Proton Magnetic Resonance and Conformational Energy Calculations of Repeat Peptides of Tropoelastin: the Pentapeptide

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The detailed conformation of a repeating pentapeptide segment, HCO-L-Val_1-L-Pro_2-Gly_3-L-Val_4-Gly_5-OMe, of tropoelastin has been investigated using theoretical conformational energy calculations and ^1H n.m.r. studies in $CDCl_3$. Theoretical conformational energy calculations suggest the existence of two broad classes of conformations. One class of conformations (A) is stabilized by a Type II β-turn, involving the Val_4 NH and the Val_1 C=O, a 14-membered hydrogen bonded ring between the Val_1 NH and the Val_4 C=O, and an 11-membered hydrogen bonded system, called a γ-turn, between the Gly_3 NH and the Gly_5 C=O. The second class of conformations (B) is stabilized by the same Type II β-turn and 11-membered hydrogen bonded ring and by a seven-membered hydrogen bonded ring between the Gly_5 NH and the Gly_3 C=O. The theoretical results correlate reasonably well with torsion angles derived from $^3J_{C^\alpha_{H-NH}}$ coupling constants obtained in the ^1H n.m.r. studies. Temperature dependence and solvent perturbation of NH proton chemical shifts support the above intramolecular hydrogen bonds.

ELASTIN is a major protein component of the vascular wall and contributes elastic properties necessary for its biological function. In order to understand the integrity and breakdown of the vascular wall, knowledge of the detailed molecular structure of elastin and the relationship of this to its biological role are required. As a first step towards this goal, Gray, Sandberg, and their co-workers [1,2] have shown tropoelastin, the soluble precursor protein,[3-6] to consist of repeating sequences, a tetrapeptide VPGG (L-Val_1-L-Pro_2-Gly_3-Gly_4), a pentapeptide VPGVG (L-Val_1-L-Pro_2-Gly_3-L-Val_4-Gly_5), and a hexapeptide APGVGV (L-Ala_1-L-Pro_2-Gly_3-L-Val_4-Gly_5-L-Val_6). The repeat peptides, their oligomers, and high polymers have been synthesized in this laboratory.[7-9] Conformational analyses based on temperature coefficient [10-13] and solvent perturbation [14-16] methods have

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demonstrated the presence of a β-turn, a 10-membered hydrogen bonded system, as well as additional secondary structural features in each monomeric unit and their polymers.[17-19] Recently, a detailed analysis of the conformation of the tetrapeptide VPGG based on ^{1}H n.m.r. coupling constant data in $CDCl_3$ and conformational energy calculations was reported.[20]

A two-fold approach based on conformational energy calculations and ^{1}H n.m.r. is well suited for conformational analysis of molecules.[21-27] Conformational energy calculations provide information on all possible conformational states in which a molecule can exist. On the other hand, ^{1}H n.m.r. provides information on a time-averaged conformational state. Both techniques may be compared in terms of the torsion angles necessary for a description of the conformation. A combined approach of conformational energy calculations and ^{1}H n.m.r. was found to be particularly successful for the valinomycin–K^+ complex.[27] The *X*-ray structure of the valinomycin–K^+ complex [28] is in satisfactory agreement with the results of combined analysis of ^{1}H n.m.r. and conformational energy calculations.[27]

Although theoretical conformational analysis is presently being extended to include environmental effects such as solvation,[29-36] such methods are as yet too complicated for an application to a molecule of the size of the pentapeptide VPGVG. Nevertheless, conformational energy calculations *in vacuo* have been related to approximate conformations observed in solvents of low polarity and such calculations provide much useful information.[36] In our present efforts conformational energy calculations for elastin peptides *in vacuo* are compared with ^{1}H n.m.r. studies of the peptides in a low polarity solvent, $CDCl_3$. In $CDCl_3$, ABX spin patterns are observed for the glycine α-CH_2 groups allowing for estimates of all C$^\alpha$H–NH torsion angles by means of ^3J coupling constants.

The β-turn, a 4 ⟶ 1 hydrogen bonded conformation,[37,38] was shown to be a dominant conformational feature in the repeat pentapeptide.[8,17] Other additional secondary structural features such as an 11-membered hydrogen bonded system involving the Gly_3 NH and Gly_5 C=O,[17] called a γ-turn, and in water at higher temperatures a Val_1 NH · · · O=C Val_4 hydrogen bonded system were shown to be present.[19] Recently, nuclear Overhauser enhancement measurements [39] and conformational energy calculations [40] also demonstrated the β-turn to be Type II. In the present study a ^{1}H n.m.r. analysis of the secondary structure of VPGVG in $CDCl_3$ is presented and values of all C$^\alpha$H–NH torsion angles are calculated from Karplus-like equations.

Conformational energy calculations describe the low energy *in vacuo* conformations which are presented in terms of torsion angle energy diagrams and profiles; these data are then compared to the n.m.r. derived preferred secondary structure and C$^\alpha$H–NH dihedral angles. A favourable comparison of these conformational details, along with Dreiding model approximated C$^\alpha$–C′ torsion angles obtained with the secondary structure and C$^\alpha$H–NH restrictions, provide for confidence in the conformations derived by theoretical means.

EXPERIMENTAL

The repeat pentapeptide of elastin, HCO-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe, was synthesized in this laboratory.[8] ^{1}H N.m.r. spectra were obtained in $CDCl_3$ at a concentration (0.05M) where no effective association was observed. To facilitate spectral analysis, 20% v/v C_6D_6 was added (see Figure 1a and b). The measurements were taken with a Varian HR-220 spectrometer operating at a probe temperature of 21 °C and equipped with an SS-100 computer system. Simulated spectra were obtained by using a Varian data machine spin simulation program. All the double resonance experiments were performed on a JEOL PS-100 spectrometer operating at a probe temperature of 22 °C and in the internal lock mode. Variable temperature experiments were made with the PS-100 spectrometer equipped with a JEOL JNM VT-3B temperature controller.

