Conformational Study of the Cyclic Hexapeptide [L-Ala-L-Pro-Gly-L-Val-Gly-L-Val], by Nuclear Magnetic Resonance Spectroscopy

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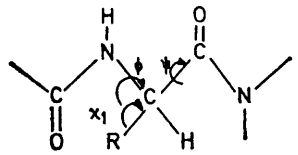
The cyclohexapeptide [L-Ala^1-L-Pro^2-Gly^3-L-Val^4-Gly^5-L-Val^6] has been synthesized and its conformational features have been derived from n.m.r. parameters in chloroform. The nuclear Overhauser effect (n.O.e) has been used to distinguish between the two glycine and valine residues in the molecule. ^{13}C Spin–lattice relaxation times (T_1) have been measured and an average T_1 value of 189 ms with a range of 158—202 ms for the α-carbon atoms has been obtained, which is indicative of a relatively rigid molecule. The n.O.e. and the geminal coupling constants [^2J] and ^3J(NH–αCH) have been utilized to obtain an estimation of the torsion angles ψ and ϕ. Two β-turns, formed between the Ala^1 NH and the Val^4 (C=O (type II') and the Val^4 NH and the Ala^1 C=O (type II), are proposed. The conformational properties of this cyclic molecule are discussed in relation to the conformational features of its linear counterpart.

STUDIES on the conformational properties of oligopeptides by physical methods have been the subject of major interest in recent years.[1-5] A number of conformations have been observed, which are designated as α-helix, 3_{10} helix, β-pleated sheet, β-helix, β-turn, *etc.* These conformations are conveniently characterized by the various combinations of the torsion angles ϕ and ψ, corresponding to the rotation at the N–C$^\alpha$ and C$^\alpha$–C′ bonds, respectively, as shown in the peptide molecular fragment (1). ^{1}H N.m.r. spectroscopy has been used to



(1)

obtain an estimate of the torsion angle ϕ by observing the ^3J(NH–αCH) coupling and using the Karplus–Bystrov relation (1),[6] where θ is related to the torsion angle ϕ by equation (2).[7]

$$^3J(\text{NH–}\alpha\text{CH}) = 9.4\cos^2\theta - 1.1\cos\theta + 0.4 \quad (1)$$

$$\theta = |\phi - 60°| \text{ for L-amino acid residues} \quad (2)$$

Methods for determining the conformational properties of the C$^\alpha$–C′ bond, *i.e.* the torsion angle ψ, are not as well developed. Barfield *et al.*[8] have derived a relation for obtaining an estimate of ψ from the geminal coupling [^2J(HH)] for the glycine residue in peptides. One of the ^{1}H n.m.r. methods, namely the nuclear Overhauser effect (n.O.e.), has been utilized particularly for the purpose of assignments and to show proton–proton proximity.[9-12] Gibbons *et al.*,[12] in a study of gramicidin S, were the first to use n.O.e. evidence for the occurrence of β-turns. In the gramicidin S β-turn, however, there is no NH_{i+2} proton (as it is a prolyl residue) with which to interact through space with the CH_{i+1} proton. This is the specific interaction utilized for the elastin peptides [10] and which can generally be used for delineation of the type of β-turn. Recently, Leach *et al.*[13] have noted that the n.O.e. results can be used to derive an estimate of the torsion angle, ψ_{i+1}. The n.O.e. in this manner, has been applied to conformational studies on the repeat hexapeptide of tropoelastin,[14] HCl·Ala^1-Pro^2-Gly^3-Val^4-Gly^5-Val^6-OMe.

Cyclic peptides, owing to the cyclic constraint, have facilitated the development of n.m.r. methods [1-3] and can facilitate characterization of the spectral behaviour and conformation of linear counterparts. While the linear repeat peptides of tropoelastin have been extensively characterized by n.m.r. methods,[4,15] we are interested in synthesizing the cyclic counterparts of repeat peptides of tropoelastin in order to compare their conformational properties in solutions. With this purpose, the cyclic analogues of the repeat hexapeptide of tropoelastin were synthesized. While the conformational properties of the cyclododecapeptide [(Ala^1-Pro^2-Gly^3-Val^4-Gly^5-Val^6)$_2$] have been reported,[16,17] for the desired complete perspective the conformational behaviours of other cyclic analogues are required. Therefore, the cyclic hexapeptide,

[L-Ala^1-L-Pro^2-Gly^3-L-Val^4-Gly^5-L-Val^6] [abbreviated as *cyclo*(APGVGV)] was synthesized, and its confirmational characteristics in chloroform, obtained by using n.m.r. methods, are reported.

