Conformations of Peptides in Solution by Nuclear Magnetic Resonance Spectroscopy. Part III.† Cyclic Dipeptide Ring Conformations

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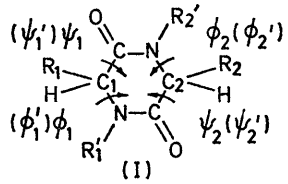
The determination of the degree of folding (β) of the diketopiperazine ring (DKP) of cyclic dipeptides from ^3J(HNCH) and ^5J(HH), the homoallylic coupling across peptide bonds, is discussed. From measurements of the 100 MHz ^{1}H n.m.r. spectra of cyclic dipeptides containing glycine or sarcosine [*i.e.* cyclo-Gly(Sar)-X where X = amino acid] it is concluded that β increases with the bulkiness of amino-acid X for both D_2O and DMSO solutions. The results are in agreement with those determined from the c.d. spectra of the same cyclic dipeptides in aqueous solution. A correlation is observed between the magnetic non-equivalence of the Gly (or Sar) methylene group and the degree of folding of cyclic dipeptides containing amino-acids not substituted by aromatic rings at the β-carbon atom.

THE conformations of cyclic dipeptides have been intensively studied in the solid state by *X*-ray crystallography [1-6] and in solution by various forms of spectroscopy.[7-13] The structure of a cyclic dipeptide (I) shows that the diketopiperazine ring (DKP) is substituted by amino-acid side chains (R_1, R_2) and that both peptide bonds are constrained in the *cis*-conformation. The



(I)

peptide bond may also be substituted (R_1', R_2' as in proline or *N*-methylated amino-acids) or unsubstituted ($R_1' = R_2' = H$). As cyclic dipeptides have limited conformational freedom they have been used to develop the criteria necessary for analysing the conformations of larger linear and cyclic peptides and proteins. In the solid state *X*-ray crystallographic studies have shown that the substituted DKP ring exists in the planar conformation for molecules with a centre of symmetry (*c*-Gly-Gly,[14] *c*-Sar-Sar,[6] and *c*-L-Ala-D-Ala [5]) but buckled conformations of the ring occur for unsymmetrically substituted derivatives (*c*-Gly-L-Tyr,[4] *c*-Sar-L-Val,[15] and *c*-L-Pro-X derivatives [1-3]).

For solutions n.m.r. studies have been used to show that the DKP ring of cyclic dipeptides exists in buckled conformations. Most results of Kopple and his coworkers [7,16,17] were analysed in a qualitative manner by relating the observed ^3J(HNCH) for cyclic dipeptides in dimethyl sulphoxide (DMSO) solution to the dihedral angle between N–H and C–H bonds using a Karplus type relation. In some cases where ^3J(HNCH) could not be observed (*i.e.* in D_2O–CF_3CO_2H solutions) the magnetic non-equivalence of the glycine residue was used to indicate whether a cyclic dipeptide had a planar (magnetic equivalence) or buckled conformation (non-equivalence).[7] The results for a number of cyclic dipeptides containing aromatic residues indicated a buckled

† Part II, ref. 18.

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DKP ring such that the side chain was stabilised in a flagpole conformation by interaction between the aromatic ring and the amide group.[16,17] An extensive study of cyclic dipeptides containing at least one cyclic imino-acid (D-pipecolic, Pip; L-proline, Pro; or D-azetidine-2-carboxylic acid, Aze) also showed that ^3J(HNCH) and Gly CH_2 magnetic non-equivalence are powerful methods for analysing the conformations of such relatively rigid molecules in solution.[10,11] The method of determining the approximate conformation of the DKP ring using ^3J(HNCH) is obviously limited to solutions in which such coupling can be observed (*e.g.* $CDCl_3$, DMSO, and H_2O but not D_2O or CD_3OD) though the magnetic non-equivalence of the glycyl methylene group can be used in the latter solvents. Neither of these methods have so far yielded a quantitative determination of the DKP ring buckling.

A recent analysis [13] of the c.d. curves of *c*-L-Pro-L-Pro and *c*-L-Ala-L-Ala in solution has shown that such measurements can be used for a quantitative determination of the degree of folding of the DKP ring. Indeed it was suggested that the DKP ring of *c*-L-Ala-L-Ala exists in solution with a fold (R_1, R_2 quasi-equatorial) which is the reverse of that observed in the solid state (R_1, R_2 quasi-axial). The c.d. analysis has been applied to symmetrical cyclic dipeptides ($R_1 = R_2$) but extension to molecules with different residues ($R_1 \neq R_2$) has not been made.