METHODS

Conformational Energy Calculations.—Total conformational energy was calculated, employing computer programs,[41] using a partitioned potential energy method

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consisting of van der Waals, electrostatic, torsion, and hydrogen bond energies [equation (1)]. $E_{\text{van der Waals}}$ was

$$E_{\text{Total}} = E_{\text{van der Waals}} + E_{\text{electrostatic}} + E_{\text{torsion}} + E_{\text{H-bonding}} \quad (1)$$

calculated using ' 6-exp ' potential function with parameters suggested by Ramachandran and Sasisekharan.[42] $E_{\text{electrostatic}}$ was calculated assuming a dielectric constant of unity, up to the monopole term [43] and using *ab initio* minimal basis set (STO-3G) net charges for *N*-formyl, glycyl, L-valyl, and L-prolyl residues.[44,45] The net charges for VPGVG were assembled from those of the fragments, taking care to preserve overall electroneutrality of the peptide. E_{torsion} was calculated using a three-fold torsional potential with torsional barriers of 0.6 and 0.2 kcal mol^{-1} [46] for the C$^{\alpha}$–N and C$^{\alpha}$–C′ bonds, respectively. Calculation of $E_{\text{H-bonding}}$ was performed in the same manner as described earlier.[20] It was assumed that the empirical potential function for hydrogen bonding taken from Ramachandran *et al.*[47] is applicable to 11- and 14-membered hydrogen-bonded systems as well as to 10-membered hydrogen bonded ring systems.

A fully extended conformation of VPGVG was constructed as described earlier.[40] The *N*-formyl end group was constructed with C′–N, C=O, and C–H bond lengths of 1.32, 1.24, and 1.09 Å, respectively, and with N–C′=O and N–C′–H bond angles of 125 and 115°, respectively.[48] The methoxy-group was constructed with a bond length of 1.42 Å for the C–O bond and a bond angle of 110° for the C–O–C bond angle.[48] The methyl group was assumed to be staggered about the C–O–C plane. Glycyl, valyl, and prolyl residues were constructed as described earlier.[20,40] The torsion angles necessary for a description of the backbone peptide chain of VPGVG are shown in Figure 5 where the ϕ_1, ψ_1 torsion angles are designated for Val_1; ϕ_2, ψ_2 for Pro_2; ϕ_3, ψ_3 for Gly_3; ϕ_4, ψ_4 for Val_4; and ϕ_5, ψ_5 for Gly_5. Rotations, ϕ and ψ, around the single bonds, *i.e.*, N–C$^{\alpha}$ and C$^{\alpha}$–C′ in the pentapeptide were systematically performed, starting with the co-ordinates of the fully extended conformation with the exception of ϕ_2 being restricted to $-60°$. As a result of rigidity of the pyrrolidine ring, the conformational energy of VPGVG is a function of nine torsion angles ϕ_1, ψ_1, ψ_2, ϕ_3, ψ_3, ϕ_4, ψ_4, ϕ_5, ψ_5, not taking into account the torsion angles necessary for describing the orientation of valyl and prolyl side chains. In an initial search for allowed conformations with the valyl side chains fixed in the trans conformation and ϕ_2 set at $-60°$, all combinations of the ϕ and ψ angles which do not violate the van der Waals criteria * were calculated at 40° intervals in order to evaluate conformational energy in a coarse grid over allowed configuration space. The low energy regions in configuration space were then examined at 20 and finally 10° intervals to obtain details of the minima. The low energy conformations were then minimized by allowing the peptide to become non-planar and the C′–C$^{\alpha}$–N bond angle to be relaxed. The resultant values are given in Table 3. Finally the valyl side chains were rotated about the C$^{\alpha}$–C$^{\beta}$ bond to find the preferred χ^1 angles. The results from the conformational energy calculations are represented by ϕ–ψ contour energy maps in which the remainder of the molecule was held in the low energy conformation while a given ϕ, ψ pair were rotated.

The expected coupling constants, $\langle {}^3J_{C^{\alpha}H-NH} \rangle$, were calculated [27] from the (ϕ_1, ψ_1), (ϕ_3, ψ_3), (ϕ_4, ψ_4), and (ϕ_5, ψ_5) conformational energy maps presented in Figure 3a—d using expression (2) where J_{ϕ} is calculated using a Karplus-like relation with the coefficients of Bystrov *et al.*[49] (see

$$\langle J_{C^{\alpha}H-NH} \rangle = \sum_{\phi} J_{\phi} e^{-E_{\phi}/RT} / \sum_{\phi} e^{-E_{\phi}/RT} \quad (2)$$

ref. 20 for the relevant expressions). The Abraham–McLauchlan equation (3) [50] was used for calculation of the

$$J_{\theta} = \begin{cases} 10.5 \text{ Hz } \cos^2 \theta - 0.28 \text{ Hz } (0—90°) \\ 13.7 \text{ Hz } \cos^2 \theta - 0.28 \text{ Hz } (90—180°) \end{cases} \quad (3)$$

$J_{C^{\alpha}H-C^{\beta}H}$ coupling constants where θ is related to χ^1. The angle θ ranges from 0 to $\pm 180°$ whereas χ^1 ranges from 0 to 360°.

RESULTS

^{1}H *N.m.r.*—The n.m.r. spectrum of the expanded α-CH region of HCO-VPGVG-OMe obtained in $CDCl_3$ is shown in Figure 1a. The assignments of all the signals were achieved by observing the fine structure, by comparing the spectrum of HCO-VPGVG-OMe with that of Boc-VPGVG-ONp in $CDCl_3$, and by double resonance experiments. The chemical shifts of the Val NH at δ 7.41 and of the Gly NH at δ 7.96 are shifted to δ 5.52 and 8.84, respectively, on going from HCO-VPGVG-OMe to Boc-VPGVG-ONp whereas the second Gly NH at δ 7.86 and the second Val NH at δ 7.89 (see Table 1) are shifted no more than 0.01 p.p.m. Since the terminal blocking groups affect the chemical shifts of the terminal amino-acid residues, the NH signals at δ 7.41 (d) and 7.96br (t) were assigned to Val_1 NH and Gly_5 NH, respectively, for HCO-VPGVG-OMe. The other NH signals at δ 7.39 (d) and 7.86br (t) were assigned to Val_4 NH and Gly_3 NH, respectively.

