Nuclear Magnetic Resonance and Conformational Energy Calculations of Repeat Peptides of Tropoelastin: Conformational Characterization of the Cyclododecapeptide

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N.m.r. data obtained in chloroform–dimethyl sulphoxide solutions and conformational-energy calculations are reported which deduce the preferred conformation of the cyclic dodecapeptide, cyclo-(L-Ala[1]-L-Pro[2]-Gly[3]-L-Val[4]-Gly[5]-L-Val[6])$_2$, having the hexapeptide repeat sequence of tropoelastin. The non-equivalence of the Gly α-CH_2 protons and the similarity of α-carbon relaxation times indicate that the molecule is quite rigid. The ^{1}H n.m.r. spectrum of the molecule resembles that of a hexamer, indicating that the molecule possesses two-fold symmetry on the n.m.r. time scale. The possible ranges of backbone torsion angles except ψ (Ala[1]) are derived from α-C*H*–N*H* coupling constants, geminal coupling constants, and nuclear Overhauser effects. From temperature-dependence studies of the peptide N*H* chemical shift and from the coupling constants, secondary structural features of the molecule are obtained. The valyl α-C*H*–β-C*H* coupling constants show that the Val[4] side chain is in a *gauche* conformation, while the Val[6] side chain is in the *trans*-conformation. A static wire model is developed using these data and containing the following solution-derived hydrogen bonds: CO(1) · · · HN(4) (β-turn), NH(1) · · · OC(4) and NH(6) · · · OC(4) (bifurcated 14- and 17-membered rings), and NH(3) · · · OC(5) (γ-turn). *In vacuo* conformational-energy calculations are performed to obtain several minimum-energy conformations. The theoretical calculations utilize the Go–Scheraga method for cyclization with exact two-fold symmetry. Only one of the low-energy structures so obtained is consistent with all the experimental data, and this structure is quite similar to the static wire model based on the n.m.r. data.

THE interest in conformational analysis of cyclic peptides arises from many aspects. Some cyclic peptides (*e.g.* valinomycin, oxytocin) are biologically active. Others may serve as a model system for linear peptides (*e.g.* enniatin B for gramicidin A).[1,2] This is due to the fact that a linear (sequential) polymer and the corresponding cyclic peptide with the same sequence may have many conformational features in common. It may even happen that all backbone torsion angles of the cyclic peptides are similar to those in the corresponding linear peptide (the linear correlate).† This is a special case, but one of particular interest.‡ The chances of its occurrence obviously improves with increasing size of the cyclic peptides. Cyclization restricts the number of possible conformations that a peptide can assume. In this respect, the work of Go and Scheraga [5] is quite significant. They have shown that cyclization itself, in general, reduces the number of degrees of freedom by six. Obviously, small cyclic peptides will be significantly affected by cyclization. However, for a large linear peptide, relatively smaller changes in torsion angles can bring about a significant decrease (or increase!) in its end-to-end length. Thus, it is reasonable to state that the linear peptide may adopt a conformation close to that of its cyclic counterpart especially in solvents that tend to prefer non-random conformations with small end-to-end lengths. This is the concept of cyclic conformations with linear conformational correlates.[1,2]

Finally, cyclic molecules are fascinating in their own way and there have been excellent reviews on conformational analysis of cyclic peptides.[6–10]

The repeat hexapeptide of tropoelastin contains the sequence L-Ala[1]-L-Pro[2]-Gly[3]-L-Val[4]-Gly[5]-L-Val[6] (hereafter abbreviated as APGVGV). This linear hexapeptide, both in its monomeric and polymeric forms, has been found to form a 10-membered hydrogen bond,[11] called a β-turn, between the Val[4] NH and the Ala[1] C=O groups [12] and an 11-membered hydrogen bond (a γ-turn) [13,14a] between the Gly[3] NH and Gly[5] C=O groups [14b,15] (see Figure 1). Consideration of the literature [6,11,16–18,§] shows that the -L-Pro-Gly- sequence in a peptide promotes the formation of a type II β-turn, since the torsion angle ϕ of the L-Pro residue is locked at *ca.* −60°. It was, therefore, anticipated that a type II β-turn may also occur in the cyclic counterpart of the linear hexapeptide, APGVGV. The cyclohexapeptide, ⌐-APGVGV-⌐, was synthesized and found to adopt a conformation [20] characteristic of cyclohexapeptides [6] containing the expected type II β-turn together with another β-turn (type II′), with the latter differing from its linear counterpart.[14b,15]

Whether or not any higher cyclic analogues of the repeat hexapeptide sequence, APGVGV, could exhibit conformational features similar to its linear counterpart is a question of interest. With this in mind, the cyclododecapeptide, ⌐-(APGVGV)$_2$-⌐, was synthesized and was

† For instance, Kopple and Go [3] have obtained a cyclic dodecapeptide during polymerization of repeating sequences of the hexapeptide Met-Val-Gly-Pro-Asn-Gly. From the readiness with which the cyclization was achieved, they suggested that the linear dodecapeptide probably has a conformation close to that of the cyclic molecule.

‡ It is found that the n.m.r. spectrum of cyclo-(VPGVG)$_3$ is strikingly similar to that of the pentamer VPGVG, and also that of the linear polymer (VPGVG)$_n$ clearly suggesting that the cyclic pentadecapeptide and the linear polypentapeptide may be expected to possess many secondary structural features in common.[4]

§ The *X*-ray crystal structure analysis [19] of cyclo-(VPGVG)$_3$ shows unambiguously that there is a type II β-turn at the Pro-Gly sequence as deduced from n.m.r. and energy calculations on elastic repeat peptides.[17]

found to undergo an inverse temperature transition leading to crystallization.[21] A similar inverse temperature transition of the polyhexapeptide, $(APGVGV)_n$, resulted in fibre formation [22] and in an increase of the intra- and inter-molecular order.[17,23] This is undoubtedly a fascinating phenomenon and it is, therefore, essential to investigate the detailed conformational properties of this cyclododecapeptide, $\overline{(APGVGV)_2}$.

studies on cyclic molecules as model systems. The conformational properties of the cyclododecapeptide, $\overline{(APGVGV)_2}$, obtained in this study in chloroform by using these experimental methods are compared with its low-energy structures, resulting from the *in vacuo* theoretical-energy calculations. On the basis of these studies we propose that the cyclododecapeptide exhibits a fairly rigid unique structure with two-fold symmetry,

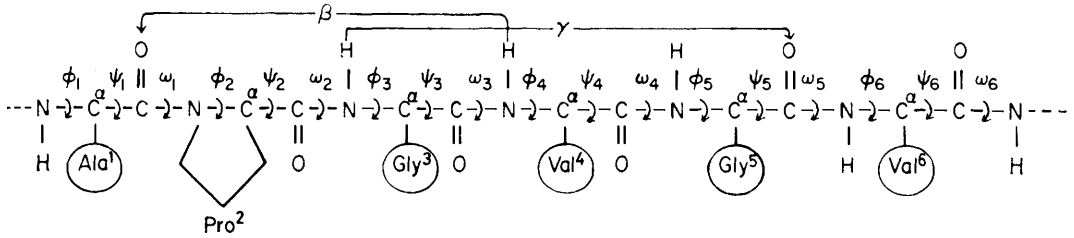


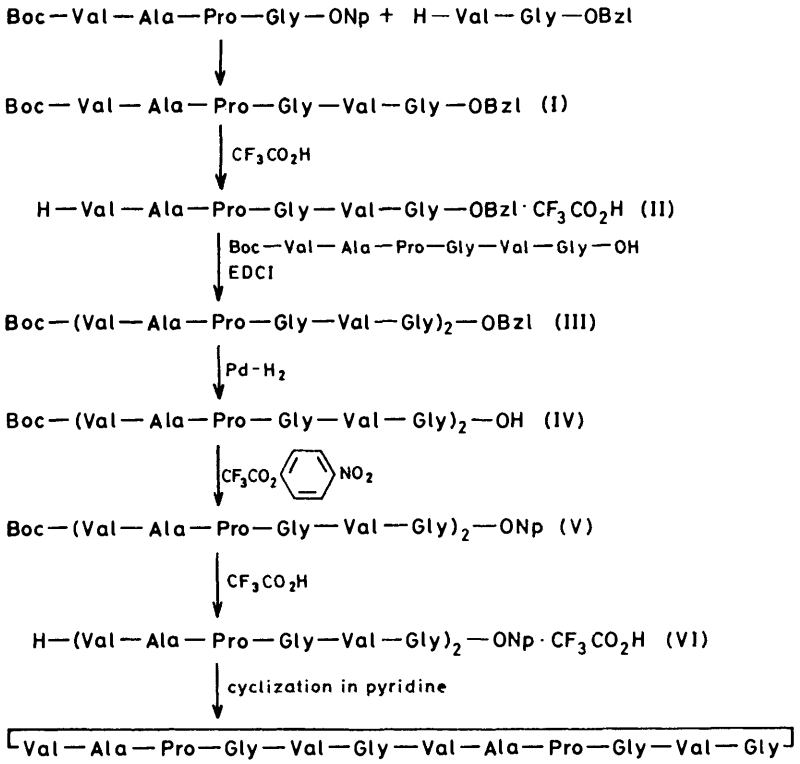
FIGURE 1 A schematic drawing of the hexapeptide sequence showing the hydrogen bonds formed in the linear hexapeptide (APGVGV)