It is obviously desirable to develop the n.m.r. analysis of cyclic dipeptides in order to provide quantitative information on the degree of folding using a technique with a number of parameters available for analysis. Five-bond long range coupling has recently been observed between α-CH protons of cyclic dipeptides [18] and analysed according to the relation (1) where A^s is a

$$^5J(\mathrm{HH}) = nA^s\sin^2\phi' \sin^2\psi' \qquad (1)$$

constant for syncoplanar α-CH groups, ϕ' and ψ' are homoallylic torsion angles for C_α–N and C′–C_α bonds, and n equals the number of equivalent coupling paths (*i.e.* $n = 2$ for cyclic dipeptides). The angles ϕ' and ψ' for L- and D-amino-acids are related to the peptide torsional angles ϕ and ψ (I) by the relationships (2) and (3).

$$\phi = 240 - \phi'_L = 120 - \phi'_D \qquad (2)$$

$$\psi = \psi'_L - 240 = \psi'_D - 120 \qquad (3)$$

Hence analysis of ^5J(HH) of cyclic dipeptides leads to determination of the conformation of the DKP ring. The results for a series of *c*-Gly-X and *c*-Sar-X compounds in DMSO, D_2O, and $CDCl_3$ solutions are compared with those found using ^3J(HNCH). It is found that the buckling of the ring depends on the bulkiness of the amino-acid X; concomitantly the observed magnetic non-equivalence of the Gly (or Sar) methylene group exhibits a linear correlation with the degree of folding of the ring.

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EXPERIMENTAL

The preparation of the cyclic dipeptides used in this study has been discussed previously.[18] N.m.r. spectroscopy was used to characterise these compounds in [$^2H_6$]DMSO solution and the parameters have been published.[18] 100 MHz ^{1}H N.m.r. spectra of the cyclic dipeptides in D_2O solution were

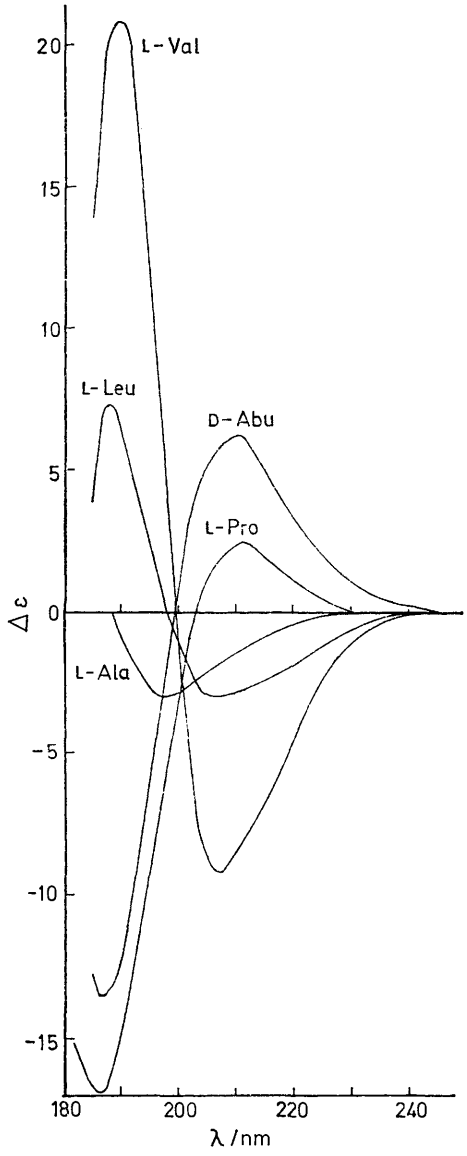


FIGURE 1 C.d. curves of *c*-Gly-X derivatives in aqueous solution, X = amino-acid

measured using a JEOL MH100 spectrometer operating in the internal lock mode at a probe temperature of 308 K. Spectra were calibrated with a Racal 801R frequency counter and proton chemical shifts {δ(p.p.m.) downfield from sodium [2,2,3,3-$^2H_4$]-3-trimethylsilylpropionate (TSP)} and spin coupling constants (J/Hz) are summarised in Table 1. Magnitudes of long-range coupling (>0.5 Hz) were observed directly from the splitting patterns of α-CH multiplets; otherwise they were determined from at least five measurements of the line-widths of coupled and decoupled signals at 54 Hz sweep width (1.5 Hz cm^{-1}). The results are summarised in Table 2, together with those for

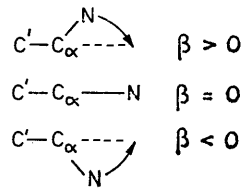
cyclic dipeptides in [$^2H_6$]DMSO solution. C.d. measurements were performed on a Cary 61 spectrometer operated by the University of London Intercollegiate Research Service. We thank Dr. P. M. Scopes, Westfield College, for help and advice. The c.d. spectra are shown in Figure 1.

DISCUSSION

The *c*-Gly-X compounds listed in Table 1 form a series in which different amino-acid residues (X) have side chains of varying size. It is expected that different buckled conformations of the DKP ring are related to the bulkiness of X. The n.m.r. parameters of cyclic dipeptides in DMSO [18] and D_2O solutions (Table 1) are analysed in terms of the conformations of the molecules. It was shown [1,18] from the results of *X*-ray crystallographic studies of cyclic dipeptides that the *cis*-peptide bond is essentially planar, that the DKP ring folds along the line joining the two α-C atoms [(I) C_1 and C_2] and that, generally, the DKP ring is buckled symmetrically; these assumptions are correct to within an accuracy of 4—5°. In this work observed ^3J(HNCH) and ^5J(α-CH, α-CH) values are related to the degree of folding (β) of the DKP ring and the results are compared for different solvents (D_2O and DMSO) and with crystal conformations where available.