The α-CH signals, labelled in Figure 1, were assigned by

* For an L-valyl residue in *N*-acetyl-L-valine-*N*-methylamide with χ^1 180°, a search of allowed or permissible conformations at 40° intervals for ϕ and ψ reveals that only five or at most six out of a total of 81 conformations are allowed on the basis of the ' hard-sphere ' approximation. In this case the ' hard-sphere ' approximation rules out a significant number of conformations due to close contact between the atoms in the side chain with the backbone. The conformation of a valyl residue in a peptide will be further restricted by the ' hard-sphere ' approximation due to close contact with the neighbouring residues, *i.e.* inter-residue contacts. During the initial scan at 40° intervals for a total of nine torsion angles, 645 321 conformations were found to be allowed on the basis of the ' hard-sphere ' approximation and therefore the energies of this number of conformations were calculated.

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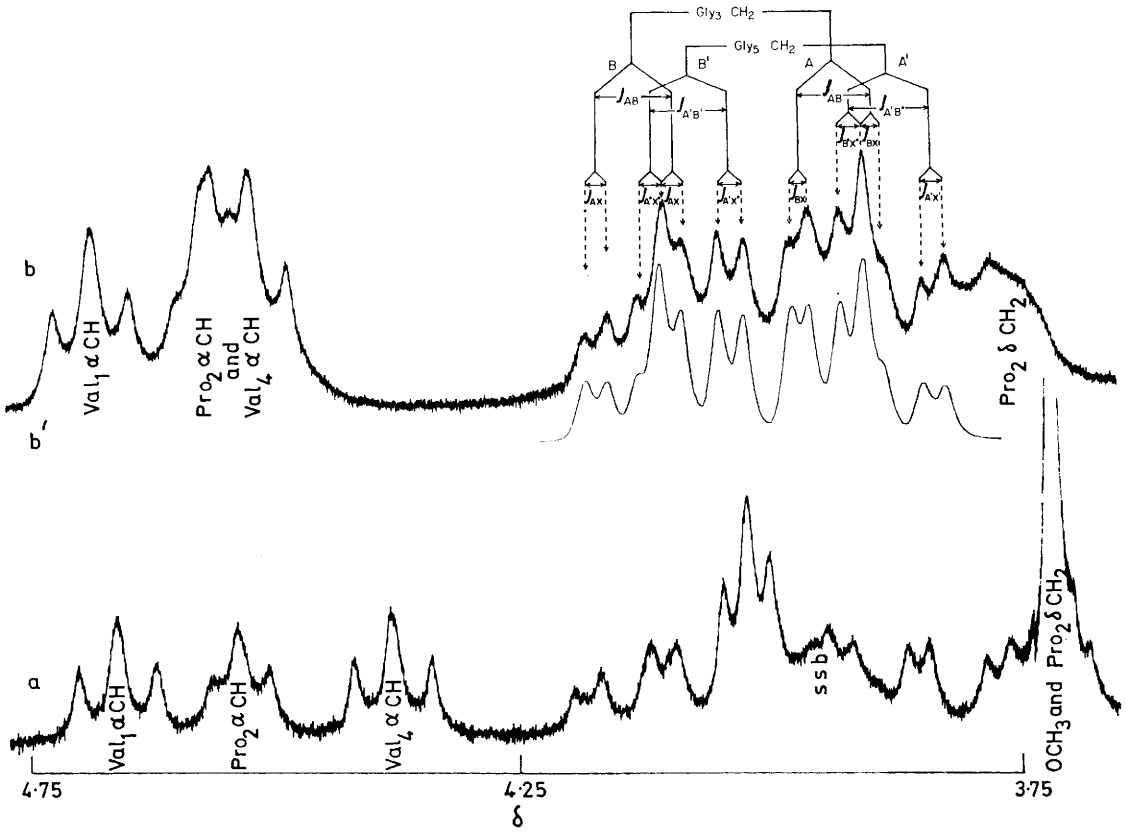

FIGURE 1 220 MHz N.m.r. spectra of HCO-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe (α-CH region only): a, in $CDCl_3$; b, 80% $CDCl_3$–20% C_6D_6 (v/v); b′, computer simulated spectrum of Gly_3 and Gly_5 CH_2 protons (analysed as an ABX spin system).

TABLE 1

N.m.r. Parameters for HCO-VPGVG-OMe in $CDCl_3$

Amino-acid residues	Proton(s)	δ (±0.01)	$^3J_{C^\alpha H-NH}$	$^3J_{C^\alpha H-C^\beta H}$	$^3J_{C^\beta H-C^\gamma H}$
HCO	CHO	8.16 (s)			
L-Val_1	1_γ-CH_3	0.89 (d)			7.0 ± 0.1
	2_γ-CH_3	0.89 (d)			7.0 ± 0.1
	β-CH	2.75 (m)		8.5 ± 0.1	7.0 ± 0.1
	α-CH	4.66 (pt)	9.0 ± 0.1	8.5 ± 0.1	
	NH	7.41 (d)	9.0 ± 0.1		
L-Val_4	1_γ-CH_3	0.97 (d)			7.0 ± 0.1
	2_γ-CH_3	1.08 (d)			7.0 ± 0.1
	β-CH	2.75 (m)		9.0 ± 0.1	7.0 ± 0.1
	α-CH	4.38 (pt)	9.5 ± 0.1	9.0 ± 0.1	
	NH	7.39 (d)	9.5 ± 0.1		
L-Pro_2	γ- and β-CH_2	1.8—2.2 (m)		*a*	*a*
	β-CH_2	*b*			
	α-CH	4.51br (t)			
				^2J/Gly CH_2	
Gly_3	α-CH(A)	3.80	4.5 [c]	−17.0	
	α-CH(B)	4.16	5.5 [c]	−17.0	
	NH(X)	786br (t)			
Gly_5	α-CH(A′)	3.99	5.5 [c]	−17.8	
	α-CH(B′)	4.07	5.5 [c]	−17.8	
	NH(X′)	796br (t)			
	CH_3	3.72 (s)			

[a] Not analysed. [b] Overlapped with OMe signal. [c] Values obtained by an ABX spin analysis of CH_2 signal. t, triplet; d, doublet; s, singlet; pt, pseudo triplet; m, multiplet.

irradiating the corresponding NH signals. The two methylene groups (Gly_3 CH_2 and Gly_5 CH_2) appeared as ABX spin patterns which partially overlapped (see Figure 1a). In order to obtain a simplified spectrum for methylene protons, 20% C_6D_6 (v/v) was added. The spectrum thus obtained is shown in Figure 1b where it can be seen that all 16 lines for two ABX spin systems, designated as ABX for Gly_3 CH_2 and as A'B'X' for Gly_5 CH_2 protons, are readily resolvable.

able coupling constants nor the temperature dependences of chemical shift of the peptide NH protons it is reasonable to assume that C_6D_6 does not alter the conformation. The values for the chemical shifts and coupling constants are given in Table 1.