There have been many experimental [6,7,19,24,25] and theoretical [6,25-29] studies made on the conformations of cyclic peptides utilizing n.m.r. spectroscopy and conformational-energy calculations. N.m.r. methods, such as the determinations of the backbone torsion angles (ϕ,ψ) from vicinal (^3J) and geminal (^2J) coupling constants [30,31] backbone motional reorientation from the relaxation behavior of α-carbon atoms,[32] and the nuclear Overhauser effect (NOE) [33] to obtain proton–proton proximity, have been developed from extensive

having many features in common with those of the linear polyhexapeptide but also having some significant differences.

EXPERIMENTAL AND METHODS

Synthesis.—cyclo-(-Val-Ala-Pro-Gly-Val-Gly-)$_2$ was synthesized *via* cyclization of the *p*-nitrophenyl (ONp) ester of the linear dodecapeptide, H-(Val-Ala-Pro-Gly-Val-Gly)$_2$-ONp·CF_3CO_2H, as shown in the Scheme. The identity and purity of all intermediate compounds were checked by t.l.c. and 220-MHz ^1H n.m.r. spectroscopy. T.l.c. was performed



SCHEME EDCI = 1-Ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride, Bzl = benzyloxycarbonyl

on silica gel plates supplied by Quantum Industries, utilizing the following solvent systems: R_F^1 choroform–methanol–acetic acid (95 : 5 : 3); R_F^2 chloroform–methanol–acetic acid (85 : 15 : 3); and R_F^3 n-butanol–acetic acid–water–pyridine (30 : 6 : 24 : 20).

Boc-Val-Ala-Pro-Gly-Val-Gly-OBzl (I).—To a solution of Boc-Val-Ala-Pro-Gly-ONp [34] (15.8 g, 28 mmol) and 1-hydroxybenzotriazole (3.8 g, 28 mmol) in dimethylformamide (80 ml), a solution of H-Val-Gly-OBzl·CF_3CO_2H [35] (10.4 g, 27.4 mmol) and triethylamine (3.92 ml, 28 mmol) in dimethylformamide (30 ml) was added at 0—5 °C. The mixture was stirred at room temperature overnight and then evaporated under reduced pressure. The residue was dissolved in chloroform; and the chloroform solution was washed successively with 10% citric acid solution, saturated sodium hydrogencarbonate solution, and water and dried over magnesium sulphate. The solvent was removed, and the residual oil was triturated with light petroleum to yield a powder (16.2 g, 85.8%); m.p. 153—155 °C (Found: C, 59.7; H, 7.55; N, 12.15. Calc. for $C_{34}H_{52}O_9N_6$: C, 59.3; H, 7.6; N, 12.2%).

H-Val-Ala-Pro-Gly-Val-Gly-OBzl·CF_3CO_2H (II).—A solution of (I) (2.1 g, 3 mmol) in trifluoroacetic acid (3 ml) was kept for 40 min at room temperature. The mixture was evaporated to dryness and solidified on addition of ether. The solid was collected by filtration, washed with ether, and dried *in vacuo* over sodium hydroxide (yield 2.1 g, 100%).

Boc-(Val-Ala-Pro-Gly-Val-Gly)$_2$-OBzl (III).—To a solution of Boc-Val-Ala-Pro-Gly-Val-Gly-OH [15] (1.8 g, 3 mmol), 1-hydroxybenzotriazole (450 mg, 3.3 mmol), (II) (2.1 g, 3 mmol), and triethylamine (0.42 ml, 3 mmol) in dimethyl sulphoxide (15 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (632 mg, 3.3 mmol) at −5 to 0 °C. The mixture was stirred overnight at room temperature and then diluted with chloroform (200 ml). The solution was washed successively with 10% citric acid, 6% sodium hydrogencarbonate solution, and water, and dried ($MgSO_4$). The solvent was removed under vacuum, and an amorphous powder was obtained on addition of ethyl acetate (yield 2.9 g, 82.6%) (Found: C, 57.9; H, 7.65; N, 14.1. Calc. for $C_{56}H_{88}O_{16}N_{12}$: C, 57.5; H, 7.6; N, 14.35%).

Boc-(Val-Ala-Pro-Gly-Val-Gly)$_2$-OH (IV).—Compound (III) (1.8 g, 1.54 mmol) was dissolved in acetic acid (50 ml) and hydrogenated at 50 lb in^{-2} in the presence of 10% Pd–C (100 mg) for 6 h. The catalyst was removed by filtration, and the solvent was distilled off under reduced pressure. The residue was triturated with ether to yield powder (1.5 g, 90.2%), m.p. 90—120 °C.

Boc-(Val-Ala-Pro-Gly-Val-Gly)$_2$-ONp (V).—*p*-Nitrophenyl trifluoroacetate (470 mg, 2 mmol) was added to a solution of (IV) (500 mg, 0.5 mmol) in a mixture of pyridine (1.5 ml) and dimethyl sulphoxide (1.5 ml). The mixture was stirred at room temperature for seven days. The solid material was collected by filtration and washed successively with chloroform, 6% sodium hydrogencarbonate solution, and water and dried at 50 °C (yield 300 mg, 49.9%), m.p. 140—150 °C, R_F^1 0.12, R_F^2 0.85. This material was used in the following reaction without further purification.

H-(Val-Ala-Pro-Gly-Val-Gly)$_2$-ONp·CF_3CO_2H (VI).—A solution of (V) (250 mg, 2 mmol) in trifluoroacetic acid (2 ml) was allowed to stand for 40 min at room temperature. The solvent was evaporated to dryness, and the residue was collected by filtration, washed with ether and dried *in vacuo* over sodium hydroxide (yield 250 mg, 100%).

cyclo-(Val-Ala-Pro-Gly-Val-Gly)$_2$ (VII).—A solution of (VI) (250 mg, 2 mmol) in a mixture of dimethylformamide (20 ml) and acetic acid (0.5 ml) was added dropwise over 5 h with stirring to pyridine (1 700 ml) at 75—80 °C. The mixture was stirred at 80 °C for an additional 12 h. The mixture was cooled and the solvents were removed under reduced pressure. Water (50 ml) was added to the residue, and the mixture was evaporated. This procedure was repeated three times. The oil thus obtained was dissolved in methanol–water (50 ml; 1 : 3 v/v) and passed through a column packed with Bio-Rad mixed bed resin, AG 501-X8(D) (2 × 30 cm). The column was washed with the same solvent (200 ml), and the effluent (250 ml) was evaporated. The resulting oil was redissolved in water and lyophilized to yield a powder, R_F^1 0.10, R_F^2 0.56, R_F^3 0.84 (Found: C, 53.45; H, 7.77; N, 16.85. Calc. for $C_{44}H_{72}O_{12}N_{12}\cdot H_2O$: C, 53.0; H, 7.7; N, 16.85%) [Found: *M* (osmometry), 958. Calc. for $C_{44}H_{72}O_{12}N_{12}$: *M*, 960].

N.M.R. Measurements.—Since the cyclododecapeptide, [-(APGVGV)$_2$-], exhibited limited solubility in $CDCl_3$, a 10mM solution of this peptide was prepared in 90% $CDCl_3$–10% [$^2H_6$]DMSO. The ^1H n.m.r. spectra were obtained on a Varian HR-220 spectrometer operating at a probe temperature of 20 °C. The simulated spectra were obtained with the SS-100 computer system using the Varian data machine spin-simulation program. A JEOL PS-100 spectrometer, operating in the continuous-wave mode, was used to measure the nuclear Overhauser effect (NOE). Since a 100-MHz spectrometer has a 50-Hz decoupler spill over, experiments were performed by offsetting the irradiation field by 50 Hz on each side of the resonance of interest in order to obtain an accurate value for the NOE. The enhancement of the signal intensity was obtained by integrating the signals several times with and without the irradiation field set on the resonance of interest. The accuracy of the % NOE values is *ca.* ±2.5%.