(i) *Degree of Folding* (β).—A measure of the buckling of the DKP ring of cyclic dipeptides in the solid state was determined by the angle between the least squares plane of the peptide bonds [1] and the results for most crystal structures have been summarised previously.[1,18] These studies did not discriminate between the two possible conformations of the substituted DKP ring for the same angle between the planes, *i.e.* keeping C_1 and C_2 fixed in (I) the peptide planes fold either into or out of the plane of the page. These conformations can be discriminated if the angles between the peptide planes are converted to the degree of folding (β) previously used in the analysis of c.d. studies of cyclic dipeptides.[13]

The degree of folding (β) which is the angle subtended between the planes containing the peptide bonds is zero for a planar conformation. For the cyclic dipeptide viewed along the line joining the two α-C atoms, β is positive (or negative) for clockwise (or anti-clockwise) rotation of the plane containing N–C_α to become coplanar with the plane containing C–C_α as shown in the Scheme. It is found from crystal structure analyses of



SCHEME

cyclic dipeptides that the sign and magnitude of β depends on the *cis*-(LL) or *trans*-(LD)-substitution of the DKP ring and also on the bulkiness of the amino-acid side chain. The range of β values from +39 (*c*-L-Pro-X derivatives) through zero (*c*-L-Ala-D-Ala, *c*-Gly-Gly, and *c*-Sar-Sar) to −26 (*c*-Sar-L-Val) are illustrated in Figure 2.

TABLE 1

^{1}H N.m.r. parameters of *c*-Gly(Sar)-X dipeptides in D_2O solution

	Gly (Sar) residue					X Residue						
	δ (p.p.m.)			J(Hz)		δ (p.p.m.)				J(Hz)		
Cyclic dipeptide	α-L	α-D	NCH_3	$^2J(\alpha\alpha)$	$^5J(NCH_3,\alpha)$	α	β	γ(β)	δ(γ)	αβ	βγ(αβ)	γδ(βγ)
Gly-Gly	4.04	4.04		*a*		4.04						
Gly-L-Ala	4.02	4.084		−18.35		4.167	1.96			6.9		
Gly-D-Abu [b]	4.12	4.01		−18.4		4.12	1.89	0.95		3.0	7.4	
Gly-L-Val	4.20	4.37		−18.3		4.10	2.18	0.97	(0.88)	3.4	7.0	(7.0)
Gly-L-Leu	4.04	4.18		−18.6		4.14	1.52	1.80	1.01	4.7	4.5	6.0
Gly-L-Pro	4.22	3.93		−17.6	0.9 (±0.1)	4.32	(2.2—2.4)		3.60	*c*	*c*	*c*
Sar-Sar	4.21	4.21	2.98	*a*	0.23 (±0.08)	4.21						
Sar-Gly	4.075	4.075	2.98	*a*	0.37 (±0.06)	4.014						
Sar-L-Val	4.09	4.36	2.87	−18.05	0.2 (±0.1)	4.05	2.46	1.18	(1.05)	4.0	6.6	(6.6)
Sar-L-Phe	3.17	2.23	2.42	−17.6	0.17 (±0.1)	4.08	2.95	(2.74)	7.80 [d]	3.7	(4.6)	−13.5 [e]

[a] Not observed due to magnetic equivalence of α-CH signals. [b] Abu = 2-Aminobutyric acid. [c] Signal overlap precludes measurement at 100 MHz. [d] Phenyl group. [e] $^2J(\beta\beta)$.

The orientation of the amino-acid side chain with respect to the DKP ring depends on the configuration (LL, DD, or LD) and conformation (β) of the cyclic dipeptide. Idealised conformations for LL-cyclic dipeptides are shown alongside the crystal conformations in Figure 2; side chains are quasi-equatorial ($\beta > 0$) or quasi-axial ($\beta < 0$) and the corresponding α-C protons are quasi-axial ($\beta > 0$) or quasi-equatorial ($\beta < 0$). The reverse relationships hold for DD-cyclic dipeptides. The relative orientation of the α-C protons for different conformations is important in determining the magnitudes of ^5J(HH) and ^3J(HNCH). The following approximate relationships indicate the dependence of both ^5J(HH) and ^3J(HNCH) on β for LL-cyclic dipeptides.

Assuming planar peptide bonds and tetrahedral α-C atoms for cyclic dipeptides, it is found that equation (4) applies where θ(HNCH) is the dihedral angle between

$$\beta = |\theta_L| - 60 = 60 - |\theta_D| \qquad (4)$$

the $HC_\alpha N$ and $C_\alpha NH$ planes and subscripts L and D refer to the amino-acid configuration. As only the magnitude of dihedral angle (θ) is important in the Karplus relation, no account is taken of the sign of θ_L and θ_D in equation (4). It was previously assumed that the Karplus relation between ^3J(HNCH) and θ(HNCH)

can be approximately represented by relation (5) for the limited range of $\theta(0 < \theta < 90)$ found for cyclic dipeptides.[18] The relation between ^3J and β derived from