The non-equivalent nature of the two glycine (Gly_3 and Gly_5) methylene protons is an indication of a constrained conformation.[20,51,52] Since the temperature dependence of peptide NH protons provides information regarding the secondary structure,[11,13,53,54] the temperature profiles for all the NH protons of VPGVG in $CDCl_3$ were obtained for the temperature range −30 to +60 °C. The temperature coefficients ($d\delta/dT$) are 0.006 8, 0.005 1, 0.003 8, and 0.009 3 p.p.m. $°C^{-1}$ for the Val_1 NH, Gly_3 NH, Val_4 NH, and Gly_5 NH protons, respectively. Studies of temperature coefficients of peptide NH chemical shifts in $CDCl_3$ using well characterized cyclic systems give values for solvent exposed

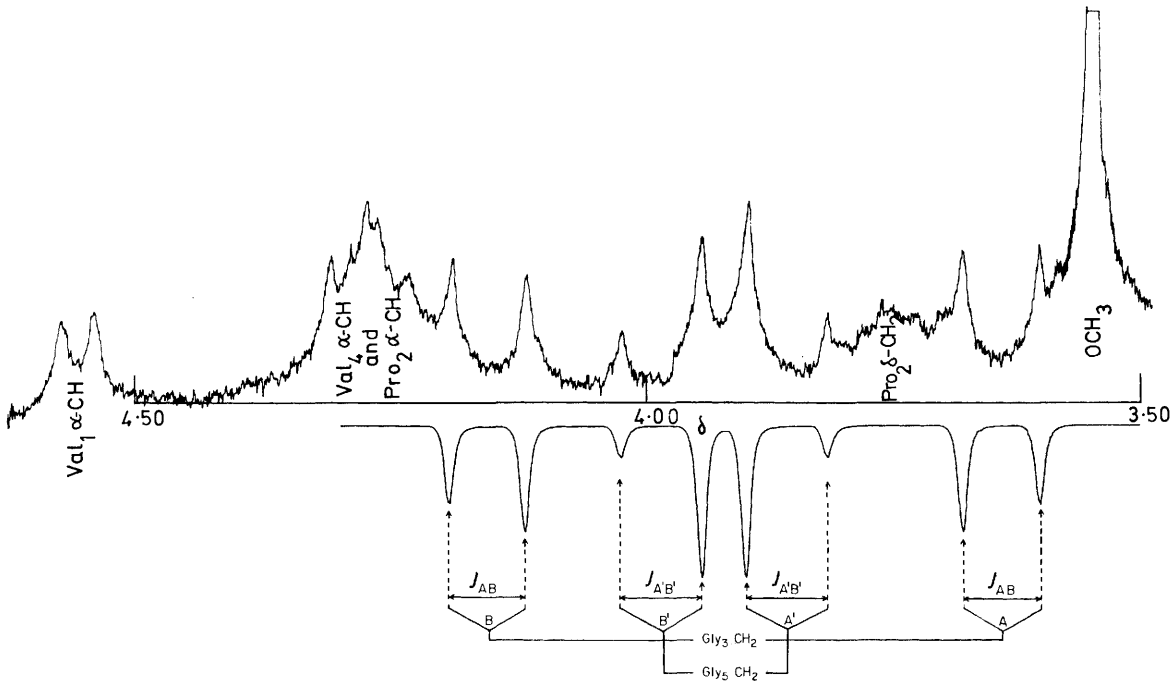

FIGURE 2 220 MHz N.m.r. spectrum of HCO-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe (pre-deuteriated to remove α-CH–NH couplings) in 70% $CDCl_3$–30% C_6D_6. The α-CH region is shown with the computer simulated spectrum for Gly_3 and Gly_5 CH_2 protons (analysed as an AB spin system)

TABLE 2

Backbone torsion angles [a] of HCO-VPGVG-OMe obtained from n.m.r. and conformational energy calculations

	L-Val_1		L-Pro_2		Gly_3		L-Val_4		Gly_5	
Method	ϕ_1(°)	ψ_1(°)	ϕ_2(°)	ψ_2(°)	ϕ_3(°)	ψ_3(°)	ϕ_4(°)	ψ_4(°)	ϕ_5(°)	ψ_5(°)
H^1 N.m.r.	−150	150 [b]	−60 [b]	120 [b]	60	30 [b]	−140	120 [b]	65	±170 [b]
Conformational Energy Calculations										
Conformation A	−140	170	−60	120	80	40	−160	140	70	−170
Conformation B	−130	30	−60	120	80	40	−10	−20	70	−170

[a] Torsion angles are given in accordance with IUPAC–IUB convention (J. C. Kendrew, S. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, D. C. Phillips, G. N. Ramachandran, and H. A. Scheraga, *Biochemistry*, 1970, **9**, 3471). [b] Angles were obtained from the Dreiding model of VPGVG which had the ϕ values fixed and the further restrictions of the three hydrogen bonds of conformation (A). With the ϕ constraints and the three hydrogen bonds, the Kendrew wire model gives much strain and a ψ_4 of +80°.

The assignments were ensured by the decoupling experiments of the respective NH protons. A computer simulated spectrum for these two ABX spin systems was obtained and shown in Figure 1b'. To verify the eight line spectrum for the two AB spin systems (AB for Gly_3 CH_2 and A'B' for Gly_5 CH_2), the corresponding NH (X) protons were exchanged. A spectrum of proton exchanged HCO-VPGVG-OMe in the solvent mixture of $CDCl_3$ and C_6D_6 (70 : 30 v/v) is shown in Figure 2 together with the computer simulated spectrum. As addition of C_6D_6 changes neither the analys-

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peptide NHs of 0.011 p.p.m. °C^{-1} (*e.g.* cyclic Gly-L-Pro at low concentration, 0.01M) and values of 0.002 p.p.m. °C^{-1} for the valinomycin–K^+ complex [27,28] in which both the C–O and NH of the peptide system are shielded from the solvent by hydrogen bonding. Over the temperature range −30—60 °C the coupling constants of the pentapeptide remained essentially unchanged.

