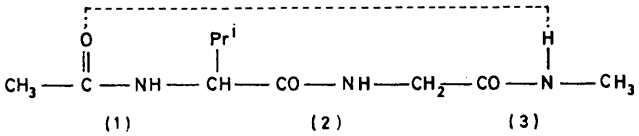
	Peptide torsion angles [a]					
	Bond (1)		Bond (2)		Bond (3)	
Crystal structures	ψ_1 (°)	ϕ_2 (°)	ψ_2 (°)	ϕ_3 (°)	ψ_3 (°)	ϕ_4 (°)
(a) Type I (LL)						
Oxytocin (*C*-terminal tetrapeptide) [b]	−68	−66	−29	−115	13	−95
Gly-Pro-Leu-Gly [c]	171	−63	−29	−106	15	−106
Cyclohexaglycyl (1 → 4) [d]	−170	−69	−29	−94	8	−114
Cyclohexaglycyl (4 → 1) [d]	−175	−69	−30	−92	4	−121
Lysozyme [e]						
Residue 55—56	172	−46	−35	−108	10	53
Residue 96—97	−39	−63	−51	−75	−18	−89
Residue 123—124	−19	−64	−38	−97	−4	−70
Antamanide [f]						
Pro-Ala-Phe-Phe | | Pro-Phe-Phe-Val	148	−69	−13	−84	−6	−123
	145	−79	−13	−90	8	−115
Mean [i]		−65 (±6)	−30 (±8)	−96 (±10)	3 (±8)	
(b) Type II (LD)						
Ferrichrome A [g]		−57	132	82	−1	
Valinomycin-K^+ Complex [h] ($\bar{3}$)	−18	−59	132	82	2	58
Valinomycin (Karle *et al.*) [i] ($\bar{4}$)	1	−66	130	89	0	63
Valinomycin (Smith *et al.*) [j] ($\bar{6}$)	0	−65	130	88	−1	64
Mean [i]	−4 (±10)	−63 (±4)	131 (±1)	86 (±7)	0 (±5)	62 (±4)
(c) Type II′ (DL)						
Valinomycin-K^+ Complex [h] ($\bar{3}$)	2	58	−131	−72	−18	−59
Valinomycin (Karle *et al.*) [i] ($\bar{4}$)	0	63	−134	−86	1	−66
Valinomycin (Smith *et al.*) [j] ($\bar{6}$)	−1	64	−135	−86	0	−65
Gramicidin S [k]		60	−137	−75	−18	
Mean [i]	0 (±5)	61 (±2)	−134 (±2)	−80 (±6)	−9 (±9)	−64 (±3)

[a] All peptide conformation angles (ϕ, ψ) presented in terms of I.U.P.A.C.–I.U.B. standard nomenclature.[47] [b] Ref. 49. [c] Ref. 50. [d] Ref. 51. [e] Ref. 52. [f] Refs. 54 and 55. [g] Ref. 56. [h] Ref. 57; average values of three similar torsion angles given, ($\bar{3}$). [i] Ref. 58; average values of four angles (two values for structures I and II) ($\bar{4}$). [j] Ref. 59; average values of six angles (two values for structures A, B_1, B_2) ($\bar{6}$). [k] Ref. 54. [i] Mean values weighted by including all angles for each structure.

[46] J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, S. Némethy, G. N. Ramachandran, and H. A. Scheraga, *J. Mol. Biol.*, 1966, **15**, 399.

[47] J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Némethy, D. C. Phillips, G. N. Ramachandran, and H. A. Scheraga, *Biochemistry*, 1970, **9**, 3471.