$$^3J(\text{HNCH}) = B\cos^2\theta + C \qquad (5)$$

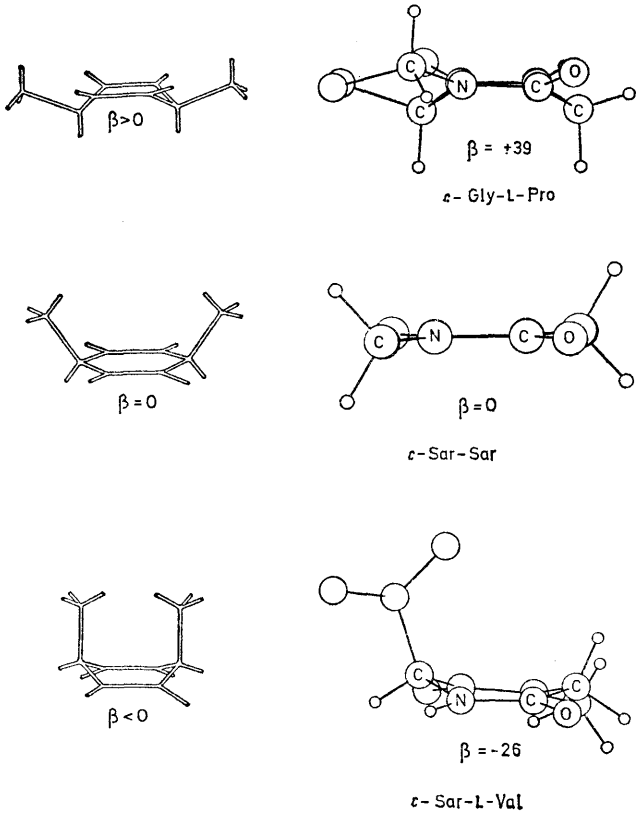


FIGURE 2 Degree of folding (β) of cyclic dipeptides shown for an idealised model and for crystal conformations

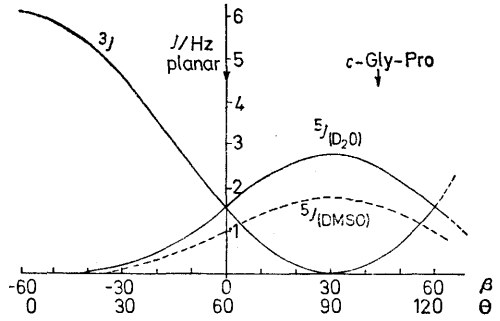


FIGURE 3 Relation of ^3J(HNCH) and ^5J(HH) with degree of folding (β) for LL-cyclic dipeptides. Similar curves for DD-cyclic dipeptides are generated by reflection of ^5J and ^3J about the β = 0 axis

equations (4) and (5) is given by equation (6) for L- and D-amino-acids. The dependence of ^3J(L) on β is plotted

$$^3J(\text{L}) = B\cos^2(\beta + 60)$$
$$^3J(\text{D}) = B\cos^2(60 - \beta) \qquad (6)$$

in Figure 3 for a range of β $(-60 < \beta < 60)$ using the values of $B = 6.1$ Hz and $C = 0$ as discussed previously.[18] For $-60 < \beta < 0$ observed ^3J(L) leads to a unique determination of β. The relation between ^3J(D) and β in equation (6) generates a curve similar to that for ^3J(L) but the curve for ^3J(D) is the reflection of the ^3J(L) curve shown in Figure 3 about the axis β = 0.

The relation between ^5J(HH) and β is determined in the following manner. For *cis*-substituted DKP rings (LL- or DD-cyclic dipeptides) it was shown[18] from crystal structure data that $\phi_1' \sim \psi_1' \sim \phi_2' \sim \psi_3'$; hence equation (1) reduces to equation (7). The relation

$$^5J = nA^s\sin^4\phi' = nA^s\sin^4\psi' \qquad (7)$$

between ϕ' and $\theta[(\phi'_{\text{L}} = 180 + \theta_{\text{L}}$ and $\phi'_{\text{D}} = 180 - \theta_{\text{D}})]$ transforms equation (7) to equation (8) and enables the

$$^5J = nA^s\sin^4\theta \qquad (8)$$

variation of ^5J with β to be calculated for L- and D-amino-acid residues by the appropriate substitution from equation (4). A^s Was found to vary slightly in different solvents.[18] Calculations of ^5J(LL) were made for cyclic dipeptides ($n = 2$) in DMSO (A^s 0.9 Hz) and D_2O (A^s 1.4 Hz) solutions and the results for $-60 < \beta < 60$ are shown in Figure 3. It can be seen that ^5J(HH) observations lead to determination of a unique conformation of the DKP ring for $-60 < \beta < 30$. The curve for ^5J(DD) also leads to determination of β as it is the reflection of the ^5J(LL) curve shown in Figure 3 about the axis β = 0. Measurements of ^3J(HNCH) and ^5J(HH) of cyclic dipeptides in DMSO solution can be used to check the reliability of the method of analysis by comparison of the values of β determined from ^3J(L), ^3J(D), and ^5J(HH) for each cyclic dipeptide measured in [$^2H_6$]DMSO solution. The results are compared with magnitudes of β determined from previous observations of cyclic dipeptides.

(ii) *Conformations of Cyclic Dipeptides.*—Magnitudes of J for *c*-Gly(Sar)-X derivatives in DMSO solution[18] (^3J and ^5J) and in D_2O solution (^5J, this work) are summarised in Table 2 together with the degree of folding (β) determined from the curves in Figure 3. There are four possible determinations of β, two from ^3J(L) observed for each amino-acid residue, one from ^3J(D) of the glycine residue, and one from ^5J(LL). The results for *c*-Gly(Sar)-X in DMSO in Table 2 show that β determined from each ^3J(L) is essentially the same and that the value is similar to that determined from ^5J(LL); greater variation is found for β determined from ^3J(D) as for many of the molecules measured in this work $\beta < 0$ and $^3J(\text{D}) \ll {}^3J(\text{L})$. Only one determination of β can be made for cyclic dipeptides in D_2O solution [from ^5J(LL)] but it is found that β(D_2O) ~ β(DMSO), *i.e.* the conformation of the DKP ring of *c*-Gly(Sar)-X derivatives is similar in DMSO and D_2O solutions.