EXPERIMENTAL

Peptide Synthesis.—The synthesis of the hexapeptide H-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-ONp·CF_3CO_2H has been reported elsewhere.[18] To achieve cyclization, a solution of H-Val-Ala-Pro-Gly-Val-Gly-ONp·CF_3CO_2H (2 g, 2.73 mmol) in dimethylformamide (15 ml) and acetic acid (1 ml) was added dropwise, during 8 h with stirring, to pyridine (1.5 l) at 70—80 °C [reaction (3)]. 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 : 9 v/v) and passed through a column (2 × 30 cm) of Bio Rad mixed bed resin, AG 501-X8(d).

$$\text{H-Val-Ala-Pro-Gly-Val-Gly-ONp}\cdot CF_3CO_2H \xrightarrow{\text{pyridine}} \textit{cyclo}(\text{-Val-Ala-Pro-Gly-Val-Gly-}) \quad (3)$$

The column was washed with the same solvent (200 ml) and the effluent (250 ml) was evaporated. The resulting oil was redissolved in water (200 ml) and lyophilized to yield a fluffy powder (940 mg, 71.7%), m.p. 187—197 °C (Found: C, 55.15; H, 7.6; N, 17.5. $C_{22}H_{36}O_6N_6$ requires C, 55.0; H, 7.55; N, 17.5%). T.l.c. on silica gel G showed R_F values of 0.28 and 0.63 for the solvent systems chloroform–methanol–acetic acid (95 : 15 : 3 v/v) and n-butanol–acetic acid–water (4 : 1 : 1 v/v), respectively.

N.M.R. Experiments.—A 0.01M solution of *cyclo*(-L-Ala[1]-L-Pro[2]-Gly[3]-L-Val[4]-Gly[5]-L-Val[6]-) in $CDCl_3$ was used. A sample for nuclear Overhauser experiments was degassed in the n.m.r. tube by freeze-thawing and then sealed *in vacuo*. The n.m.r. spectra were obtained on a Varian HR-220 spectrometer operating at a probe temperature of 22 °C. The simulated spectra were obtained with the SS-100 computer system of the spectrometer by means of a Varian Data Machine spin-simulation program.

The n.O.e. was measured on a JEOL PS-100 spectrometer operating in the internal lock mode at 100 MHz and 26 °C. The enhancement of signal intensity was obtained by the electronic integration provided with the spectrometer. The signal intensity was recorded at least five times. At least 1 min was allowed between the successive integrations to allow recovery of the magnetization vector, M_z. To check the 100 MHz decoupler spill-over, experiments were performed by offsetting the irradiation field 50 Hz on each side of the resonance of interest to demonstrate that no appreciable change in intensities of the observed signals was found. The accuracy of the n.O.e. values is about ±2.5%.

The torsion angles ϕ of the Val and Ala residues were approximated from the experimentally derived coupling constant, ^3J(NH–αCH) using equations (1) and (2). The ϕ torsion angles for the glycine residues were also approximated by means of a Karplus–Bystrov expression (4),[6] where ϕ is determined directly from this equation.

^{13}C Spin–lattice relaxation times (T_1) were obtained from a 0.05M sample of *cyclo*(APGVGV) dissolved in $CDCl_3$. In order to remove the dissolved oxygen, dry argon gas was passed through the sample in the microcell for about 15 min. A JEOL FX-100 n.m.r. spectrometer equipped with a multinuclear probe was used to measure the T_1 values for the ^{13}C nuclei at 25.15 MHz (2.35 T) using the inversion recovery method (180–τ–90 pulse sequence) where a 38-μs pulse was used for the 180° tilt while a 19-μs pulse was used for the 90° tilt. A Texas Instruments 980-B computer was used to calculate the T_1 values using 4—7 data 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 measurements after the relaxation times had been determined.

$$^3J(\text{NH–}\alpha\text{CH}) + {}^3J(\text{NH–}\alpha\text{CH}) = -9.8\cos^2\phi - 1.3\cos\phi + 15 \quad (4)$$