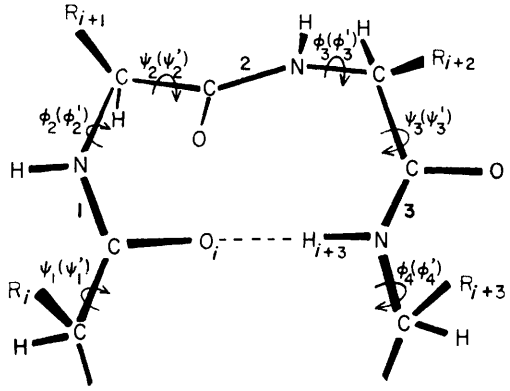
(iii) β-*Turn:* N-*Acetyl*-L-*Val-Gly-methylamide* (*DMSO Solution*).—The molecular formula of *N*-acetyl-L-Val-Gly-NMe consists of three amide or peptide bonds which are labelled (1)—(3). N.m.r. studies [28] indicate that a hydrogen bond exists between the acetyl carbonyl



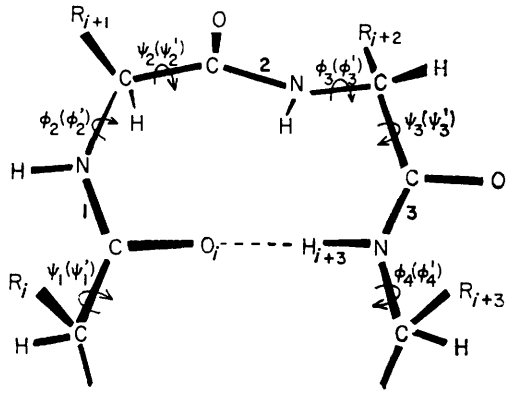
group and the amide amino-group to form a ten-membered ring (β-turn). A number of β-turn conformations have been suggested from theoretical considerations [48] (*e.g.*, Types I, II, and II′) and the present work investigates the role of ^5J(HH) and ^3J(HNCH) in discriminating between these conformations by n.m.r. spectroscopy.

The β-turn conformations defined by Venkatachalam [48] are investigated from the results of structures of a number of linear and cyclic peptides; [49-56] in particular, Figure. The different β-turn conformations depend on the configurations of residues $i + 1$ and $i + 2$ *viz.* Type I (LL), Type II (LD), and Type II′ (DL). The crystal structure results in Table 4 indicate that molecules within a β-turn type exhibit similar values of peptide angles for residues $i + 1$ (ϕ_2,ψ_2) and $i + 2$ (ϕ_3,ψ_3) and that the mean values of angles are characteristic of each conformer type. These angles are compared with those determined by theoretical calculation,[48] and the results of solution studies.[60,61] It can be seen, from the data listed in Table 5, that within the average error limits for each angle determined from crystal structures (except ψ_2 for Types II and II′), the mean values are the same as those proposed originally [48] by theoretical considerations.

Magnitudes of ^5J(HH) are predicted for each conformational model. In order to compare results for each peptide bond, calculations were performed for molecules of the type *N*-acetyl-X-Y-*N*-methylamide where X and Y are amino-acids. In these molecules it is likely that free rotation occurs about the C–CH$_3$ [bond (1), ψ_1'] and N–CH$_3$ [bond (3), ϕ_4'] bonds which is confirmed for ϕ_4' by the value of ^3J(HNCH$_3$) of 4.5 Hz for *N*-acetyl-L-Val-Gly-NMe observed in this work. It is found that ^5J(HH) predicted from theoretical calculations and crystal structure average conformations each exhibit similar magnitudes for peptide bonds (1) (DMSO, 0.30—0.34; D_2O, 0.67—0.75 Hz), (2) (0—0.06; 0—0.12 Hz), and (3) (0.27—0.36; 0.60—0.79 Hz) for