An approximately planar conformation is found for cyclic dipeptides with a pseudo-centre of symmetry (*c*-Gly-Sar) similar to that observed for cyclic dipeptides with a centre of symmetry (*c*-Gly-Gly and *c*-Sar-Sar) in both the solid state and in solution. In this conformation both C_α–H bonds are *gauche* to the neighbouring carbonyl and NH(Gly) or NCH_3(Sar) groups. For unsymmetrical cyclic dipeptides a buckled conformation is

found for the DKP ring ($0 < \beta > 0$) such that the bulky C_α–R bond minimises interactions with adjacent N–H–(CH_3) and C=O groups at the expense of the C_α–H bond interactions. The side chain R group exists in the quasi-axial position ($\beta < 0$ for LL- and $\beta > 0$ for DD-cyclic dipeptides). Magnitudes of β depend on the nature of the amino-acid side chain; increasing bulkiness of X in the series H (Gly), CH_3 (Ala), CH_2CH_3 (Abu = 2-aminobutyric acid), $CH(CH_3)_2$ (Val), and $CH_2CH(CH_3)_2$ (Leu) leads to greater folding of the DKP ring (β increases). It is found that the amino-acid side chain preferentially exists in a quasi-axial relationship with respect to the DKP ring similar to the 'flagpole' conformation previously found for aromatic derivatives.[7]

The degree of folding is also calculated for cyclic dipeptides measured previously.[7,8,11] The results are listed in Table 2 where it can be seen that the error in β is greater than in the present work as, in some cases, ^3J(HNCH) only is reported and, in other cases, ^3J and ^5J are reported as approximate values. However, these results also show that bulky side chains cause greater buckling of the DKP ring even though large discrepancies are sometimes found with the present results, *e.g.* variations in β for *c*-Gly-L-Leu [$-14(\pm4)$ and $-7.5(\pm7)$] in DMSO solutions are at the limits of the errors involved in the previous measurements. It should be noted that a number of cyclic dipeptides contain racemic mixtures of D- and L-residues.[7] The single values of δ and J for these compounds indicate that the buckled conformation of each cyclic dipeptide in solution (L and D) exist as mirror images, *i.e.* degrees of folding of $-\beta$ for L- and $+\beta$ for D-cyclic dipeptides.

(iii) *C.d. Spectra.*—The degree of folding determined from the c.d. measurements of *c*-Gly-X derivatives shown in Figure 1 agree qualitatively with the results of n.m.r. measurements. The c.d. curve of *c*-Gly-L-Pro resembles that observed previously [13] for *c*-L-Pro-L-Pro in aqueous solution in having a negative lobe at *ca.* 187 nm ($\Delta\varepsilon$ −17 corresponds to M_θ 5.6×10^4) † and a maximum at *ca.* 211 nm ($\Delta\varepsilon$ 2.4). The c.d. curves indicate that the two cyclic dipeptides containing L-proline have similar conformations ($\beta > 0$) in aqueous solution as well as in the solid state [1-3] (β *ca.* +39). On the other hand inverse behaviour is found for *c*-Gly-L-Leu and *c*-Gly-L-Val where the c.d. curves exhibit positive lobes at 188 ($\Delta\varepsilon$ 7.2) and 190 nm ($\Delta\varepsilon$ 20.7), respectively, and negative lobes at 206 ($\Delta\varepsilon$ 3.0) and 207 nm ($\Delta\varepsilon$ −9.2) respectively. This behaviour is similar to that found for *c*-L-Ala-L-Ala ($\beta < 0$).[13] Thus both the n.m.r. and c.d. results indicate that *c*-Gly-L-Leu and *c*-Gly-L-Val have buckled DKP ring conformations in aqueous solution with $\beta < 0$. The similarity in shape of the negative lobe of the c.d. curve of *c*-Gly-L-Ala to that for *c*-Gly-L-Leu indicates that the DKP ring of *c*-Gly-L-Ala is buckled with $\beta < 0$ in agreement with the results of n.m.r. measurements. The minimum of the negative lobe at 197 nm for *c*-Gly-L-Ala is similar to that observed for *c*-L-Ala-L-Ala (200 nm) in aqueous solution.[13] Assuming the relation between β and rotatory strengths of the $n \longrightarrow \pi^*$ and $\pi \longrightarrow \pi^*$ transitions (Figure 7 of ref. 13), the c.d. curves in Figure 1 indicate that in aqueous solution *c*-Gly-L-Val is buckled appreciably greater than L-Leu or L-Ala derivatives. A similar conclusion is determined from n.m.r. measurements (Table 2) though the degree of buckling of the L-Val derivative (−9.5) is only slightly greater than the L-Leu (−8) and L-Ala (−6) derivatives.

The c.d. curve for *c*-Gly-D-Abu in aqueous solution is

† $M_\theta = 3\,300 \times \Delta\varepsilon$.