RESULTS

A 220-MHz n.m.r. spectrum of the α-proton region of the *cyclo*(APGVGV) is shown in Figure 1(a). The peptide NH protons were exchanged with deuterium, and the simplified spectrum of the α-protons is depicted in Figure 1(b). The signals for the alanine, proline, glycine, and valine residues could readily be recognized from their splitting pattern, by comparing the Figures 1(a) and 1(b),

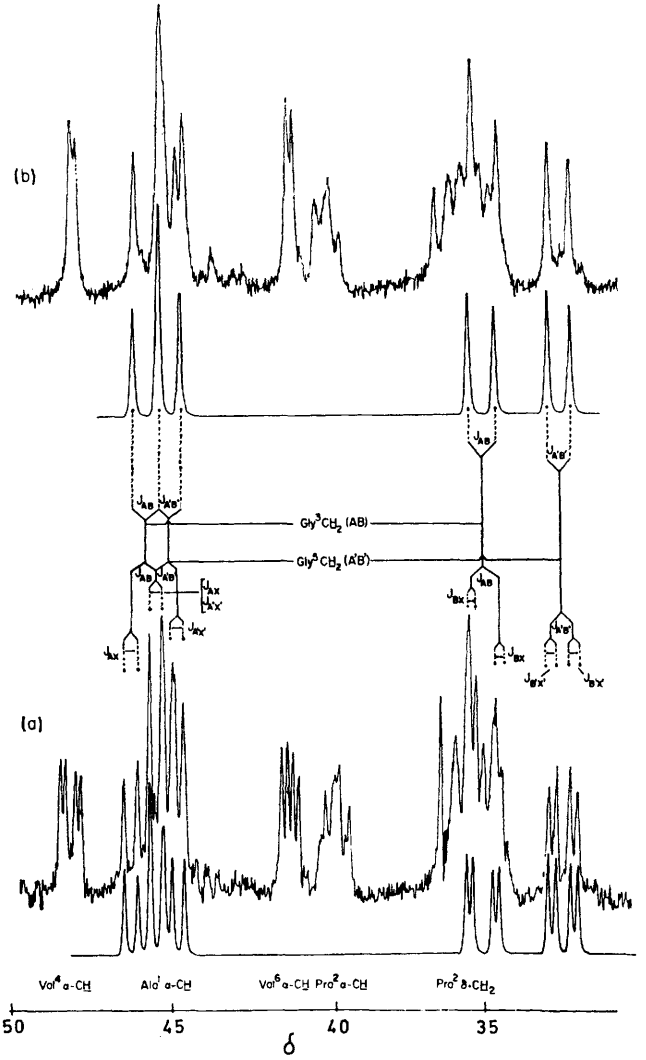


FIGURE 1 220-MHz ^1H n.m.r. spectrum of *cyclo*(-Ala[1]-Pro[2]-Gly[3]-Val[4]-Gly[5]-Val[6]-) in $CDCl_3$: (a) α-proton region matched with the simulated spectrum of Gly[3] and Gly[5] CH_2 protons, analysed as an ABX spin system; (b) α-proton region where all the peptide NH protons were exchanged with deuterium. (Note that the AB spin-pattern of Gly[3] and Gly[5] CH_2 protons are also matched with the computer-simulated spectrum)

and by spin-decoupling. However, it was relatively difficult to distinguish between the Gly[3] and Gly[5] and the Val[4] and Val[6] resonances. In many instances it has been shown that the -L-Pro-Gly- fragment in a peptide sequence promotes the formation of a type II β-turn (a 10-membered H-bonded ring).[3,4,16,19] If such a turn is formed, it brings the Pro[2] α-CH and Gly[3] NH protons into juxtaposition. The n.O.e. was used to show this proton proximity in repeat peptides of tropoelastin.[10] Therefore, the n.O.e. was

TABLE 1

^{1}H N.m.r. parameters of *cyclo*(-Ala^1-Pro^2-Gly^3-Val^4-Gly^5-Val^6-) in $CDCl_3$ (Me_4Si was used as an internal standard)