The conformational torsion angles, ϕ, for VPGVG were obtained from the observed $^3J_{C^\alpha H-NH}$ coupling constants using the Karplus-like relations of Bystrov *et al.*,[49] for the peptide α-CH–NH bond system of valyl and of glycyl residues. The calculated values of ϕ are given in Table 2 where they may be compared to the values obtained from the conformational energy calculations for the lowest energy state (see below). The Bystrov *et al.* equations [49] provide four possible values of ϕ for each value of $^3J_{C^\alpha H-NH}$. As may be seen in Table 2 in each case one of the four solutions comes within 20° or less of the theoretical values of ϕ obtained for the lowest energy conformation (A).

Low Energy Conformations.—Ramachandran plots for the pairs of angles ϕ_1, ψ_1; ϕ_3, ψ_3; ϕ_4, ψ_4; and ϕ_5, ψ_5 are presented in Figure 3 and the conformational energy as a function of ψ_2 for the Pro_2 residue is shown in Figure 4a. The theoretically predicted values of the torsion angles,

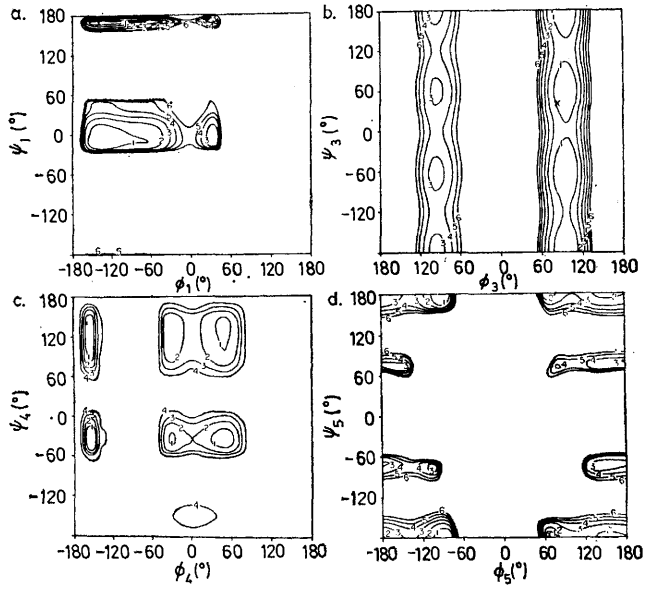

FIGURE 3 a, ϕ_1–ψ_1 Energy surface for Val_1 in kcal mol^{-1} relative to the global minimum marked ×. Torsion angles ϕ_2, ψ_2, ϕ_3, ψ_3, ϕ_4, ψ_4, ϕ_5, and ψ_5 were assumed to be locked in preferred values for conformation (A) listed in Table 2. b, ϕ_3–ψ_3 Energy surface for Gly_3 in kcal mol^{-1} relative to the global minimum marked ×. Torsion angles ϕ_1, ψ_1, ϕ_2, ψ_2, ϕ_4, ψ_4, ϕ_5, and ψ_5 were assumed to be locked in preferred values for conformation (A) listed in Table 2. c, ϕ_4–ψ_4 Energy surface for Val_4 in kcal mol^{-1} relative to the global minimum marked ×. ϕ_1, ψ_1, ϕ_2, ψ_2, ϕ_3, ψ_3, ϕ_5, and ψ_5 were assumed to be locked in preferred values for conformation (A) listed in Table 2. d, ϕ_5–ψ_5 Energy surface for Gly_5 in kcal mol^{-1} relative to the global minimum marked as ×. Torsion angles ϕ_1, ψ_1, ϕ_2, ψ_2, ϕ_3, ψ_3, ϕ_4, and ψ_4 were assumed to be locked in preferred values for conformation (A) listed in Table 2.

ϕ and ψ, for conformations A and B, respectively, are listed in Table 2. During the minimization [55] of the trial atomic co-ordinates obtained for the two low energy conformations all backbone bond angles were allowed to relax from the starting values obtained as described earlier in connection

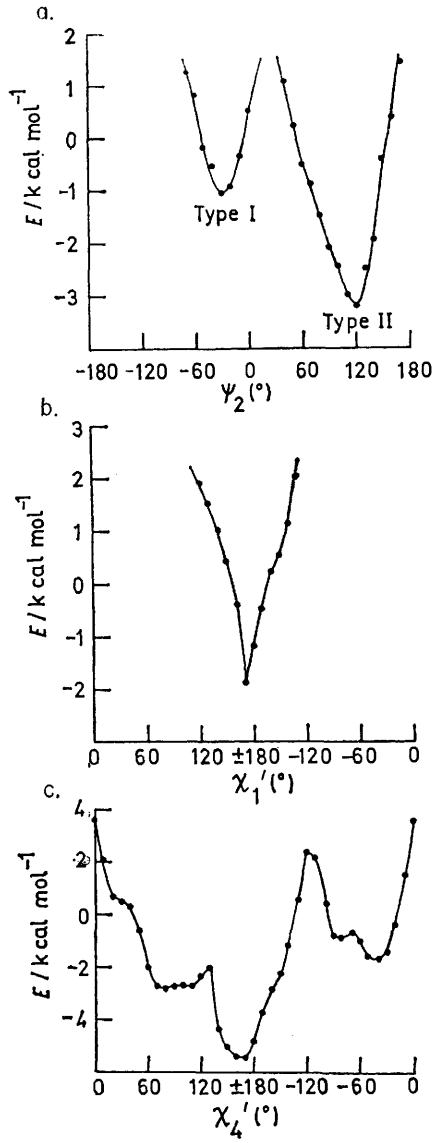

FIGURE 4 a, Energy in kcal mol^{-1} as a function of ψ_2 for Pro_2 assuming ϕ_2 −60°. Type I and II β-turns occur at the minima. b, Energy in kcal mol^{-1} as a function of χ^1 for Val_1 side chain rotation in conformation A. c, Energy in kcal mol^{-1} as a function of χ^1 for Val_4 side chain rotation in conformation (A).

with the generation of atomic co-ordinates for the fully extended conformation. In Table 3 the values of bond angles τ, *i.e.* N–C^α–C′ and torsion angles ω and θ_N necessary to describe the non-planarity of peptide groups [56] are listed for conformations A and B, respectively. The side chain torsion angles were found to assume values only slightly different from the *trans*-conformation. A plot of energy as a function of χ^1 for Val_1 and Val_4 residues are shown in Figure 4b and c. The torsion angle χ^1 for the C^α–C^β bond of the valyl side chains is taken as 180° when the N–C^α–C^β–

[55] V. Renugopalakrishnan, M. Renugopalakrishnan, and B. Sarkar, *Internat. J. Quantum Chem.*, 1975, **QBS2**, 109.