TABLE 2

Degree of ring folding (β) for cyclic dipeptides

c-Gly-X	DMSO solutions									D_2O solution	
X	$^3J_{1L}$	β_{1L}	$^3J_{1D}$ [c]	β_{1D}	$^3J_2$	β_2	$^5J_{1.2}$	$\beta_{1.2}$	$\bar{\beta}$	^5J	β
Gly (L)	2.2	−7	2.2	+7	2.2	−7	*a*			*a*	
Gly (D)	2.2	+7	2.2	−7	2.2	+7	*a*		0 (±7)	*a*	
L-Ala	2.2	−7	2.2	+7	2.0	−5	0.9	−2	−2 (±4)	1.2	−6
D-Abu [b]	1.8	−3	2.4	+9	*d*		0.7	+7	+4 (±4)	1.0	+9.5
L-Val	2.9	−13.5	1.0	−6	3.0	−14.5	0.4	−15.5	−12 (±3)	1.0	−9.5
L-Phe	2.8	−13	0.7	−10	2.6	−11	0.46	−14	−12 (±2)	*i*	
L-Leu	3.2	−16.5	1.0	−6	3.0	−14.5	0.3	−19.5	−14 (±4)	1.1	−8
L-Pro	0.2	+39	4.4	+28			1.65	+39	35 (±5)	2.6	+39
Gly [e]	2.2	±7	2.2	±7	2.2	±7	*a*		0 (±7)		
D-L-Leu [e]	3.0	±14.5	1.5	±0.5	*f*		*f*		±7.5 (±7)		
D-L-Val [e]	3.0	±14.5	~1	±6.5	*f*		*f*		±10.5 (±4)		
D-L-PhGly [b,e]	3.0	±14.5	⩽0.5	⩽±13	3.0	+14.5	*f*		±14 (±1)		
D-L-His [e]	2.5	±9.5	1.5	±0.5	*f*		*f*		±5 (±4)		
D-Pip [g]	2.2	−7	2.2	+7			0.8	4.5	1.5 (±5.5)		
L-Pro [g]	≠0 < 1		4.0	+24			*f*		*ca.* +24		
D-Aze [g]	5.0	−34	0	−30			1.3	−8 to −52	*ca.* −32		
D-L-Pro [h]	4.5	±30	1.5	±1.5			*f*		*ca.* 30		
c-Sar-X derivatives [b]											
Gly					2.0	±5	1.1	2.5	1 (±4)	1.6	0
L-Val					2.6	−11	0.5	−12.5	−12 (±1)	1.0	−9.5
L-Phe					2.8	−13	0.37	−17.5	−15 (±2)	0.8	−13

[a] Not observed due to magnetic equivalence of α-CH groups. [b] Abu = 2-Aminobutyric acid; PhGly = 2-phenylglycine; Sar = sarcosine, *N*-methylglycine. [c] Values of β_D determined by reflecting β_L curves in Figure 3 about axis $\beta = 0$. [d] Signal overlap precludes measurement. [e] Ref. 7. [f] Value not published. [g] Ref. 11. $CDCl_3$ Solutions. [h] Ref. 8. [i] Not sufficiently soluble in aqueous solution to measure ^5J(HH).

approximately the inverse of those found for the L-Val and L-Leu derivatives. The magnitudes of the positive and negative lobes of the D-Abu derivative are midway between those found for the L-Val and L-Leu derivatives. These results indicate a buckled ring conformation for *c*-Gly-D-Abu ($\beta > 0$) with the side chain (ethyl group) in a quasi-axial conformation between those found for L-Leu (isobutyl) and L-Val (isopropyl) derivatives. It was also concluded from the n.m.r. observations of *c*-Gly-D-Abu in aqueous solution that the DKP ring is buckled ($\beta > 0$) and the degree of buckling ($\beta + 9.5$) is found to be similar to the valine derivative ($\beta - 9.5$). Analysis by n.m.r. of *c*-Gly(Sar)-X derivatives cannot distinguish between optical isomers or racemic mixtures of amino-acid (X) whereas the advantage of using one isomer is confirmed by comparison of the results with example, a number of *c*-Gly-X (X = Val, His, PhGly) with buckled conformations in DMSO solution [different ^3J(HNCH) and non-equivalent Gly α-CH] were concluded to exhibit planar conformations in trifluoroacetic acid (TFA) as the Gly α-CH signals showed magnetic equivalence.[7] Similar behaviour was found for *c*-Gly-Pro which was concluded to exhibit buckled conformations in DMSO and D_2O [non-equivalent Gly α-CH] and a planar conformation in TFA [equivalent Gly α-CH].[9] The widespread use of the magnetic equivalence of the glycine methylene group as a criterion for planar ring conformations of *c*-Gly-X derivatives suggests that a relation might exist between the magnetic non-equivalence of the glycine methylene group and the degree of buckling of the DKP ring. Many of the previous observations of chemical shifts are rationalised with the