Parameter	Ala^1	Pro^2	Gly^3	Val^4	Gly^5	Val^6
Chemical shifts (δ values) (±0.01)						
NH	7.47		7.08	7.78	7.60	7.31
α-CH	4.55	4.01	3.57,[a] 4.60 [a]	4.82	3.33,- 4.53 [a]	4.16
β-CH	1.31	*b*		2.64		2.43
γ-CH		*b*		1.03, 0.92		0.74, 0.92
δ-CH		3.61				
Coupling constants in Hz (±0.1)						
^3J(αCH–NH)	7.0		9.0, 3.5	10.0	8.0, 4.0	7.5
^3J(αCH–βCH)	7.0	*c*		3.0		4.0
^3J(βCH–γCH)		*c*		7.0		7.0
^2J(αα)			−17.5 [a]		−15.0 [a]	
Temp. coeff.[d] ($\Delta\delta/\Delta T$) in p.p.m./°C						
Peptide NH	0.0023		0.0067	0.0029	0.0050	0.0042
Torsion angle in degrees						
ϕ	−160, −80 [e]	−60 [f]	125	−140	60	−70
ψ	140 [f]	100 [g]	−30	140 [g]	−80	−40 [f]

[a] Values obtained from an ABX spin analysis. [b] Overlapped between δ 2.22 and 1.90. [c] Not analysed. [d] Values obtained in the temperature range 20—60 °C and at a sample concentration of 0.01M. [e] This range of values could be obtained from Table 2 of ref. 14. [f] From Dreiding model. [g] From n.O.e. and Dreiding model.

utilized initially to show the proximity between the Pro^2 α-CH proton and its adjacent Gly^3 NH proton. An 18% signal enhancement of the glycine NH proton at δ 7.08 was observed on irradiation of the Pro^2 α-CH proton. This is shown in Figure 2(b). This NH signal was, therefore, assigned to the Gly^3 residue. The remaining Gly NH signal at δ 7.60 was then assigned to the Gly^5 residue.

Consideration of the literature [3,20-22] shows that the existence of two β-turns is a general conformational characteristic of cyclic hexapeptides. Since the foregoing data suggest that the -Pro^2-Gly^3- sequence comprises one of two possible β-turns in *cyclo*(APGVGV), -Gly^5-Val^6- would be expected to be the sequence of the second β-turn. An n.O.e. between these two residues would therefore be expected but this is experimentally precluded owing to the multiplet ABX spin pattern of the Gly^5 CH_2 protons. How-