[56] V. Renugopalakrishnan and R. Rein, *Biochim. Biophys. Acta*, 1976, **434**, 164.

$C^{\gamma 1}$ atoms are all in the same plane. This $C^{\gamma 1}$ is chosen such that this orientation is also *trans*, θ 180°, for the H–C^{α}–C^{β}–H atoms. With this definition $\theta = \chi^1$ for χ^1 0—180°

TABLE 3

Bond angles τ and torsional angles ω and θ_N for the peptide groups in conformations (A) and (B) of HCO-VPGVG-OMe

	Conformation (A)			Conformation (B)		
	τ [a] (°)	ω [b] (°)	$[\theta_N]$ [c] (°)	τ [a] (°)	ω [b] (°)	$[\theta_N]$ [c] (°)
Val_1	112	178	5	112	179	6
Pro_2	114	177	11	115	178	12
Gly_3	109	180	3	108	170	3
Val_4	115	174	7	106	174	5
Gly_5	107	173	13	114	172	12

[a] Bond angle τ refers to the angle C′–Cα–N. [b] Torsion angle ω is the angle between the planes $C_1{}^{\alpha}C'N$ and $C'NC_2{}^{\alpha}$.[56] [c] Torsion angle θ_N is the angle between the planes $C'NC_2{}^{\alpha}$ to C′NH.[56]

whereas $\theta = \chi^1 - 360$ for χ^1 180—360°. θ is the dihedral angle of interest in the n.m.r. experiment.

A stereoscopic perspective of conformation (A), with a 10-membered hydrogen bonded system involving Val_1 C=O and Val_4 NH, a 14-membered hydrogen bonded system involving Val_1 NH and Val_4 C=O, and an 11-membered hydrogen bonded system involving Gly_3 NH and Gly_5 C=O is shown in Figure 5a. The other low energy conformation (B) with the 10-membered hydrogen bonded system involving Val_1 C=O and Val_4 NH, an 11-membered hydrogen bonded system involving Gly_3 NH and Gly_5 C=O and a seven-membered hydrogen bonded system involving Gly_3 C=O and Gly_5 NH groups is shown in Figure 5b. In Table 2 the torsion angles of the minimum energy conformation for conformation (A) in Figure 5a are compared with torsion angles derived from n.m.r. studies on VPGVG.

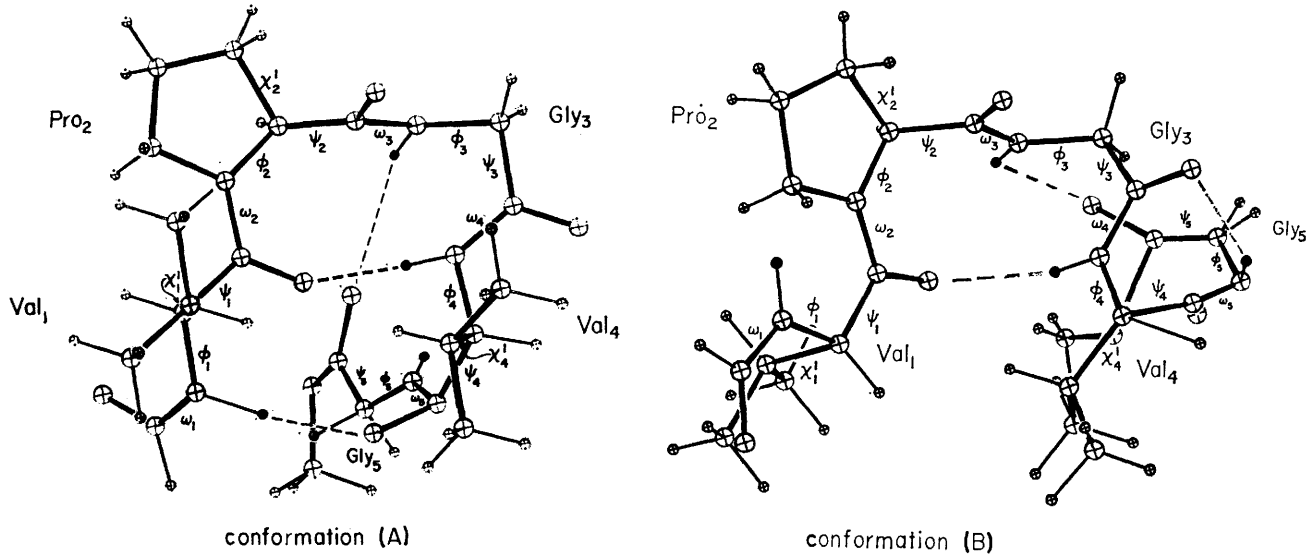

FIGURE 5 a, A stereoscopic perspective of conformation (A). b, A stereoscopic perspective of conformation (B). Intramolecular hydrogen bonds discussed in the text are shown by broken lines

DISCUSSION

The conformation of VPGVG is discussed from the results of a combined analysis of n.m.r. and theoretical studies. The Val_1 residue may be seen in Figure 3a to assume two regions in the conformational energy map. The minimum energy conformation occurs at ϕ_1 —140 and ψ_1 170°. However, additional local minima are found to occur in regions near ψ_1 0 and ϕ_1 —120 and 40° and near ψ_1 170 and ϕ_1 30°. The formation of a hydrogen bond between Val_1 NH and Val_4 C=O further stabilizes the minimum energy conformation of Val_1 at ϕ_1 —140 and ψ_1 170° which seems to arise from the interaction of valine and the proline side chains. The observations on the conformation of a valyl residue preceding a proline are in line with similar observations for an alanyl residue preceding a proline.[57]

The proline residue with its pyrrolidine ring was assumed to fix ϕ_2 at —60°.[57] This fixed geometry of the pyrrolidine ring significantly limits the allowed values of ψ_2. In general, ψ_2 values for proline are found to occur from —10 to —60° and 120 to 160°.[57] These two different conformations, in conjunction with the immediately succeeding residues, *i.e.* Gly_3 and Val_4 gives rise to Type I and Type II β-turns as originally suggested by Venkatachalam[37] and Geddes *et al.*[38] A plot of energy as a function of ψ_2 for Pro_2 is given in Figure 4a which shows the two minima.