TABLE 3

Comparison of magnetic non-equivalence of Gly (and Sar) methylene protons with degree of folding (β) for cyclic dipeptides [a]

c-Gly-X	DMSO solutions				D_2O solutions				
X	δ_L	δ_D	$\Delta\delta$	β	X	δ_L	δ_D	$\Delta\delta$	β
1 L-Pro	4.071	3.591	0.48	+35 (±5)	8 L-Pro	4.22	3.93	0.29	+39
2 D-Abu [b]	3.789	3.70	0.089	+4 (±4)	9 D-Abu	4.12	4.01	0.11	+9.5
3 Gly	3.850	3.850	0	0 (±7)	10 Gly	4.04	4.04	0	0
4 L-Ala	3.73	3.73	0	−2 (±4)	11 L-Ala	4.022	4.084	−0.062	−6
5 L-Val	3.614	3.805	−0.191	−12 (±3)	12 L-Val	4.20	4.37	−0.17	−9.5
6 L-Leu	3.597	3.818	−0.221	−14 (±4)	13 L-Leu	4.04	4.18	−0.14	−8
7 L-Phe	3.357	2.777	0.580	−12 (±2)					
14 Gly [c]	3.70	3.70	0	0 (±7)					
D-L-HomoPhe [b,c]	3.78	3.78	0	*f*					
15 D-L-Leu [c]	3.63	3.83	±0.2	±7.5 (±7)					
16 D-L-PhGly [b,c]	3.73	3.93	±0.2	±14 (±1)					
17 D-L-Val [c]	3.63	3.81	±0.18	±10.5 (±4)	D-L-Val	4.58	4.74	±0.16	*f*
18 D-L-His [c]	3.33	3.57	±0.24	±5 (±4)	D-L-His	3.58	4.13	±0.55	*f*
19 D-L-Phe [c]	3.37	2.79	±0.58	±14 (±1)					
20 D-L-Trp [c]	3.35	2.86	±0.49	±14 (±1)					
21 D-L-Tyr [c]	3.32	2.72	±0.60	±14 (±1)					
22 D-Pip [d]	4.02	4.02	0	1.5 (±5.5)					
23 L-Pro [d]	4.10	3.87	0.23	*ca.* 24					
24 D-Aze [d]	3.73	4.05	−0.32	*ca.* −32					
25 D-L-Pro [e]	4.02	3.63	±0.39	*ca.* 30	D-L-Pro	4.16	3.86	±0.30	
c-Sar-X derivatives									
26 Gly	3.957	3.957	0	1 (±4)	29 Gly	4.075	4.075	0	0
27 L-Val	3.88	4.064	−0.184	−12 (±1)	30 L-Val	4.09	4.36	−0.27	−9.5
28 L-Phe	3.409	2.487	0.922	−15 (±2)	31 L-Phe	3.17	2.23	0.94	−13

[a] Measurements of chemical shifts by different workers are in the temperature range 300—315 K. [b] Abbreviations: Abu = 2-aminobutyric acid; Sar = sarcosine, *N*-methylglycine; HomoPhe = homophenylalanine; PhGly = 2-phenylglycyl. [c] Ref. 7; DMSO solutions. [d] Ref. 11; $CDCl_3$ solutions. [e] Ref. 8; DMSO and D_2O solutions. [f] No information given from which β could be determined.

those determined from c.d. measurements. In general the conclusions from both methods of analysis are similar for each molecule within the error limits of each observation and the limitations of each type of analysis.

(iv) *Non-equivalence of Glycine Residue.*—The relative chemical shifts of glycine α-CH signals have been used to indicate planar or buckled conformations of the DKP ring.[7,9,11] Magnetic equivalence of Gly-CH_2 signals was found for the planar DKP ring in *c*-Gly-D-Pip [planarity determined from ^3J(HNCH) 2.2 Hz] whereas markedly different values of δ(α-CH) and ^3J(HNCH) were found for the glycyl protons in *c*-Gly-L-Pro and *c*-Gly-D-Aze which have buckled DKP rings.[11] The same criterion was used to indicate changes in conformation of the DKP ring of cyclic dipeptides in different solvents. For present results by showing that a relation exists between the degree of folding of the DKP ring and the chemical shift difference of the glycine methylene group of *c*-Gly-X in different solvents. It is likely that the absolute values of observed chemical shifts of glycine residues vary with solvent and substitution of the DKP ring (*i.e.* nature of X) though the chemical shift difference is less dependent on different environments. Accordingly chemical shift differences ($\Delta\delta = \delta_L - \delta_D$) of the glycine residue in *c*-Gly-X derivatives have been calculated for the present results and the results of other workers [7,11] and compared with the degree of folding (β) of the DKP ring. The results which are summarised in Table 3 indicate that glycine magnetic non-equivalence increases ($\Delta\delta$ increases) with greater folding of the DKP ring (β

increases). It should be noted that, for cyclic dipeptides containing L–D mixtures of amino-acids, $\Delta\delta$ and β exhibit both positive and negative values; also, β determined from the results of other workers [usually ^3J(HNCH) but sometimes ^5J(HH)] exhibits far greater variation than the present results. Using the most accurate available results a correlation is found to exist between $\Delta\delta$ and β as shown in Figure 4. The key to compounds plotted in Figure 4 is given in Table 3. An