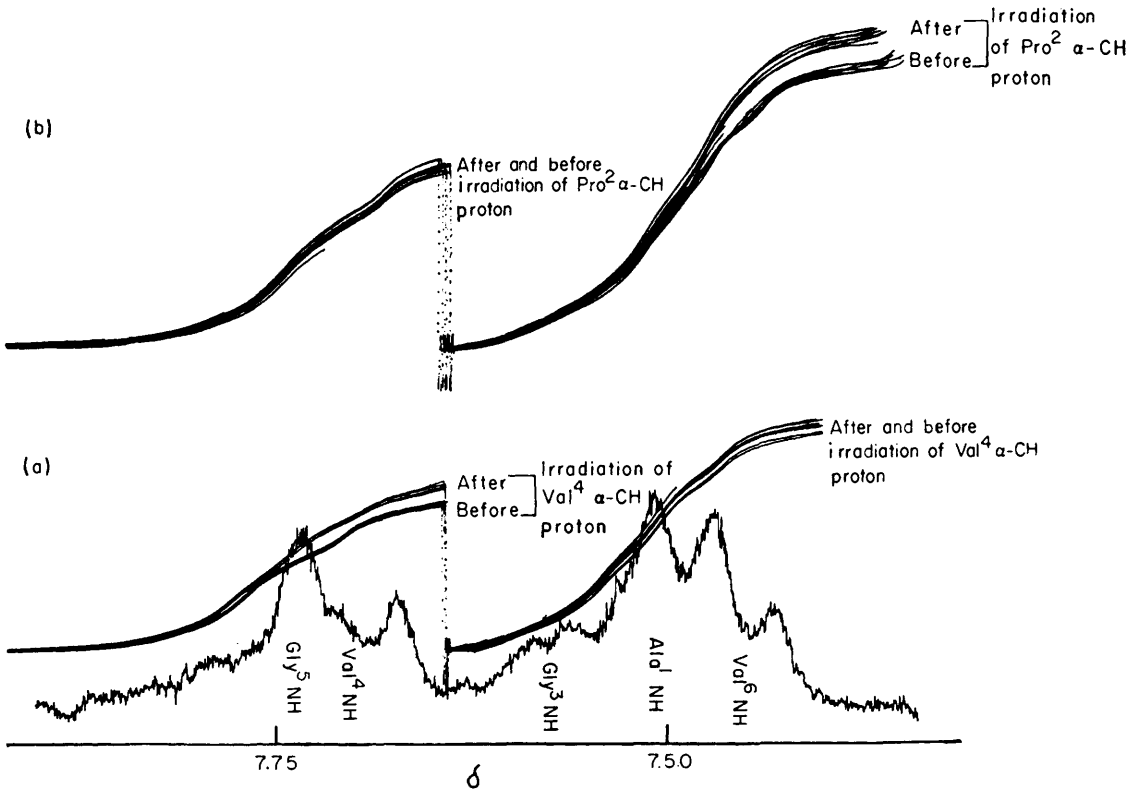


FIGURE 2 N.O.e. observed at 100 MHz and at a concentration of 0.05M of *cyclo*(-Ala^1-Pro^2-Gly^3-Val^4-Gly^5-Val^6-) in $CDCl_3$: (a) 15% enhancement of the Gly^5 NH proton signal intensity on irradiation of the Val^4 α-CH proton; (b) 18% enhancement of the Gly^3 NH proton signal intensity on irradiation of the Pro^2 α-CH proton. (Larger downfield shifts of Gly^3 NH, Gly^5 NH, and Val^6 NH protons could be observed here at a higher concentration of 0.05M, which is consistent with solvent exposure of these protons; conversely, the Ala^1 NH and the Val^4 NH protons are solvent shielded or intramolecularly H-bonded)

ever, in accordance with the foregoing conformational properties, models of this molecule show a proximity of the Val[4] α-CH and the Gly[5] NH protons; irradiation of the Val α-CH proton at δ 4.82 gave a 15% signal enhancement on the Gly[5] NH at δ 7.60 [see Figure 2(a)], whereas no n.O.e. was observed on irradiation of the Val α-CH proton at δ 4.16 (not shown in this Figure). Therefore, the α-CH proton signal at δ 4.82 was assigned to the Val[4] α-CH proton. The ABX spin pattern of each glycine residue was assigned by spin-decoupling of the respective NH protons. The chemical shifts and coupling constants of all the residues are in Table 1.

After completion of the assignments of all the peptide NH resonances, the behaviour of these resonances was observed with variation of temperature. A plot of the chemical shifts of the peptide NH protons *vs.* temperature in the range 20—60 °C is shown in Figure 3. The temperature

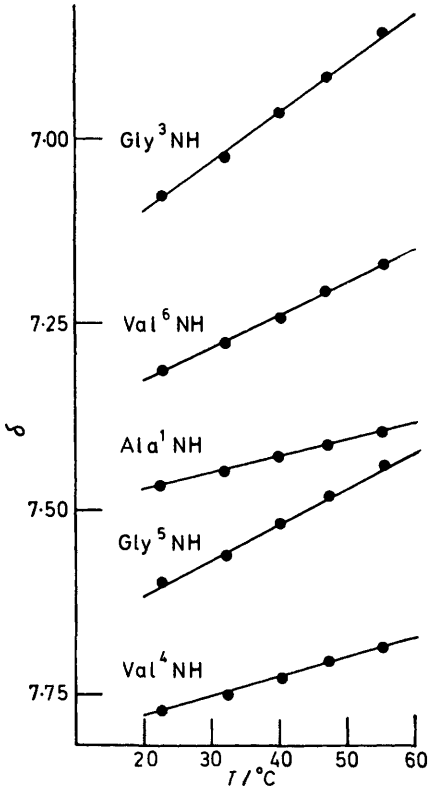


FIGURE 3 Variation of chemical shifts of peptide NH protons of *cyclo*(APGVGV) in $CDCl_3$ as a function of temperature

coefficients ($\Delta\delta/\Delta T$) of these peptide NH protons are also listed in Table 1. During the peptide NH proton exchange with deuterium, a slow exchange rate was observed for the Ala[1] NH and Val[4] NH protons. From the smaller temperature dependence of Ala[1] NH (0.002 3 p.p.m./°C) and Val[4] NH (0.002 9 p.p.m./°C) protons (see Figure 3) and from their slow exchange rate with deuterium, these two peptide NH protons are likely to be involved in intramolecular H-bonds. On the basis of analogy with other cyclic hexapeptide conformations,[3] a Dreiding molecular model of *cyclo*-(APGVGV) was constructed (Figure 4). If two intramolecular H-bonds, between the Ala[1] NH and the Val[4] C=O and between the Val[4] NH and the Ala[1] C=O (see Figure 4) are formed, the structure appears to be fairly rigid and as such its relationship to the observed ^1H n.m.r. parameters can be readily considered.