The conformational map for Gly_3 is presented in Figure 3b where a number of local minima may be observed. The global minimum at ϕ_3 80 and ψ_3 40° corresponds to a Type II β-turn, positioning the Val_4 NH in a hydrogen-bonding configuration with the Val_1 C=O, thereby giving rise to a 10-membered hydrogen bonded system. The theoretical results correlate well with earlier n.m.r. studies on VPGVG,[8,17] as well as with the present n.m.r. studies on VPGVG in $CDCl_3$. N.m.r. studies of the temperature dependence of peptide protons of VPGVG in $CDCl_3$ show the Val_4 NH to be the most

[57] B. Pullman and A. Pullman, *Adv. Protein Chem.*, 1974, **28**, 347.

shielded proton ($d\delta/dT$ 0.003 8 p.p.m. °C^{-1}). The value for a completely solvent exposed peptide NH in chloroform is *ca.* 0.009 p.p.m. °C^{-1}. Furthermore, the appearance of Gly_3 CH_2 as an ABX spin system is consistent with a constrained structure as occurs when stabilized by a hydrogen bond involving the Val_4 NH. By means of a ^{13}C n.m.r. solvent study, it was previously shown [58] that the Val_1 C=O is similarly most shielded, giving rise to a 10-membered β-turn containing Pro_2 and Gly_3 at the corners. The $^3J_{C^\alpha H-NH}$ coupling constants provide an approximate mean value for the torsion angle ϕ_3 of 60° which is in agreement with the theoretically predicted value of 80° and the previously predicted value of 60° for a Type II β-turn.[59] On the other hand, utilizing the (ϕ, ψ) map for geminal coupling constants, derived recently by Barfield *et al.*[60] for Gly_3 CH_2, a value of ^2J of −17 Hz gives rise to ϕ_3 80—90 and ψ_3 20—30° which is in agreement with the theoretically predicted values. From Figure 3b a Type I β-turn with ϕ_3 *ca.* −90 and ψ_3 *ca.* 0° may be observed with an energy *ca.* 3.4 kcal mol^{-1} higher than the conformation corresponding to a Type II β-turn.

In Figure 3c the conformational energy map of Val_4 is presented. It should be observed that the map for Val_4 is quite different from that of Val_1 (see Figure 3a). The minimum energy conformation for Val_4 occurs at ϕ_4 −160 and ψ_4 140°. A torsion angle of ψ_4 140° positions the carbonyl group in a favourable conformation for forming a hydrogen bond with the Val_1 NH, giving rise to a 14-membered hydrogen bonded system. The formation of the 1 ⟶ 4 type 14-membered hydrogen bonded system is reasonable from the temperature dependence of the Val_1 NH chemical shift ($d\delta/dT$ 0.006 8 p.p.m. °C^{-1}) and from the conformational angles for the Val_1 and Val_4 residues obtained from $^3J_{C^\alpha H-NH}$ coupling constants of 9 and 9.5 Hz, respectively. The latter values indicate a near *trans*-orientation of the $C^\alpha H$–NH dihedral angle. The presence of a 14-membered hydrogen bonded system has been observed in the *X*-ray structure of (*Z*)-Gly_1-L-Pro_2-L-Leu_3-Gly_4-L-Pro_5 by Ueki *et al.*[61] between the Gly_1 NH and the Gly_4 C=O. The allowed region in the Val_4 conformational map centred around ϕ_4 −60—80 and ψ_4 60—170 is responsible for conformation (B), one of the two conformations predicted to be stable from theoretical studies, in which Val_1 NH · · · O=C Val_4 is absent. In conformation (B), Val_1 adopts a conformation with ϕ_1 30 and ψ_1 170°. In this connection it may be noted that $d\delta/dT$ for the Val_1 N*H* indicates a probability of occurrence of *ca.* 50% for the 14-membered hydrogen-bonded ring, *i.e.* for conformation (A).

The Gly_5 conformational energy map is presented in Figure 3d where it may be observed that the allowed region is found to be significantly reduced compared to a normal Gly residue. The Gly_5 residue assumes the torsion angles of ϕ_5 70 and ψ_5 −170 which gives rise to the 11-membered hydrogen bonded γ-turn involving Gly_5 C=O and Gly_3 NH. N.m.r. observations are in line with the above conclusion since the second most shielded NH proton is that of Gly_3 ($d\delta/dT$ 0.005 1 p.p.m. °C^{-1}). It was also previously observed from the temperature dependence and solvent perturbation [17] that the Gly_3 NH was almost totally shielded in dimethyl sulphoxide and proposed that there occurs an 11-membered ring stabilized by a hydrogen bond between the Gly_3 NH and the Gly_5 C=O. This type of ring formation, which is called a γ-turn,[62] was also observed in thermolysin.[63] The appearance of the Gly_5 CH_2 protons as an ABX spin pattern (see Figure 1) can result from a constrained structure as occurs on formation of the 11-membered ring γ-turn utilizing the Gly_3-Val_4-Gly_5 sequence of Val_1-Pro_2-Gly_3-Val_4-Gly_5. The conformational angles for Gly_3, Val_4, and Gly_5 (see Table 2) for VPGVG differ from those predicted for the γ-turns of Gly-Gly-Gly [62] and of Gly-Val-Gly [64] as isolated systems. Using the (ϕ,ψ) map of Barfield *et al.* [60] for ^2J, the ϕ_5 and ψ_5 values for Gly_5 are consistent with the theoretical calculations (Table 2) but differ from the previous values.[62,64] This variance is easily understood when one considers the other structural features of VPGVG such as the presence of a β-turn (discussed earlier) and the formation of a 14-membered hydrogen bond ring between the Val_1 NH and Val_4 C=O.