^{13}C Spin–lattice relaxation times were obtained from a 0.026M sample, a concentration similar to that in the NOE experiments, in 90% $CDCl_3$–10% [$^2H_6$]DMSO. The sample was placed in a micro-cell, and dissolved oxygen was removed by passing argon gas through the solution for 25 min. A JEOL FX-100 pulse n.m.r. spectrometer was used to measure the T_1 values with the ^{13}C resonance frequency at 25.15 MHz (23.482 kG field strength). The inversion recovery method (180°–τ–90° pulse sequence) was used with a 32-μs pulse for 180° tilt of the ^{13}C magnetization vector and a 16-μs pulse for 90° tilt. A Texas Instruments 980-B computer with 16K memory was used to operate the spectrometer by the Fourier-transform method and also to calculate the T_1 values using 4—7 points in the least squares method for each value reported. The probe temperature was 27 ± 1 °C. Tetramethylsilane was added as an internal reference for the chemical-shift values after the relaxation time had been determined.

Conformational-energy Calculations.—The method of approach employed in computing the low-energy conformations of the cyclododecapeptide is based on the information concerning the salient features of the molecule gleaned from n.m.r. studies. Rather than undertake a complete search of the entire conformational space available for this molecule, the search was initiated in a subspace derived from ranges of values obtained from analysis of n.m.r. parameters. The purpose of the energy calculations described is to determine the minimum energy conformations that are consistent with the n.m.r. data.

N.m.r. data presented below clearly shows that the mole-

cule possesses two-fold symmetry on the n.m.r. time scale: only a hexamer spectrum is observed. This, combined with the rigidity of the molecule inferred from the non-equivalence of the Gly α-CH_2 protons and from the similarity of all the α-carbon relaxation times, provides a sound basis for the assumption of C_2 symmetry. Thus, in this cyclic peptide, the conformation exhibits the symmetry of the chemical sequence. In all the energy calculations, therefore, the molecule was assumed to possess exact C_2 symmetry. The backbone conformation of the molecule is essentially characterized by 24 backbone torsion angles, ϕ and ψ (Figure 1).[36] But the imposition of C_2 symmetry reduces the total independent angles to 12, and the condition of cyclization further limits the independent variables to 10, since two angles will have to be adjusted so as to ensure cyclization.[5] Further, the prolyl ring closure fixes the torsion angle ϕ_2 to be *ca.* $-70°$.[37] Thus we only need nine independent angles to specify the gross backbone conformation of the molecule. In general, the trigonometry of the cyclization conditions allows one to choose any two of the backbone torsion angles of the asymmetric unit to be the dependent variables with which to achieve cyclization. The nine independent variables have been so chosen as to minimize the number of conformations that have to be searched to start with, consistent with the expected ranges of values for the torsion angles from the n.m.r. data. Accordingly, ψ_1 and ψ_6 were chosen as dependent variables during the initial stages of the energy calculations since for these two angles we obtained the least restricted estimate from n.m.r.

In computing conformational energies, we followed largely the recent method of Scheraga and his co-workers.[38,39] All geometric parameters, the non-bonded potential function and partial atomic charges are taken from Scheraga *et al.*[38,39] The Cγ atom of the proline was fixed with $\Gamma -30°$,[40,*] corresponding to the ' down ' conformation of the prolyl ring, during the initial stages of the energy minimization.

One of the most difficult aspects of the conformational analysis on a molecule of this size is the location and identification of all the low-energy regions in the conformation space. It is indeed a tedious and challenging task to make certain that the conformation space that is accessible to the molecule has been thoroughly searched. In order to minimize computing time, suitable algorithms have to be devised to search efficiently the multi-dimensional conformation space for energy minima.

The strategy employed for locating minimum energy regions consistent with the n.m.r. data may be classified into three stages, as described elsehwere,[41] in a manner similar to the method employed by Madison.[42] In the first stage, using constant bond lengths and bond angles and planar *trans*-peptide units, the nine independent backbone torsion angles (ϕ and ψ) are systematically varied in steps of 30° within the ranges suggested by n.m.r. analysis (see Table 3). For the resulting sets of angles, ψ_1 and ψ_6 are adjusted in order to achieve cyclization with two-fold symmetry. For each set of torsion angles, this resulted in two sets of values for the pair (ψ_{Ala^1}, ψ_{Val^6}) whenever the cyclization succeeded. About one-third of the sets failed to cyclize and they were discarded. Those solutions, for which (ψ_{Ala^1}, ψ_{Val^6}) fell well outside the Ramachandran contact map [43] for alanine, were also rejected. At this stage, the set of starting structures was further expanded by including conformations in which parts of the molecule are constructed so as to contain specific secondary structural features (such as the β-turn at Pro^2-Gly^3, the $Gly^3 \longrightarrow Gly^5$ γ-turn, *etc.*) expected from n.m.r. (see Results). For the resulting 50 000 conformations, the total conformational energy was obtained. To minimize computing time, in this stage, the valyl side chain atoms except Cβ and Hα were omitted in computing the energy. The low-energy regions were identified. The second stage consisted of a systematic stepwise exploration of the conformation space near the low-energy regions, still, as before, ignoring the side-chain atoms of the valyl residues. This stage resulted in obtaining a cluster of conformations on a 10° grid near the low energy regions. The final stage consisted of a complete energy minimization using a quasi-Newton algorithm by Fletcher.[44] During the energy minimization, all independent torsion angles and the six ω values (thus allowing the peptide units to become non-planar) were allowed to vary. In the final stages of the minimization, the bond angles τ(N–Cα–C) were also allowed to vary.† During the entire minimization procedure, the adjustment of ψ_1 and ψ_6 was in effect, obviously, thereby ensuring perfect cyclization with two-fold symmetry. However, in several cases the minimization was terminated by cyclization failures just as observed by Scheraga and his co-workers for cyclohexaglycyl.[28] Whenever this happened the two dependent angles of cyclization had to be redefined until the minimization could be successfully continued.

All atoms of the valyl side chains were included during the Fletcher minimization. In general, three different conformations were considered for the valyl side chain corresponding to ideal values of $\chi^1 = 60$, 180, and 300°. For each low-energy region, the combinations of Val^4, Val^6 side-chain conformations leading to very high energies, were rejected. Fletcher minimization was repeated for each combination of Val^4, Val^6 side-chain types. During the minimization, the torsion angles $\chi^1(Val^4)$ and $\chi^1(Val^6)$ were allowed to depart from the ideal values given above, which had corresponded to perfectly staggered arrangements about the Cα–Cβ bond. All the methyl hydrogens were also allowed to deviate from staggered conformations. It was found, in particular, that $\chi_{Val}^{2,1}$ and $\chi_{Val}^{2,2}$ coupled rather strongly with $\chi^1{}_{Val}$ as has been observed by Scheraga and his co-workers.[39]

A flexible proline was employed during the Fletcher minimization in the following manner: Both torsion angles, ϕ_{Pro^2} and L, were also allowed to vary. The bond-angle distortion energy of the proline was taken from Venkatachalam *et al.*[40] For each low-energy structure obtained from the second stage, Fletcher minimization was carried out starting from both ' down ' conformations of Cγ atom (Γ *ca.* $-30°$) as well as the ' up ' conformation (Γ *ca.* $+30°$).

* The angle Γ is a pseudorotation of the Cγ atom about the Cβ · · · Cδ line such that the bond lengths Cβ · · · Cγ, Cδ · · · Cγ are held constant (see ref. 38). Γ and ϕ completely define the puckering of the proline ring. The γ-puckered geometries with the Cγ atom displaced towards the side of the carbonyl carbon is termed – or ' down ' puckered while if it is displaced away from the carbonyl, it is + or ' up '-puckered. Defining Γ as in ref. 40, a negative (or positive) value of Γ refers to ' down ' (or 'up ') puckering of the Cγ atom provided ϕ is close to $-60°$. See also ref. 39.

† The energy of peptide non-planarity was computed by taking the intrinsic torsional potential for the ω-rotation to be $V_\omega = \frac{1}{2}V_0(1 - \cos 2\omega)$ with $V_0 = 20$ kcal mol^{-1}. The bond angle deformation energy was estimated as $V_\tau = \frac{1}{2}K_\tau(\Delta\tau)^2$ with $K_\tau = 80$ kcal mol^{-1} rad^{-2}. It must be pointed out that the consistency of these expressions with the potential functions [38,39] used for non-bonded energy have not been studied.