Type I β-Turn



Type II β-Turn

Definition of β-turn: Type I β-turn, residues $i + 1$ and $i + 2$ both of L configuration; Type II β-turn, residue $i + 1$ of L configuration and residue $i + 2$ glycine or of D configuration; Type II′ β-turn, residue $i + 1$ glycine or of D configuration and residue $i + 2$ of L configuration

recent determinations of valinomycin [57-59] and the valinomycin–K^+ complex [14,57] enable Types II and II′ β-turns to be characterized. The peptide torsion angles (ϕ,ψ) defining the different β-turn conformations are compared in Table 4. The definitions of β-turn conformations (Types I, II, and II′) and the corresponding peptide bonds (1)—(3), peptide torsional angles (ϕ,ψ), and homoallylic angles (ϕ',ψ') are shown in the

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[49] (*a*) A. D. Rudko, F. M. Lovell, and B. W. Low, *Nature, New Biol.*, 1971, **232**, 18; (*b*) D. W. Urry and R. Walter, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 956.
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[60] G. Boussard, M. Marraud, and J. Néel, *J. Chim. phys.*, 1974, **71**, 1081.
[61] M. A. Khaled, V. Renugopalakrishnan, and D. W. Urry, *J. Amer. Chem. Soc.*, 1976, **98**, 7547.

the different conformational types (I, II, II') in the same solvent. However, the small predicted differences in ^5J(HH) for bond (2) are sufficient to differentiate Types I (DMSO, 0.04—0.06; D_2O, 0.08—0.12 Hz) and II (0—0.01; 0—0.03 Hz) particularly if the $i + 2$ residue is glycine. The results indicate that Types II and II' cannot be differentiated by ^5J(HH) observations for conformations defined by theory and crystal structure averages. The major variation occurs for Type II conformational models between theory [48] and crystal structure average, on the one hand, and solution conformations determined by spectroscopy,[60,61] on the other hand. The differences are most marked for bond (3) values of ^5J(HH) could only be evaluated on irradiation of the methylene protons and observation of line-width changes of α-CH (Val) [bond (2)] and N-CH_3 [bond (3)]. In each case the sum of ^5J(HH) is observed. Results for bond (2) for both solvents are consistent with Type II rather than Type I conformations whereas the results for bond (3) ([$^2H_6$]DMSO, 0.5 Hz) are closer to the solution conformational models [60,61] (ψ_3 30—40°; ^5J 0.49—0.56 Hz) rather than crystal structure average and theoretical conformations (ψ_3 0, ^5J 0.68 Hz). On the other hand, the observed ^5J(HH) of 0.7 Hz for bond (3) of *N*-acetyl-L-Val-Gly-NMe in D_2O solution differs from the predicted values (Boussard *et al.*,[60] 1.09;

TABLE 5

Comparison of observed and predicted ^5J(HH) for β-turn conformations of CH_3-CO-NH-CHR-CO-NH-CHR'-CO-NMe