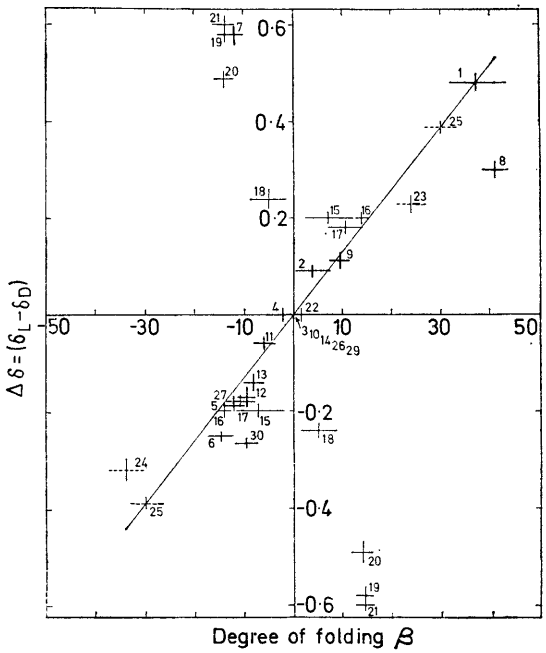


FIGURE 4 Correlation between magnetic non-equivalence of Gly (Sar) methylene protons ($\Delta\delta = \delta_L - \delta_D$) and degree of folding (β) of the substituted diketopiperazine ring. Key to data for different compounds in Table 3. Slope *ca.* 0.013 p.p.m. degree^{-1}. Results from this work are plotted as bolder points

approximately linear correlation between $\Delta\delta$ and β is found for cyclic dipeptides containing cyclic imino-acids (data 1, 8, and 22—25) or acyclic residues not substituted by aromatic rings in the β-carbon atom (data 2—6, 9—17, 26, 27, 29, and 30) but not for acyclic residues substituted by aromatic rings at the β-carbon atom (data 7, 18—21, 28, 31). Within the large error limits of the present results the correlation is observed for *c*-Gly-X derivatives in different solvents, *i.e.* D_2O, DMSO, and $CDCl_3$ solutions. Hence the magnetic non-equivalence of the glycine methylene group can be used to estimate the degree of folding of the DKP ring in cyclic dipeptides containing cyclic imino-acids or acyclic residues not substituted by aromatic rings in the β-carbon according to equation (9) where k is the slope of the linear correlation shown in

$$\Delta\delta = (\delta_L - \delta_D) = k\beta \qquad (9)$$

Figure 4, *i.e. ca.* 0.013 p.p.m. per degree. Equation (9) is valid for cyclic dipeptides with β in the range $-30 < \beta < 30$. The relation shows that planar molecules ($\beta = 0$) exhibit magnetic equivalence of the glycine residue ($\Delta\delta = 0$) and that buckled molecules exhibit increasing magnetic non-equivalence with greater degree of folding of the DKP ring ($0 < \beta > 0$).

For cyclic dipeptides containing imino-acids or acyclic residues not substituted by aromatic rings on the β-C atom, the glycine chemical shift anisotropy depends to a large extent on the conformation of the DKP ring rather than the properties of the amino-acid side chain or solvent effects for D_2O, DMSO, and $CDCl_3$ solutions. Thus it was previously found [11] for *c*-Gly-X (X = imino-acid) that the quasi-axial α-proton is downfield compared to the quasi-equatorial one and that with increasing axial nature of X (X = Pip, Pro, or Aze) the magnetic non-equivalence increases. It was concluded [11] that these results are opposite in trend from models of magnetic anisotropy of the amide group where it is assumed that protons lying above and below the plane of the amide bond are shielded more than protons lying in the same plane.[19] Similar results were obtained in the present work where it is found for L-Val and L-Leu derivatives that the quasi-axial proton (H_D) is downfield compared to the quasi-equatorial one ($\beta < 0$, $\Delta\delta < 0$, $\delta_L < \delta_D$) and for *c*-Gly-D-Abu ($\beta > 0$, $\Delta\delta > 0$, $\delta_L > \delta_D$) where the quasi-axial proton (H_L) is also downfield with respect to the quasi-equatorial proton. These results suggest that the glycine α-CH chemical shift correlation (Figure 4) reflects their spatial relationship to the two planar *cis*-peptide bonds.

Acyclic residues substituted by aromatic rings in the β-carbon atom (X = Phe, Tyr, Trp, His) do not conform to the $\Delta\delta$–β correlation shown in Figure 4. The DKP rings of these molecules are buckled ($\beta < 0$ for L and $\beta > 0$ for D derivatives) such that the side chain exists in a quasi-axial relation to the DKP ring, the ' flagpole ' conformation.[7] For a predominantly *gauche-gauche*-conformation for rotation about the C_α–C_β bond the aromatic ring projects over the glycyl α-proton in a quasi-axial conformation causing a significant upfield shift compared to an acyclic side chain.[7] From the relative position of the His residue (compound 18) to the aromatic residues (compounds 7 and 19—21) plotted in Figure 4, it can be seen that the ring current effect of the imidazole ring (His) on the glycine protons is less than for the phenyl (Phe, Tyr) or indole ring (Trp). On the other hand the phenyl ring of the 2-phenylglycyl derivative (PhGly, compound 16) has little effect on the glycine protons. Hence the $\Delta\delta$–β correlation can be used to investigate the effect of side chain interactions of cyclic dipeptides containing amino-acids with aromatic residues at the β-carbon atom as well as monitor the conformation of the diketopiperazine ring of cyclic dipeptides not containing aromatic residues at the β-atom.

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