TABLE 1

^{1}H N.m.r. parameters of the cyclododecapeptide, (Ala^1-Pro^2-Gly^3-Val^4-Gly^5-Val^6)$_2$, in $CDCl_3$–[$^2H_6$]DMSO (9 : 1)

Parameters	Ala^1	Pro^2	Gly^3 [a]	Val^4	Gly^5 [a]	Val^6
Chemical shift (δ) (±0.01)						
NH	8.04		7.64	7.86	8.59	7.88
CαH	4.77	4.23	3.84, 4.50	4.58	3.47, 4.29	4.32
CβH	1.07	2.20—1.90		2.20—1.90		2.20—1.90
CγH		2.23—1.93		0.95—0.85		0.95—0.85
CδH		3.80—3.60				
Coupling constants (J/Hz) (±0.1)						
^3J(NH-CαH)	7.3		2.7, 5.2	10.0	5.0, 7.5	8.5
^3J(CαH-CβH)	7.0	*b*		4.0		8.5
^3J(CβH-CγH)		*b*		7.0		7.0
^2J(HH)		*b*	−17.5		−16.7	
Temperature coefficient ($\Delta\delta/\Delta T$) (p.p.m./°C)						
Peptide NH × 10^{-3}	−2.7		−3.07	−3.60	−6.50	−4.77

[a] Values are obtained by an ABX spin analysis of the Gly-CH_2 protons. [b] Not analysed.

RESULTS

N.M.R.—The 220-MHz ^{1}H n.m.r. spectrum of the α-CH region of *cyclo*-(Ala^1-Pro^2-Gly^3-Val^4-Gly^5-Val^6)$_2$ is shown in Figure 2a. Figure 2b represents the same spectrum when all the peptide NH protons were exchanged with deuterium by adding a drop of 100% D_2O. Comparison of these two

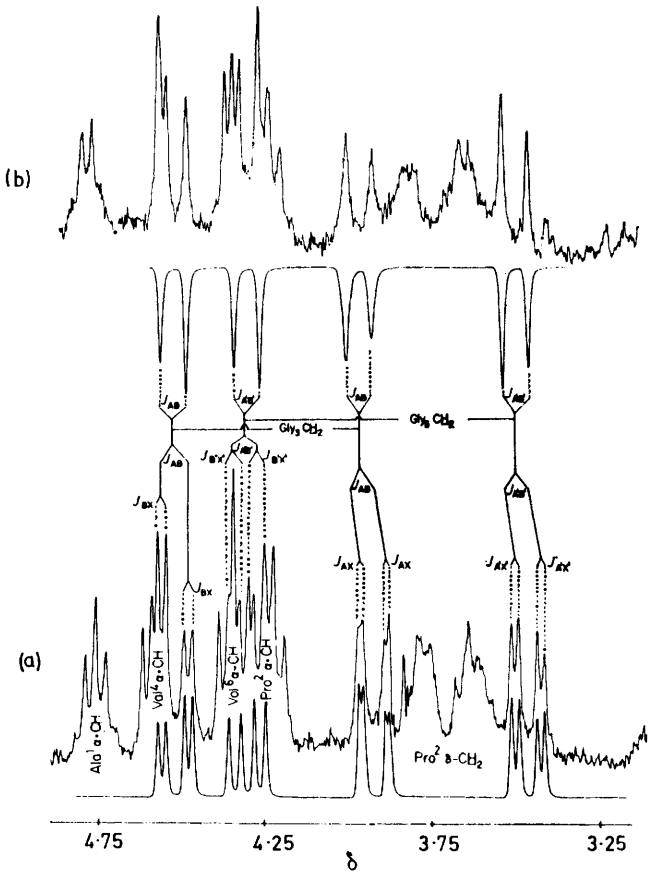


FIGURE 2 220-MHz ^{1}H N.m.r. spectrum of *cyclo*-(APGVGV)$_2$ in 90% $CDCl_3$–10% [$^2H_6$]DMSO: (a) α-proton region matched with the simulated spectrum of Gly_3 and Gly_5 CH_2 protons; (b) α-proton region when all the peptide NH protons are perdeuteriated (note that the AB spin-pattern of Gly_3 and Gly_5 CH_2 protons are also matched with the computer-simulated spectrum)

Figures readily reveals the resonance pattern of alanine, proline, valine, and glycine residues which were also confirmed by spin-decoupling experiments. The delineation of Val^4, Val^6, Gly^3, and Gly^5 was made tentatively on the basis of their previous assignments in dimethyl sulphoxide.[45] These assignments were later verified through their spectral properties (*vide infra*). The values for the chemical shifts and coupling constants are listed in Table 1.

Since temperature-dependence of the peptide NH protons provides information on the inter- and intra-molecular hydrogen-bonding, the temperature coefficients ($\Delta\delta/\Delta T$) for all the NH protons of *cyclo*-(APGVGV)$_2$ were obtained and are given in Table 1. If the -Pro-Gly- peptide sequence induces a β-turn, one would then expect Val^4 NH to be hydrogen-bonded to the Ala^1 C=O and therefore the Val^4 NH proton should show less temperature-dependence which is in agreement with a value of $\Delta\delta/\Delta T = -3.6 \times 10^{-3}$ p.p.m. per °C for the peptide proton assigned to the Val^4 residue (see Table 1). The nuclear Overhauser effect (NOE) has been used to obtain information on the type of β-turns,[33,46,47] whether type I or II. Such an experiment was performed and is depicted in Figure 3. The irradiation of the Pro^2 α-CH proton enhances the signal intensity (33%) of the glycine peptide NH proton at δ 7.64 (see Figure 3). This peptide NH resonance was therefore assigned to the Gly^3 residue. Similar irradiation of Val^4 α-CH proton gave a 24% NOE for the Gly^5 NH proton signal. On the other hand, on saturation of the Val^6 α-CH proton no appreciable NOE was observed on the Ala^1 NH proton. Since Ala^1 α-CH has no corresponding NH proton in the Pro^2 residue and since both the Gly CH_2 protons are overlapped with a widely spread ABX spin pattern (see Figure 2), observation of these NOEs was not possible.

An inter-proton distance of <2.9 Å, between an α-CH proton of one residue and the NH proton of the succeeding residue, is obtained if the torsion angle ψ is in the range 35—200°.[48] Bell and Saunders [49] have derived the relationship (1) between NOE and the distance r (in Å) between two protons within a rigid molecule where $K = 1.8 \times 10^{-2}$ Å^{-6}.

$$\% \text{ NOE} = 100\, r^{-6}/K \qquad (1)$$

To calculate absolute internuclear distances between all the protons, one needs to measure the NOE between all of the spins and then measure the ^{1}H and ^{13}C nuclear spin–lattice relaxation times (T_1) for all the spins. However, we are here interested only in ranges of torsion angle ψ from the

NOE between two spins and, therefore, equation (1) can be utilized to calculate the approximate internuclear distances, provided the rigidity of the molecule is first established. Since identical T_1 values of the backbone α-carbons indicate

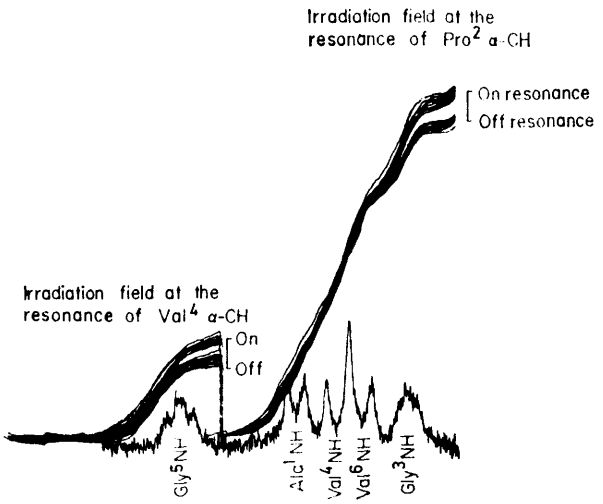


FIGURE 3 NOE observed at 100 MHz: 33% enhancement of the Gly_3 NH proton signal intensity was obtained when Pro^2 α-CH proton was irradiated; whereas 24% enhancement of the Gly^5 NH proton was recorded when the Val^4 α-CH proton was irradiated

the rigidity of a molecule [32] tumbling isotropically in solution, the T_1 values for *cyclo*-$(APGVGV)_2$ were measured in $CDCl_3$–$[^2H_6]$DMSO (9 : 1) and are listed in Table 2. The measured α-carbon T_1 values range from 117 to 125 ms except for glycine α-carbons where T_1 values are slightly higher (160 ms) presumably due to the greater flexibility of the α-carbon expected for a glycine residue.[50] The observed T_1 values are very similar and consistent with the assumption of a relatively rigid molecule undergoing isotropic motion. On the other hand, the α-carbon T_1 values of a flexible linear molecule, undergoing segmental anisotropic motion, have been shown to vary from 450 to 200 ms' approximately, on going from the end residue to the residue at the centre of the molecule.[16] In the case of a molecule undergoing isotropic rotational reorientation, and in the