	Peptide angles				Homoallylic torsion angles and $^5J_{calc}$								
					(1)			(2)				(3)		
β-Turn	(1) ϕ_2 (°)	(2) ψ_2 (°)	(2) ϕ_3 (°)	(3) ψ_3 (°)	ϕ_2' (°)	^5J(DMSO)/ Hz	^5J(D_2O)/ Hz	ψ_2' (°)	ϕ_3' (°)	^5J(DMSO)/ Hz	^5J(D_2O)/ Hz	ψ_3' (°)	^5J(DMSO)/ Hz	^5J(D_2O)/ Hz
Type I (LL)					L			L	L			L		
Theory (Venkatachalam [48])	−60	−30	−90	0	300	0.34	0.75	210	330	0.06	0.12	240	0.34	0.75
Crystal structure average [a]	−65	−30	−96	3	305	0.30	0.67	210	336	0.04	0.08	243	0.36	0.79
Gly (D proton)									(216) [d]	(0.08) [d]	(0.17) [d]	(123) [d]	(0.32) [d]	(0.70) [d]
Gly = ^5J(L) + ^5J(D)										0.12	0.25		0.68	1.49
Type II (LD)					L			L	D			D		
Theory (Venkatachalam [48])	−60	120	80	0	300	0.34	0.75	0	40	0	0	120	0.34	0.75
Crystal structure average [a]	−63	131	86	0	303	0.31	0.70	11	34	0.01	0.02	120	0.34	0.75
Gly (L proton)									(154) [d]	(0) [d]	(0.01) [d]	(120) [d]	(0.34) [d]	(0.75) [d]
Gly = ^5J(L) + ^5J(D)										0.01	0.03		0.68	1.50
Type II' (DL)					D			D	L			L		
Theory (Venkatachalam [48])	60	−120	−80	0	60	0.34	0.75	0	320	0	0	240	0.34	0.75
Crystal structure average [a]	61	−134	−80	−9	59	0.33	0.73	346	320	0.02	0.05	231	0.27	0.60
Type II solution structures								L	D			D		
Boussard *et al.*[b] (i.r.)	−60	120	60	40	300	0.34	0.75	0	60	0	0	160	0.05	0.12
Gly (L proton)									(180) [d]	(0) [d]	(0) [d]	(280) [d]	(0.44) [d]	(0.97) [d]
Gly = ^5J(L) + ^5J(D)										0	0		0.49	1.09
Khaled *et al.*[c] (Theory and n.m.r.)	−60	120	55	30	300	0.34	0.75	0	65	0	0	150	0.11	0.25
Gly (L proton)									(185) [d]	(0) [d]	(0) [d]	(270) [d]	(0.45) [d]	(1.00) [d]
Gly = ^5J(L) + ^5J(D)										0	0		0.56	1.25
N-Acetyl-L-Val-Gly-NMe (n.m.r., this work)						0.10 (±0.02)	0.15 (±0.02)			<0.03	<0.03		0.50 (±0.02)	0.70 (±0.02)

[a] Table 4, this work. [b] Ref. 60. [c] Ref. 61. [d] Calculations performed for other proton of Gly residue (R' = H) using crystal structure average data.

when a glycine residue is present. The results are compared with those observed for *N*-acetyl-L-Val-Gly-NMe in [$^2H_6$]DMSO and D_2O solutions.

In principle, the inclusion of a glycine residue in a β-turn enables Types I and II and the different Type II conformational models to be differentiated if ^5J(HH) and ^3J(HNCH) can be observed for non-equivalent glycine methylene protons. For example differences occur between ^5J(HH) of Types I and II conformations and in the observed sums of ^5J(L) and ^5J(D) for those cases where the glycine methylene protons exhibit magnetic equivalence or where the individual ^5J(HH) for each methylene proton cannot be observed. The different Type II conformations can be differentiated in a similar manner by measurements of ^5J(HH) for bond (3).

Although the glycine methylene group of *N*-acetyl-L-Val-Gly-NMe exhibits magnetic non-equivalence,[28] Khaled *et al.*,[61] 1.25 Hz; crystal structure average, 1.5 Hz) and suggests that the same conformations are not found in [$^2H_6$]DMSO and D_2O solutions. It is likely that the 1,4-type hydrogen bonding is less stable in aqueous solutions and so ^5J(HH) depends on a number of different conformations populated to different extents rather than from a unique conformation.

No ^5J(HH) was observed for bond (2) of *N*-acetyl-L-Val-Gly-NMe in either [$^2H_6$]DMSO or D_2O solutions which is consistent with all Type II conformational models, *i.e.*, ψ_2 120—130, ϕ_3 50—80°. The observed ^3J(HNCH) of 7 and 4 Hz for the glycine residue in [$^2H_6$]DMSO solutions are consistent with ϕ_3 *ca.* 55—60° with the smaller coupling on the upfield glycine methylene signal (ψ_3 *ca.* 30—40°) as this proton is closer to the plane of the peptide carbonyl group.[61-63]

The observed ^5J(HH) for bond (1) of *N*-acetyl-L-Val-

[62] G. J. Karabatsos, G. C. Sonnichsen, H. Nsi, and D. J. Fenoglio, *J. Amer. Chem. Soc.*, 1967, **89**, 5067.