A comparison of torsion angles ϕ and ψ obtained from theoretical calculations and from the n.m.r. studies for conformation (A) are given in Table 2. N.m.r. derived torsion angles pertain to an averaged conformation and, therefore, may be expected to compare favourably with the angle obtained from the expected coupling constant of equation (2). The n.m.r. results lead to the conclusion that in an inert solvent such as $CDCl_3$ the conformation of VPGVG is stabilized by a Type II β-turn (type 4 ⟶ 1), a 14-membered hydrogen bonded system (type 1 ⟶ 4) and a γ-turn involving the Gly_3 NH and the Gly_5 C=O. This same conformation is suggested on the basis of ^1H and ^{13}C magnetic resonance as the conformation of the high polymer of VPGVG at higher temperatures in water.[19] In general, theoretical studies point to two types of conformations, shown schematically in Figure 5, which are designated as conformations (A) and (B). Conformation (A) corresponds to the experimentally observed conformation in $CDCl_3$ and in water at elevated temperatures, whereas conformation (B) corresponds to the preferred conformation for the high polymer in water at lower temperatures.[19] On an average *in vacuo*, conformation (A) is predicted to be more stable than (B) by 2.5 kcal mol^{-1}.

As discussed earlier, average coupling constants

[58] D. W. Urry, L. W. Mitchell, and T. Ohnishi, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 3265.
[59] G. Boussard, M. Marraud, and J. Neel, *J. Chim. Phys.*, 1974, **71**, 46.
[60] M. Barfield, V. J. Hruby, and J. P. Meraldi, *J. Amer. Chem. Soc.*, 1976, **98**, 1308.
[61] T. Ueki, S. Bando, T. Ashida, and M. Kakudo, *Acta Cryst.*, 1971, **B27**, 2219.
[62] G. Nemethy and M. P. Printz, *Macromolecules*, 1962, **5**, 755.
[63] B. W. Mathews, *Macromolecules*, 1972, **5**, 818.
[64] M. A. Khaled, D. W. Urry, and K. Okamoto, *Biochem. Biophys. Res. Comm.*, 1976, **72**, 162.

$\langle {}^3J \rangle$, were calculated from the conformational maps presented. $J_{C^{\alpha}H-NH}$ for Val_1 and Val_4 were found to be 8.8 and 9.1 Hz, respectively. Similarly, $J_{C^{\alpha}H-NH}$ for Gly_3 and Gly_5 residues were calculated to be 5.47 and 5.53 Hz, respectively. The calculated coupling constants are found to be in good agreement with experimental coupling constants presented in Table 1.

In a later stage of the calculation, the side-chain torsion angle χ^1 was varied for the Val_1 and Val_4 side chains. Calculations were performed for conformations (A) and (B). The Val_1 and Val_4 side chains for conformation (B) were found to prefer a slightly off-*trans*-conformation, with the Val_1 side chain having more flexibility than the Val_4 side chain. A plot of energy as a function of χ^1 is presented in Figure 4b and c for Val_1 and Val_4 side chains of conformation (A). In the case of conformation (A) the side chains were also found to prefer a slightly off-*trans*-conformation, with much less flexibility than in the case of conformation (B).

Using the Abraham–McLauchlan coefficients mentioned earlier, the $J_{C^{\alpha}H-C^{\beta}H}$ coupling constant for the side chains of Val_1 and Val_4 residues both in conformations (A) and (B) were calculated, using the conformational energies as a function of χ^1 presented in Figure 4b and c, respectively. For conformation (A) of the pentapeptide coupling constants 8.3 and 8.8 Hz were obtained for the $J_{C^{\alpha}H-C^{\beta}H}$ of residues 1 and 4, respectively. Whereas for conformation (B), coupling constants of 7.8 and 8.0 Hz were obtained respectively for the Val_1 and Val_4 residues. In conformation (B) the Val_1 and Val_4 side chains behave somewhat differently although both prefer a slightly off-*trans*-conformation. The theoretical coupling constants for conformation (A) are in accord with the experimental results presented in Table 1.

In Table 3, bond angles τ, $C'-C^{\alpha}-N$ and the two torsion angles ω and θ_N necessary to describe the non-planarity [56] of the peptide group are presented for VPGVG. The bond angles τ may be observed to deviate somewhat from ideal tetrahedral values. The peptide groups are also observed to assume a non-planar geometry with the nitrogen atom adopting a slightly pyramidal configuration. The torsion angle θ_N is a measure of the pyramidality at the nitrogen atom. The deviations observed in ω and θ_N for VPGVG are in line with similar theoretical [65] and experimental (from ^{15}N n.m.r.) [66] observations on a number of other peptide systems.[65]

Conclusions.—The conformational energy calculations for the pentapeptide discussed in this paper were initiated using standard geometries for Gly, Val, and Pro residues as well as for the end groups. With this starting assumption, it is interesting to observe that the *in vacuo* theoretical calculations are able to predict two stable conformations which correlate well with extensive experimental solution studies reported earlier from this laboratory [8,17,19] and with the present detailed n.m.r. studies in $CDCl_3$.

The solution studies indicate that in a polymer each monomeric unit preserves its conformational integrity.[17,19] Longer range interactions giving rise to preferred β-spiral conformations [19] in the polypentapeptide appear to result from non-hydrogen bonded interactions between the repeating units. The relative orientation of repeating pentamers is currently under investigation in this laboratory.

Although theoretical studies are considered in terms of static states, it is to be appreciated that in solution the molecular system of interest is a dynamic entity. Under the influence of environmental factors such as temperature, solvent, *etc.*, an interconversion between the two molecular conformers shown in Figure 5a and b may take place. In this context, it is interesting to note that experimental studies have suggested that conformation (A) is preferred in water above 50 °C whereas conformation (B) is the preferred conformer in water below 50 °C.[19]

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[7/627 *Received*, 13*th April*, 1977]

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