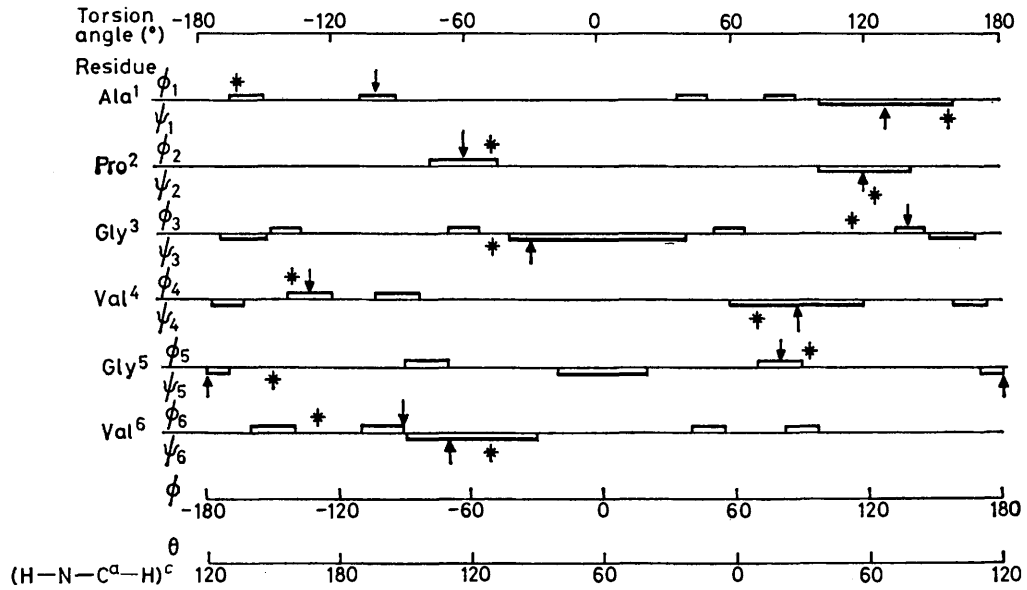
TABLE 2

^{13}C Chemical shifts (δ) and spin–lattice relaxation times (T_1) of *cyclo*-$(Ala^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5\text{-}Val^6)_2$ in $CDCl_3$-$[^2H_6]$DMSO (9 : 1)

Peptide residues	Carbon atom	Chemical shift [δ(p.p.m.) from Me_4Si]	Relaxation time T_1/s
Ala^1	α-CH	46.44	0.117
	β-CH_3	17.25	0.414
	C=O	171.24	2.449
Pro^2	α-CH	61.11	0.125
	β-CH_2	29.09	0.125
	γ-CH_2	25.49	0.132
	δ-CH_2	47.85	0.099
	C=O	172.70	1.637
Gly^3	α-CH_2	43.37	0.080
	C=O	168.75	1.561
Val^4	α-CH	59.50	0.127
	β-CH	30.90	0.204
	γ-CH_3	(19.49, 18.03)	(0.371, 0.441)
	C=O	170.36	2.145
Gly^5	α-CH_2	43.37	0.080
	C=O	169.33	1.819
Val^6	α-CH	58.53	0.123
	β-CH	30.65	0.204
	γ-CH_3	(19.15, 18.03)	(0.324, 0.441)
	C=O	171.92	1.875

TABLE 3

Ranges of ϕ_i [a] and ψ_i [b] values obtained from n.m.r. data. (Arrows indicate the values obtained from the constrained model, while asterisks show the values of structure B from energy calculations)



[a] Values obtained using Bystrov equations. [b] ψ For Gly^3 and Gly^5 are derived from the ^2J analysis, for Pro^2 and Val^4 from NOE and for Ala^1 and Val^6 from the Dreiding model. [c] Dihedral angle θ is related to the torsion angle φ by the equation $\theta = |\phi - 60|$ for L-amino-acids.

absence of significant internal motion, the rotational correlation time (τ) of the molecule can be obtained from the observed T_1 values by using equation (2) where N is the

$$\frac{1}{NT_1} = \frac{\hbar^2\gamma_H^2\gamma_C^2}{10r^6}\left[\frac{\tau}{1+(\omega_H-\omega_C)^2\tau^2} + \frac{3\tau}{1+\omega_C^2\tau^2} + \frac{6\tau}{1+(\omega_H+\omega_C)^2\tau^2}\right] \quad (2)$$

number of directly bonded proton(s), γ_H and γ_C are the gyromagnetic ratios of ^1H and ^{13}C respectively, r is the C–H bond length, ω_H and ω_C are the resonance frequencies, in rad s^{-1}, of ^1H and ^{13}C, respectively. Using the arithmetic mean of the six α-carbon T_1 values (130.4 ms) in equation (2), one obtains two solutions for τ: 3.7×10^{-10} and 0.45×10^{-7} s. The second solution is rejected on the basis that satisfies the extreme narrowing limit and according to expression (3) [51] one expects to observe undiminished

$$\text{NOE} = \frac{5 + \omega^2\tau^2 - 4\omega^4\tau^4}{10 + 23\omega^2\tau^2 + 4\omega^4\tau^4} \quad (3)$$

positive NOEs. Use of equation (3) also demonstrates that the choice of $\tau = 3.7 \times 10^{-10}$ s is quite resonable, since it agrees with the observation of positive values of NOE, while for $\tau = 0.45 \times 10^{-7}$ s a negative NOE should have been observed. The T_1 measurements, therefore, lend support to a rigid structure for *cyclo*-(APGVGV)$_2$ rotating isotropically in solution and equation (1) can be utilized in order to obtain approximate interproton distances, between two spins. Substituting the observed NOE values of 33% for the Pro^2-Gly^3 moiety, and 24% for the Val^4-Gly^5 moiety, one

TABLE 4

Minimum energy conformations of the cyclododecapeptide [a]

Structure			A	B	C	D	E	F	G	H	I
Total energy (kcal mol^{-1} per molecule), E			63.4	63.5	63.9	64.8	66.4	67.1	72.8	74.1	76.8
Backbone torsion angles (°) [a]	Ala^1	ϕ	−107	−163	−153	−142	−154	−157	−165	−166	−166
		ψ	147	157	71	96	77	79	162	142	152
		ω	179	170	157	178	−178	179	161	180	171
	Pro^2	ϕ	−65	−48	−73	−52	−69	−53	−54	−65	−57
		ψ	142	123	126	127	137	132	108	109	120
		ω	−179	−166	176	−169	178	−176	−167	−170	−172
	Gly^3	ϕ	82	115	116	123	81	103	132	140	145
		ψ	−79	−46	−67	−64	−68	−64	−46	−48	−58
		ω	−179	176	174	178	−177	180	177	−173	−177
	Val^4	ϕ	−96	−135	−85	−158	−87	−89	−146	−133	−136
		ψ	103	72	91	140	99	85	111	55	47
		ω	175	−179	−168	−175	178	−177	−167	178	177
	Gly^5	ϕ	−75	94	74	81	70	84	77	80	80
		ψ	135	−149	−174	67	−164	144	−159	142	153
		ω	−178	180	175	179	176	178	180	176	179
	Val^6	ϕ	−86	−128	−131	−120	−141	−133	−107	−127	−115
		ψ	−45	−50	97	107	158	101	−50	74	77
		ω	179	−174	172	−179	178	177	−175	177	180
Side-chain torsion angles (°)	Ala^1 [b], Pro^2 [c]	$\Delta\chi$, Γ	1, −22	−7, 20	−1, −15	−4, 24	−2, −18	−3, 19	−6, 21	−5, −20	−4, 9
	Val^4–Val^6 type [d]		2,2	3,2	2,2	1,2	2,3	2,2	1,2	3,2	3,2
	Val^4 [e]	$\Delta\chi^1$	−1	−20	−1	6	−3	0	−23	−12	−11
		$\Delta\chi^{2,1}$	1	1	−5	11	−4	−4	−11	2	3
		$\Delta\chi^{2,2}$	4	−12	7	−6	5	7	−16	−15	−13
	Val^6 [e]	$\Delta\chi^1$	0	3	5	1	−7	3	3	10	10
		$\Delta\chi^{2,1}$	−11	−10	−7	−11	9	−8	−11	−2	−1
		$\Delta\chi^{2,2}$	5	10	9	6	−10	9	8	11	11
Bond-angle deformations at Cα atoms		$\Delta\tau_1$	0	−3	6	−2	2	0	1	0	1
		$\Delta\tau_2$	6	5	5	7	6	5	2	1	1
		$\Delta\tau_3$	−1	2	−1	−1	4	0	3	1	0
		$\Delta\tau_4$	−6	3	−8	−3	−5	−7	−6	1	0
		$\Delta\tau_5$	0	1	1	1	3	1	0	0	−1
		$\Delta\tau_6$	7	9	4	5	6	4	8	0	3