[63] J. W. ApSimmon, P. V. Demarco, D. W. Mathieson, W. S. Craig, A. Karim, L. Saunders, and W. B. Whalley, *Tetrahedron*, 1970, **26**, 119.

Gly-NMe does not conform to any of the conformational models which all predict the same values. It is expected that the C–CH_3 bond exhibits free rotation so that ^5J(HH) depends only on ϕ_2; hence there are two parameters [^3J(HNCH) and ^5J(HH)] which must be satisfied by ϕ_2. The observed ^3J(HNCH) L-Val of 6 Hz predicts ^5J(HH) of 0.17 Hz according to equations (7) and (4) with D 9.8 Hz and E 0.* The value is somewhat greater than that observed (0.10 Hz, DMSO) but not far greater than the error limits involved in the determination of ^5J(HH) and calibration of A^a (Table 2). On the other hand the observed ^5J(HH) of 0.15 Hz for *N*-acetyl-L-acetyl-L-Val-Gly-NMe in D_2O solutions suggests that different conformations exist in DMSO and D_2O solutions with greater flexibility existing in aqueous solutions.

Conclusion.—Five-bond long range coupling has been observed across amide and peptide bonds. The coupling was analysed in terms of homoallylic coupling previously characterized for cyclic dipeptides and so depends on the peptide conformational angles (ϕ and ψ). The homoallylic coupling parameter for *trans* peptide bonds (A^a, antiperiplanar groups) was calibrated for various solvents ($CDCl_3$, CD_3OD, DMSO, and D_2O) using *N*-methylacetamide and *NN*-dimethylacetamide as model compounds. Analysis of ^5J(HH) observed between α-CH groups of adjacent amino-acids in linear peptides was used, together with ^3J(HNCH) magnitudes when available, to determine the conformations of peptides in solution. The method is illustrated for two peptide conformations (C_7 structure and β-turns) which have been intensively studied previously and for which various conformational models exist. The scope of using ^5J(HH) to differentiate the various conformational models is discussed.

* These values were adapted from the Karplus–Bystrov relation (A = 9.4, B = −1.1, and C = 0 Hz).[3]

Measurements were made on *N*-acetyl-L-Ala-NMe and *N*-acetyl-L-Val-Gly-NMe which were expected to exhibit the C_7 structure and β-turn, respectively. The results show that *N*-acetyl-L-Ala-NMe in $CDCl_3$ or D_2O solutions does not exist in the conformation observed in the solid state. Previous solution models by Bystrov *et al.*[3] have suggested a conformational equilibrium between a hydrogen-bonded C_7^{ax} structure (ϕ 60, ψ −60°) and an extended conformation (−60, −60°) with a predominant C_7^{ax} structure (*ca.* 80%) in $CDCl_3$ decreasing to *ca.* 50% in D_2O. The results for *N*-acetyl-L-Ala-NMe are consistent with this conformational model proposed for non-polar solvents ($CDCl_3$) but not for polar solvents (D_2O).

The different β-turn conformations (Types I, II, and II′) were characterized by averaging the peptide torsional angles observed in a number of recent crystal structure analyses. It was shown that Type I and II conformations may be differentiated by observation of ^5J(HH) between α-CH groups across bond (2), particularly if the $i + 2$ residue is glycine, and that distinctions can be made between different Type II conformational models by observations of ^5J(HH) for groups across bond (3). The results for *N*-acetyl-L-Val-Gly-NMe suggest that a Type II β-turn exists in [$^2H_6$]DMSO solution with conformational characteristics similar to the models suggested by solution studies[60,61] (ϕ_3 55—60, ψ_3 30—40°) rather than crystal structure averages or theoretical considerations.[48] The results for aqueous solutions again indicate that quite different conformations exist in which the structure, stabilized by hydrogen bonds, is less significant than in [$^2H_6$]DMSO solutions.

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