Using equations (1), (2), and (4), four sets of values for the torsion angles ϕ for Ala[1], Gly[3], Val[4], Gly[5], and Val[6] could be obtained from the observed ^3J(NH–αCH) coupling constants, while ϕ for Pro[2] is fixed at $-60°$ since it is part of the pyrrolidine ring. Only one set of values for ϕ, which is compatible with the Dreiding model, is listed in Table 1. An estimate of ψ_3 for Gly[3] and ψ_5 for Gly[5] could be obtained from the geminal coupling constants (^2J), using the Barfield *et al.*[8] ϕ—ψ map of ^2J values. ψ Values thus obtained for Gly[3] and Gly[5], which are comparable again with the Dreiding model, are given in Table 1.

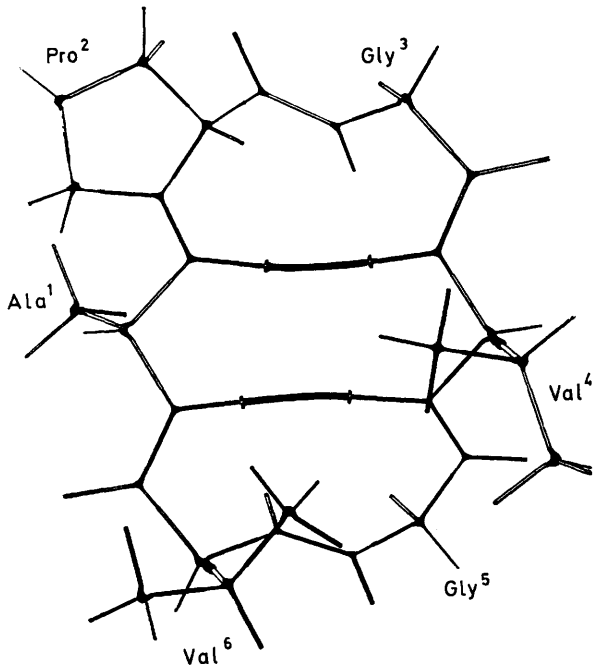


FIGURE 4 Dreiding model of the molecular conformation of *cyclo*-(APGVGV) based on the n.m.r. parameters in $CDCl_3$. (The two intramolecular H-bonds, between the Ala[1] NH and Val[4] C=O groups and the Val[4] NH and Ala[1] C=O groups, are shown by the flexible spring connections)

Recently Leach *et al.*[13] have argued that the backbone torsion angle, ψ, could be evaluated from the inter-proton distance between the α-CH proton of a given residue and the peptide NH proton of the following residue. Bell and Saunders [23] have parameterized expression (5) for the inter-proton distance within a rigid molecule, and have found

$$\% \text{ n.O.e.} = 100/Kr^6 \qquad (5)$$

$K = 1.8 \times 10^{-2}$ where r is the inter-proton distance in Å. Since common T_1 values for α-carbon atoms indicate the rigidity of a molecule [24] tumbling isotropically in solution, the T_1 values for *cyclo*(APGVGV) were measured in $CDCl_3$ in order to establish the utility of equation (5). Experimentally observed T_1 values of *cyclo*(APGVGV) in $CDCl_3$ are given in Table 2. Tentative assignments are made on the basis of data for its linear counterpart.[18] Table 2 shows that the T_1 values for the six α-carbon atoms range from 168 to 202 ms; these are very similar and consistent with the assumption of a 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 on going from the end residue to the residue at the centre of the molecule.[5] In the case of a molecule undergoing isotropic rotational re-orientation and in the 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 (6), where N is the number of

TABLE 2

Carbon-13 n.m.r. parameters of *cyclo*(-Ala[1]-Pro[2]-Gly[3]-Val[4]-Gly[5]-Val[6]-) in $CDCl_3$