[a] All angles are rounded off to the nearest degree. The conformational energies E have been rounded off to the first decimal place. Conventions and nomenclature employed follow the recommendations of IUPAC–IUB.[36] [b] $\Delta\chi$ here refers to the change in torsion angle about the Cα–Cβ bond with $\Delta\chi = 0$ for a staggered conformation of the methyl hydrogens. [c] Γ is the pseudo-rotation of the Cγ atom about the Cβ · · · Cδ line. See text and ref. 40. [d] The valyl side-chain conformation is here classified into three types based on the value of the torsion angle χ^1 (N–Cα–Cβ–Cγ^1) of the Cα–Cβ bond. Types 1, 2, and 3 refer to the values for χ^1 of 60, 180, and 300° respectively. The perfectly staggered conformations are taken as ' standard ' conformations from which departures may be measured. [e] All torsion angles $\Delta\chi$ denote the amount of rotation from a perfectly staggered conformation. $\Delta\chi^1$ is the change in the torsion angle χ^1 (N–Cα–Cβ–Cγ^1) from the staggered conformations for the given type of valine. $\Delta\chi^{2,1}$ and $\Delta\chi^{2,2}$ are similarly departures of γ-methyl hydrogens from the perfectly staggered conformation about the C –Cγ bond.

the ^{13}C resonance line-width corresponding to this value is *ca.* 150 Hz, whereas the experimentally observed line width is *ca.* 5 Hz after considering instrumental broadening. The rotational correlation time, τ, for *cyclo*-(APGVGV)$_2$ in 90% $CDCl_3$–10% [$^2H_6$]DMSO and at 27 °C is, therefore, 3.7×10^{-10} s. This value at our magnetic field of 23.48 kG obtains inter-proton distances of 2.35 and 2.48 Å, respectively. As mentioned before,[48] these distances limit the ranges of ψ_2 for Pro^2 and of ψ_4 for Val^4 as shown in Table 3. Absence of an NOE between the Ala^1 N*H* proton and the Val^6 α-CH proton also limits the range of ψ^6 for Val^6. This is also included in Table 3.

The ϕ torsion angles for the alanine and valine residues in *cyclo*-(APGVGV)$_2$ are approximated from the observed

$$^3J_{\alpha\text{-CH-NH}} = 9.4\cos^2\theta - 1.1\cos\theta + 0.4 \quad (4)$$

coupling constants, $^3J_{\alpha\text{-CH-NH}}$ by using the Karplus–Bystrov expression (4) [52] where the dihedral angle θ is related to ϕ (see Table 3). The ϕ torsion angles of the glycyl residues are obtained from equation(5) [52] where ϕ is directly related to the $^3J_{\alpha\text{-CH-NH}}$ coupling constant.

$$^3J_{\alpha\text{-CH(A)-NH}} + {}^3J_{\alpha\text{-CH(B)-NH}} = -9.8\cos^2\phi - 1.3\cos\phi + 15.0 \quad (5)$$

Analysis of the geminal couplings for Gly^3 and Gly^5 residues (−17.5 and −16.7 Hz, respectively) using the relation given by Barfield *et al.*[31] with the ranges of values for ϕ_3 and ϕ_5, gives a range of ψ_3 for Gly^3 and of ψ_5 for Gly^5.

Table 3, therefore, summarizes the ranges of possible ϕ and ψ values for all the amino-acid residues in *cyclo*-(APGVGV)$_2$ as derived from the n.m.r. data and from the Dreiding model of the molecule for an estimation of the torsion angle ψ for Ala^1 since this could not be determined from NOE. Based on the consideration of the intramolecular hydrogen-bonds as indicated by the temperature dependence of the peptide NH protons one develops a static wire model (see Discussion) of the molecule taking into account the ranges of ϕ and ψ values. The torsion angles

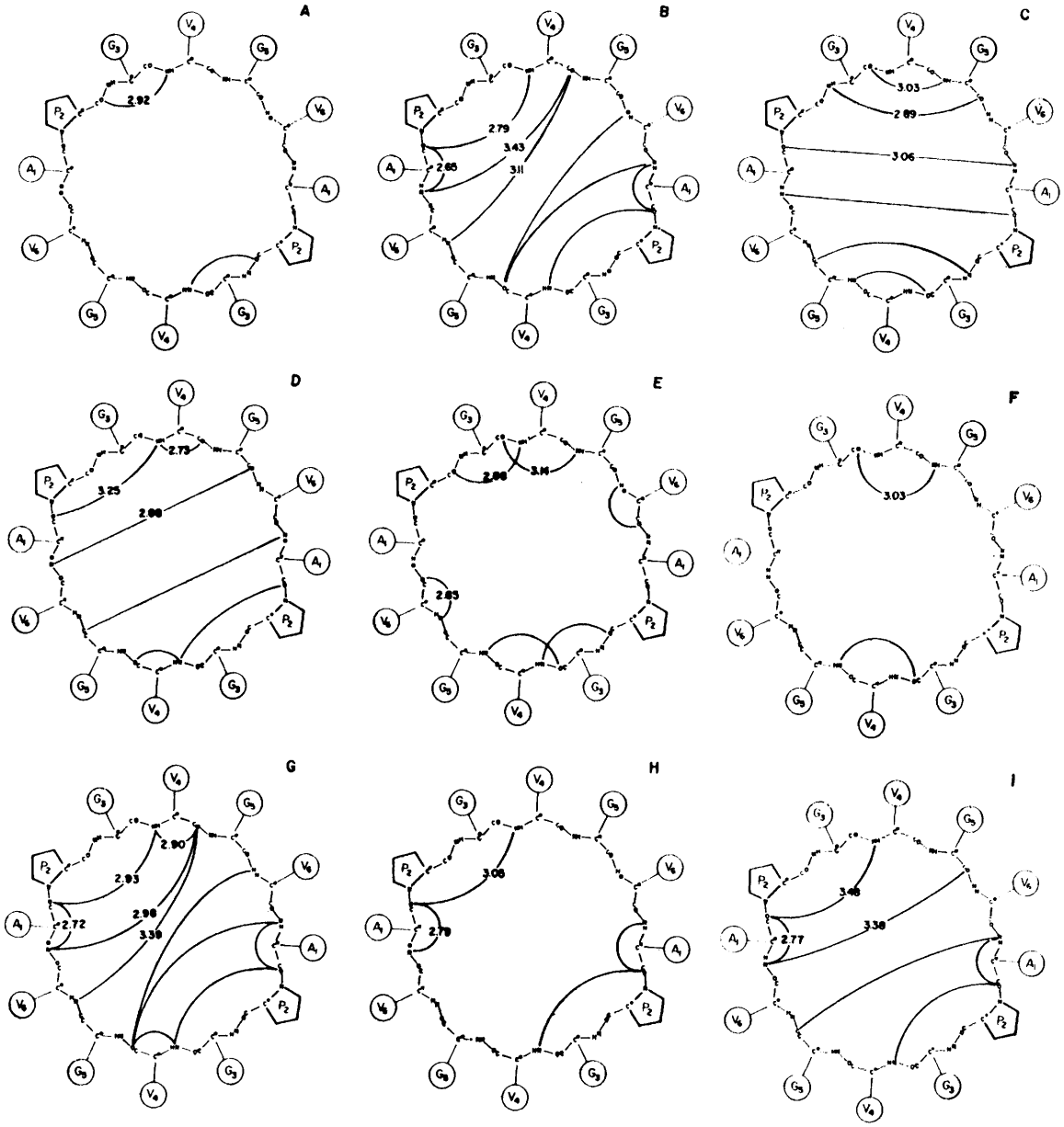


FIGURE 4 A schematic representation of the nine minimum energy conformations of the cyclododecapeptide showing the hydrogen bonds formed. For the purpose of this illustration, we have chosen to show all N · · · O interactions for which the H · · · O distances are <2.6 Å. The N · · · O distances (in Å) are marked. The symbols, A, B, *etc.*, identify the various structures listed in Table 4

estimated from the resulting wire model are indicated by arrows in Table 3.

Calculated Conformations.—Conformational-energy calculations results in several minimum-energy conformations. The structural details of the nine lowest-energy conformations are given in Table 4. A variety of conformations is exhibited by the minimum-energy structures, though they are generally consistent with the torsion-angle ranges deduced from n.m.r. The various structures show different three-dimensional folding, side-chain conformations and intramolecular hydrogen bonds. The interesting network of hydrogen bonds seen in these structures are delineated schematically in Figure 4. The topology of the folding in structures A—D may be appreciated from their perspective drawings in Figure 5.

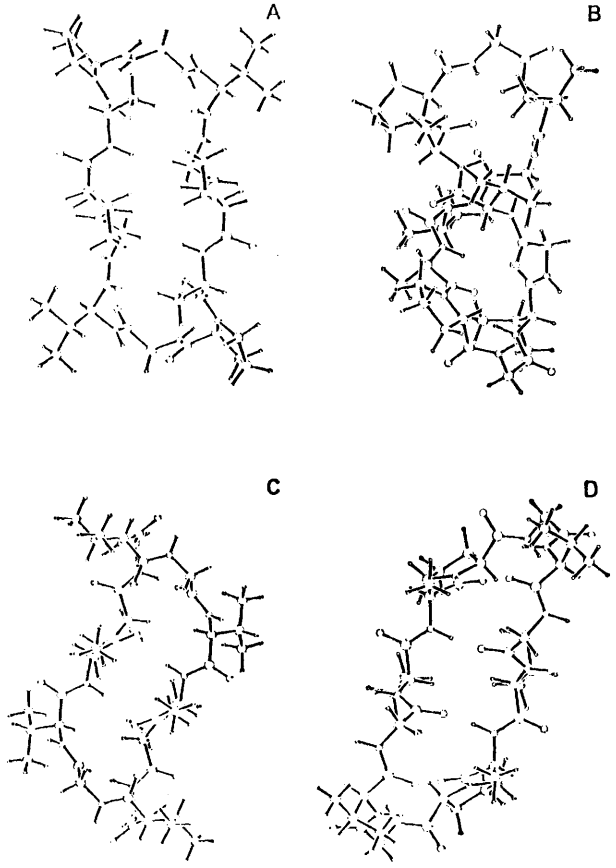


FIGURE 5 Perspective plots of the first four minimum-energy structures listed in Table 4. Except for structure B, all other views shown are down the symmetry axis. For structure B, the view is along a line *ca.* 70° away from the two-fold axis. This is done merely to show its twisted ' 8 ' appearance

Structure C displays an *anti*-parallel arrangement held together by the two 19-atom hydrogen bonds across the alanines with $Gly^3 \longrightarrow Gly^5$ γ-turns at each end. Structure B in this perspective roughly resembles a twisted ' 8 ' form. In this structure, type II β-turns are formed in the Pro^2-Gly^3 sequences. It also shows a pair of bifurcated hydrogen bonds, the C=O of Val^4 bonding with the NH groups of the preceding Val^6 and Ala^1 (17- and 14-atom hydrogen-bonded rings). Structure G is very similar to B, differing primarily in the valyl side-chain orientations. Structure D also has the two Pro^2-Gly^3 β-turns. In addition it shows two 17-atom rings, the NH of Ala^1 hydrogen bonded to the C=O of Gly^5. The remaining structures show several 5- and 7-atom hydrogen bonds.

DISCUSSION

Since single-resonance lines are observed for all chemically-equivalent protons, the conformation of *cyclo*-$(APGVGV)_2$ in $CDCl_3$–$[^2H_6]$DMSO (9 : 1) reflects, within the n.m.r. time scale, the C_2 symmetry of its covalent structure. The overall conformational properties of the molecule may, therefore, be discussed in terms of its repeating unit APGVGV. At this point it would be interesting to compare the spectral properties of this unit with its linear counterpart and with its cyclic counterpart, cyclohexapeptide ⌜-(APGVGV)-⌝.

The Ala^1 NH and the Val^4 NH protons of cyclohexapeptide ⌜-(APGVGV)-⌝ showed the highest shielding [19] on raising the temperature in $CDCl_3$. With this and dihedral angle data, it was concluded,[20] that this cyclohexapeptide forms an *anti*-parallel β-pleated sheet structure containing two β-turns (type II and II'), between the Ala^1 C=O and Val^4 NH groups and between the Ala^1 NH and the Val^4 C=O groups. This is a conformation characteristic of cyclohexapeptides.[6] On the other hand, the linear NF-APGVGV-OMe in $CDCl_3$–$[^2H_6]$DMSO (9 : 1) showed that the Gly^3 NH and Val^4 NH protons are most shielded whereas the Ala^1 NH proton is highly exposed.[53] The solution conformation of this linear counterpart was found to contain a β-turn (type II), stabilized by a 10-membered ring hydrogen-bond between the Ala^1 C=O and the Val^4 NH groups and a γ-turn, an 11-membered ring hydrogen-bond formed between the Gly^3 NH and Gly^5 C=O groups. Interestingly, however, the repeating unit of the *cyclo*-$(APGVGV)_2$ in $CDCl_3$–$[^2H_6]$DMSO (9 : 1) retains spectral properties common to both these linear and cyclohexapeptides. For example, the shielding of Ala^1 NH, Gly^3 NH, and Val^4 NH as indicated by their temperature coefficients ($\Delta\delta/\Delta T$) of −2.70, −3.07, and -3.60×10^{-3} p.p.m. per °C, respectively (see Table 1), reveals that there could occur a conformation containing a β-turn (between Ala^1 C=O and Val^4 NH), a γ-turn (between Gly^3 NH and Gly^5 C=O), and a 14-membered hydrogen-bonded ring (between the Ala^1 NH and Val^4 C=O). The latter stabilized a type II' β-turn in the cyclohexapeptide ⌜-(APGVGV)-⌝, placing -Gly^5-Val^6- at the corners.[20] Similar structural features of the *cyclo*-$(APGVGV)_2$ were also observed in dimethyl sulphoxide and trifluoroethanol from a secondary structural study using both ^1H and ^{13}C n.m.r.[45] Although the partial shielding of Val^6 NH (see Table 1), which was also observed in trifluoroethanol, needs further analyses, inspection of the Kendrew wire model suggests an interaction of the Val^6 NH of one repeating unit with the Val^4 C=O of the second repeating unit of this molecule.

The CPK model of the overall structure of *cyclo*-$(APGVGV)_2$, maintaining a C_2 symmetry, appears very compact. This compactness is substantiated by the

observation of T_1 values of 130 ms for the backbone α-carbon atoms (see the Results section) indicating the rotation of a rigid structure. The magnitude of the non-equivalence of the two glycine methylene protons requires a rigid structure in the absence of aromatic side-chains with the spatial orientation of the planes of the peptide bonds proximal to glycine CH_2 fragments being the decisive factor for inducing non-equivalence. Experimentally, indeed, the AB pattern of the Gly[3] CH_2 protons is split by 0.66 p.p.m. and that of the Gly[5] CH_2 protons by 0.82 p.p.m. (see Table 1 and Figure 2), which is consistent with the rigidity of this molecule as observed from the T_1 results. The interconversion of a mixture of conformations, in principle, can be checked by observing the changes in resonance line-width on lowering the temperature. Our n.m.r. measurements at temperatures as low as −20 °C did not show any appreciable changes in line-width which is again in agreement with the above considered symmetry.