Residue	Carbon atom	Chemical shift in p.p.m. from Me_4Si	Spin–lattice relaxation time in s (NT_1)
Ala[1]	Cα	46.8	0.168
	Cβ	16.9	1.686
	C=O	172.0	1.988
Pro[2]	Cα	63.1	0.202
	Cβ	28.9	0.489
	Cγ	25.8	0.266
	Cδ	45.1	0.227
	C=O	173.2	1.588
Gly[3]	Cα	43.7	0.189
	C=O	170.6	1.704
Val[4]	Cα	57.2	0.183
	Cβ	30.6	0.207
	Cγ	18.9, 19.6	1.280, 1.280
	C=O	171.3	2.137
Gly[5]	Cα	43.7	0.189
	C=O	169.8	2.234
Val[6]	Cα	59.6	0.201
	Cβ	30.6	0.207
	Cγ	17.5, 19.3	1.055, 1.189
	C=O	171.3	2.137

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

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 (usually 1.09 Å); and ω_H and ω_C are the resonance frequency, in rad s^{-1}, of ^1H and ^{13}C, respectively. Using the arithmetic average of the six α-carbon T_1 values of 189 ms in equation (6), two solutions for τ are obtained: 2.5×10^{-10} and 1×10^{-7} s. The line-width corresponding to $\tau = 2.5 \times 10^{-10}$ gives a value of 5 Hz while $\tau = 1 \times 10^{-7}$ gives a value of about 180 Hz. Based on the observed line-width of 6—8 Hz for the α-carbon resonances, 2.5×10^{-10} s is, therefore, taken as the correlation time (τ) of *cyclo*(APGVGV) in $CDCl_3$ at 27 °C. This value of τ at our magnetic field of 23.48 kG satisfies the extreme narrowing limit and according to the expression [25] (7) one expects to observe

$$\text{n.O.e.} = \frac{5 + \omega^2\tau^2 - 4\omega^4\tau^4}{10 + 23\omega^2\tau^2 + 4\omega^4\tau^4} \quad (7)$$

undiminished positive n.O.e.s. Use of equation (7) also shows that the choice of $\tau = 2.5 \times 10^{-10}$ is quite reasonable, since it agrees with the observation of a positive n.O.e., while for $\tau = 1 \times 10^{-7}$ a negative n.O.e. should have been observed. Therefore, the experimentally observed n.O.e. values in this study could be used in equation (5), neglecting the cross-correlation contribution,[9] in order to obtain the inter-proton distance. The experimentally observed n.O.e. of 18% on the Gly[3] NH proton (see above) gives an inter-proton distance of 2.59 Å between the Gly[3] NH proton and the Pro[2] α-CH proton. Similarly, an inter-proton distance of 2.68 Å between the Val[4] α-CH proton and the Gly[5] NH proton is obtained from the observed n.O.e. value of 15%. Maintaining these distances in the Dreiding model, approximate values of ψ_2 for Pro[2] and ψ_4 far Val[4] were obtained and these are also listed in Table 1.

The multiplet pattern of the Pro[2] δCH_2 proton resonances and the overlapping of the Ala[1] α-CH proton precluded the observation of an n.O.e. for estimating the torsion angle ψ_1 for Ala.[1] Approximate values of ψ_1 for Ala[1] and ψ_6 for Val[6] were obtained from the Dreiding model and are given in the Table 1. It should be noted in the model (Figure 4) that the Ala[1] NH and Val[6] α-CH protons are furthest apart (*ca.* 3.8 Å) and no n.O.e. would be expeeted. As already noted, this n.O.e. was not observed.

DISCUSSION

The observation of a single resonance for each group of proton(s), *e.g.* peptide NH, α-CH (see Figure 1), β-, γ-, and δ-CH protons, indicates a single time-averaged conformation of the *cyclo*(APGVGV) in chloroform on the ^1H n.m.r. time-scale. The absence of any additional detectable signals precludes to within a few percent the existence of other conformations due to the *cis–trans* isomerism at the -Ala[1]-Pro[2]- peptide bond, as has been observed previously in *cyclo*(-Ser-Pro-Gly-)$_2$ [26] and in *cyclo*(-Val-Pro-Gly-)$_2$.[27] Such a static conformation, as indicated by the α-carbon T_1 values (see Table 2), could be well represented by its Dreiding model as depicted in Figure 4. The low temperature dependence of Ala[1] NH and Val[4] NH protons (Figure 3 and Table 1) and their slow exchange rates with deuterium (already noted) could be correlated with the two intramolecular H-bonds as shown in Figure 4. These two 10-membered H-bonds, between the Ala[1] NH and the Val[4] C=O, and the Val[4] NH and the Ala[1] C=O, are called β-turns.[1,28] This is a common conformational characteristic of cyclic hexapeptides.[3]