Stepwise Analysis of ^{1}H *N.M.R. Parameters and Development of a Static Model.*[53]—Utilizing the Kendrew wire model with the hydrogen-bond constraints and the torsion angles (ϕ,ψ) restrictions leads to a satisfactory description of the conformational properties of all six residues in the repeating unit of the molecule. For example, the β-turn, stabilized by the 10-membered hydrogen-bonded ring between the Ala[1] C=O and Val[4] NH groups, limits the conformational freedom of Pro[2] and Gly[3] residues involved in the turn. As mentioned in the Introduction, ϕ_2 for Pro_2 was assumed to be fixed at −60° while the ψ_2 could be approximated by using the observed NOE value of 33% in equation (3). A distance of 2.35 Å between the Pro[2] C-H and Gly[3] NH protons as obtained from the NOE gives either 130 or 110° for ψ_2, an average of which is 120° where the ψ_2 was locked. ϕ_3 for Gly[3], as derived from the ^3J values using equation (4), gives a range of 60 to 140°. Since Gly[3] is also involved in the γ-turn (an 11-membered hydrogen-bonded ring between the Gly[3] NH and the Gly[5] C=O groups), this contraint was taken into account before the torsion angles of Gly[3] were obtained from the model. ϕ_3 was measured on the model to be 150° which is closest to the experimentally observed value of 140° and so ϕ_3 was locked at 140°. Although the determination of ψ_3 is rather ambiguous from the existing ^{1}H n.m.r. data, an estimation could be obtained by using Barfield *et al*'s. expression [31] for ψ from the observed geminal (^2J) coupling value. The measured ψ_3 value of −30° from the model falls within range of experimentally derived values (see Table 3). The observed $^3J_{NH\text{-}C\alpha H}$ value of 10 Hz (see Table 1) for Val[4] limits the range of ϕ_4 which falls within the limited range of −140 to −100° (see Table 3) according to the Bystrov expression.[52] The ϕ_4 value that describes the model best is −130°. Use of the observed NOE of 24% between the Val[4] CαH and the Gly[5] NH protons was made as described above to obtain an approximated value for ψ_4 which agrees with the model at 90°. Similar analyses of the ^{1}H n.m.r. parameters as given for Gly[3] (*vide supra*) was made for Gly[5] and the experimentally derived torsion angles, ϕ_5 and ψ_5, which describe the model best are 80 and ±180°, respectively. The third hydrogen-bonding constraint was imposed by the 14-membered hydrogen-bonded ring formed between the Ala[1] NH and Val[4] C=O groups. The $^3J_{NH\text{-}C\alpha H}$ coupling of 7.3 Hz for Ala[1] (Table 1) gives a wide range for the torsion angle ϕ_1 (see Table 3). The value that appears to fit the wire model best is −100°. Although the lack of an NH group with proline following Ala[1] precludes observation of an NOE to obtain information on ψ_1 of Ala[1], a value of 130° seems to be appropriate for the wire model. Val[6] is the only residue that remains outside of all these ring constraints and torsion angles restrictions. The $^3J_{NH\text{-}C\alpha H}$ value of 8.5 Hz for Val[6] (see Table 1), again according to Bystrov,[52] gives a wide allowable range for ϕ_6 (see Table 3). The wire model indicates the torsion angle ϕ_6 to be near −90° which falls within the experimental range. No direct experimental evidence could be put forward for the determination of the ψ_6 torsion angle except that the absence of an NOE between the Val[6] CαH and Ala[1] NH protons (see the Results section) restricts ψ_6 to the range of −10 to −120° (see Figure 4 in ref. 53). A value of $\psi_6 = -70°$ is in good agreement with the Kendrew wire model.

The side-chain orientations of the two valine residues in *cyclo*-$(APGVGV)_2$ are of particular interest. The $^3J_{C\alpha H\text{-}C\beta H}$ value of 4.0 Hz for Val[4] indicates the predominance of a *gauche* conformation for the Val[4] side-chain, while the Val[6] side-chain prefers a *trans*-orientation as indicated by its $^3J_{C\alpha H\text{-}C\beta H}$ coupling value of 8.5 Hz (see Table 1). In the cyclohexapeptide, $\overline{\text{-(APGVGV)-}}$, both valine residues adopted off-*gauche* conformations for their side chains [20] whereas in linear HCO-APGVGV-OMe the preferred conformation of both valine side chains was *trans*.[53] The side-chain orientations of the valine residues in these peptide systems may, therefore, indicate a close correlation with the backbone conformations as Benedetti [54] has shown from a collection of oligopeptide crystal structures.

The overall structural characteristics of this cyclododecapeptide appears to retain conformational features common to both the linear and cyclic hexpeptides, but does not in all its details represent the conformation of polyhexapeptide $(APGVGV)_n$. The repeating unit in cyclo-$(APGVGV)_2$ maintains in common with the polyhexapeptide the conformational features of the β- and γ-turns, but it has a 14-membered hydrogen-bonded ring and no 23 atom hydrogen-bonded ring which has been proposed for the polyhexapeptide.[15] Possibly with more repeats, a cyclic structure can be formed which will exhibit all of the essential properties of the proposed β-spiral of the linear polyhexapeptide and thereby represents a cyclic correlate.

Of all the minimum-energy conformations obtained from calculations, only structure B seems to be consistent with most of the n.m.r. data. All the backbone torsion angles for this conformation (denoted by asterisks in Table 3) lie within or near n.m.r. acceptable ranges. In this context, it may be noted that a large difference

between B and C is seen in the torsion angle ψ_6. A value of *ca.* 90° for this angle, found in C, is not acceptable, since such a value should result in a large NOE. Since this was not observed, structure C has to be rejected on the basis of this torsion angle itself. Structure A has an acceptable value of ψ_6; also all the torsion angles are within the acceptable ranges. In this case, however, the structure prefers the valines in the *trans*-conformation, while the n.m.r. analysis described earlier shows that the Val[4] should be in a *gauche* conformation. Also, A does not show any of the secondary structural features expected.

On the other hand, structure B displays most of the hydrogen bonds expected with the exception of a minor discrepancy. The γ-turn hydrogen bond expected from n.m.r. is not strictly found in this minimum energy structure, as the distance $O_{Gly}5 \cdots N_{Gly}3$ is found to be 3.99 Å. Nevertheless, this structure shows the type of chain reversal expected from a combination of β- and γ-turns reported for the elastin repeat peptides. Exploration of the conformational space near this structure did not yield a lower energy structure with a stronger γ-turn hydrogen bond. Figure 6 shows a pair of stereographic

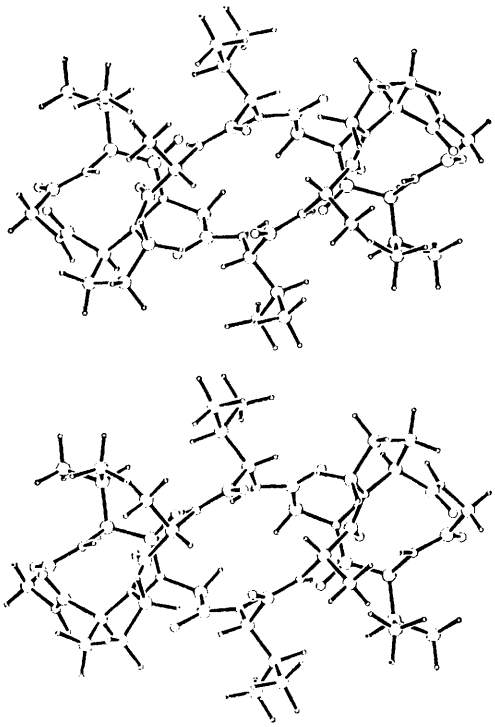


FIGURE 6 A pair of stereo-ORTEP plots of the structure of the cyclododecapeptide down the two-fold axis. This is the structure that shows the basic features expected from the n.m.r. studies. Rotate Figure 90° for stereoview

plots of this structure. The proline ring in this structure is ' up '-puckered. The Val[4] side-chain is −20° away from *gauche*$^+$ (χ^1 *ca.* 60°) orientation, while Val[6] is in the *trans*-conformation. A comparison between structures B and G is interesting. The backbone folding and the hydrogen bonding in these structures are quite similar. However, Val[4] has a *gauche*$^-$ (χ^1 *ca.* +60°) orientation in structure G. It may be seen that this change from *gauche*$^+$ orientation results in an increase in total energy of *ca.* 9 kcal mol^{-1} molecule^{-1}. While the magnitude of χ^1 (Val[4]) was inferred from the α-CH-β-CH coupling constant, the sign of the side-chain can thus be determined from energy considerations for this structure.

The facts that a wire model can be built satisfying simultaneously the torsion angle ranges and the intramolecular hydrogen bonds expected from the experimental studies reported here, and that the energy calculations result in a low energy conformation quite similar to the wire model, illustrate once again the self-consistency and the usefulness of the ' n.m.r.-energy ' approach. N.m.r. has provided valuable reliable information regarding the torsion-angle ranges and the secondary structural features, thus considerably simplifying (what is otherwise) a complex problem of a complete search of the multi-dimensional conformational space. In turn, the conformational-energy calculations, with all the inevitable approximations therein, help to refine the torsion angles of the wire model, thus effecting a synthesis of the experimental data and theoretical principles governing the detailed structure of a cyclic molecule.

The cyclododecapeptide presents a particularly interesting challenge. Since it appears to be rigid and since the crystallization appears dominated by intermolecular hydrophobic side chain contacts (*i.e.* the inverse temperature transition for crystallization from water), the likelihood that the crystal and solution structures are the same is substantial. Accordingly, future comparisons of the present report with a detailed crystal structure should provide a most informative guide as to the reliability of the present approaches and should define useful refinements.

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