The low temperature dependence of chemical shift of the peptide NH protons indicates inaccessibility to the solvent which could occur, intramolecularly, either by the energetically most favourable pairwise interaction of the NH proton with a carbonyl oxygen or by an energetically less favourable close contact with any of the other atoms or groups of atoms within the small molecule. A good model for the latter shielding has yet to be demonstrated in small molecules. Past studies with this particular linear hexapeptide have demonstrated that the Val[4] NH shielding required the carbonyl of residue one and required a *trans* residue one–Pro[2] peptide bond.[4] These previous studies, the small size of the cyclic peptide and an initial qualitative correspondence with n.m.r. limited torsion angles (see below) provides the basis for consideration of the two above noted β-turns.

If the peptide unit involved in the β-turn contains LD or DL sequences, this could give rise to a type II or type I and type II′ β-turns, respectively.[28,29] Glycine at any corner of a β-turn could behave as a D-amino-acid residue. Therefore, the -L-Pro[2]-Gly[3]-(LD) and the -Gly[5]-L-Val[6]-(DL) fragments at the corners of the two β-turns (see

Figure 4) could be designated as type II and type II′, respectively.[28,29] The experimentally observed 18% n.O.e. between the Pro[2] α-CH and Gly[3] NH protons supports this expectation.[10] The spectral complexity of the Gly[5] CH_2 protons, owing to their ABX spin-system (Figure 1), precluded checking for the presence or absence of this n.O.e. However, the torsion angles, ϕ and ψ (see Table 1) for Pro[2] (−60, 100) and Gly[3] (125, −30), and for Gly[5] (60, −80), and Val[6] (−70, −40), are the conformational characteristics of the type II and type II′ β-turns, respectively.[28,29] The total conformational properties, hydrogen bonding, and torsion angles of this molecule in chloroform are consistent with the previously observed conformational properties of other cyclic hexapeptides.[30] The side-chain conformations of the two valine residues are interesting, however (Figure 4). The ^3J(αCH–βCH) coupling constants for Val[4] and Val[6] are 3.0 and 4.0 Hz, respectively (see Table 1). Using Abraham and McLauchlan's expressions [31] for the αCH–βCH dihedral angle, the two valine residues show predominantly *gauche* conformations. Such conformational features of valine in linear peptides are uncommon, but they are observed in some cyclic peptides.[17,27,32,33] It has been observed in valine-containing diketopiperazines that the γ-CH_3 groups of valine in the *gauche* conformation tended to stay away from the peptide C=O group and their preferred orientations are more towards the peptide NH group.[27,32,33] Accordingly, the side-chain orientations of the γ-CH_3 groups of Val[4] and Val[6] in this molecule were set in the *gauche* forms as shown in Figure 4.

Previously derived conformational features of linear HCO-APGVGV-OMe showed [14] the occurrence of a type II β-turn similar to one in *cyclo*(APGVGV), and a γ-turn,[34] an 11-membered H-bonded ring between the Gly[3] NH and Gly[5] C=O groups. Although this γ-turn could not be observed in the *cyclo*(APGVGV), owing to the severity of the ring constraint, such a turn was observed in the cyclododecapeptide, $\overline{\text{(APGVGV)}_2}$, in addition to the common β-turn and a 14-membered H-bond between the Ala[1] NH and the Val[4] C=O groups.[17] This 14-membered ring H-bond, however, is compatible with the second β-turn (type II′) of *cyclo*(APGVGV) since this turn is also stabilized by the same H-bonding pair, *i.e.*, Ala[1]NH · · · O=C Val[4] (see Figure 4). In conclusion, we argue that the cyclic hexapeptide *cyclo*(APGVGV) retains the expected conformational feature of a Pro[2]-Gly[3] peptide unit containing a type II β-turn and the second hydrogen bond in common with the 14-atom hydrogen-bonded system of the linear peptide, which in the cyclic peptide defines the type II′ β-turn.

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