Conformations of Peptides in Solution by Nuclear Magnetic Resonance Spectroscopy. Part II.† Homoallylic Coupling in Cyclic Dipeptides

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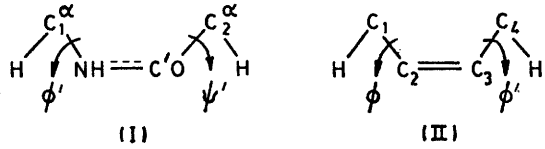
Using the crystal structure data of numerous cyclic dipeptides the scope of analysing ^5J(HH) of peptides in terms of homoallylic coupling is discussed. From the observed correlation between ^3J(HNCH) and ^5J(HC$^\alpha$NC′C$^\alpha$H) of cyclic dipeptides in DMSO and $CDCl_3$ solutions it is shown that five bond coupling can be rationalised in terms of homoallylic coupling. Assuming the same angular dependence for ^5J(HH) for peptides in all solvents, the conformational properties of the substituted diketopiperazine rings are determined for cyclic dipeptides in different solvents; in particular, detailed conformational properties are discussed for cyclic dipeptides in D_2O solutions where ^3J(HNCH) cannot be observed. The analysis is extended to explain changes with conformation in observed ^5J(α-CH,NR) of *N*-substituted cyclic dipeptides (R = CH_3 or δ-CH_2 of proline).

A NUMBER of methods have been used to determine the backbone conformations of linear and cyclic peptides in solution by n.m.r. spectroscopy.[1-4] One method of widespread use is ^3J(HNCH) which is related to the dihedral angle θ(HNCH) between vicinal protons by the appropriate Karplus relation [5] and hence to ϕ(NC$^\alpha$) for peptide systems.[6-9] The use of this parameter is restricted to situations in which NH exchange (usually with solvent) is sufficiently slow to observe the coupling. The method is not applicable to peptides in D_2O solution nor for *N*-substituted peptides such as sarcosine and proline. Even though ^3J(HNCH) has been used extensively to determine the conformation about peptide N–C$^\alpha$ bonds such information for the C$^\alpha$–C′ bond is not presently available. Recent work [10-13] on ^{15}N enriched amino-acids has shown that $^3J(^{15}NC'C^\alpha H)$ can be related to ψ(C′C$^\alpha$) though the method is not likely to develop widespread use because of the cost of materials and specialised nature of the necessary instrumentation. Methods which are widely applicable are still necessary for the determination of the conformations of peptides in solution.

Recently long range proton coupling has been observed between α-carbon atom protons of peptide bonds and between α-CH and NCH_3 of methylated peptides.[14] The observation of ^5J(HH) was used to determine the *cis*- and/or *trans*-conformations of the peptide bond. Similar coupling was observed by Kopple and Ohnishi in cyclic dipeptides [15] between the α-CH and CH_2 protons of cyclic-Gly-L-Val and cyclic-Gly-L-Phe though the values of the coupling constants were not published nor the phenomenon explained. An extensive survey [16] of cyclic dipeptides containing at least one cyclic imino-acid (pipecolic acid, Pip; proline, Pro; and/or azetidine-2-carboxylic acid, Aze) showed that five-bond long range coupling exists between the α-carbon protons and that the magnitudes of ^5J(HH) for $CDCl_3$ solutions varied from the limits of detection up to 1.3 Hz. In cyclic dipeptides each peptide bond is constrained in the *cis*-conformation. Substitution at the α-carbon atom has also been designated *cis* (substituents on the same side of the diketopiperazine ring, DKP, which encompasses LL and DD configurational isomers) and *trans* (encompasses LD isomer). It was found for the cyclic dipeptides containing Pip, Pro, or Aze that ^5J(HH) is somewhat greater in the *cis*- compared with the *trans*-isomer.[16] The various observations can be rationalised if $^5J(HC_1^\alpha NC'C_2^\alpha H)$ of peptides (I) is treated in the same manner as homoallylic



(I) (II)

coupling ^5J(HH) in but-2-ene derivatives (II).[17-23] Although the N–C′ bond is not a formal double bond, the

† Part I, reference 14.

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pseudo-double bond character of the peptide bond has been invoked to explain the planarity of the peptide bond and the high energy of activation compared with single bonds. It is expected that ^5J(HH) in peptides is smaller than in but-2-ene derivatives. An example of the difference is shown by a comparison of ^5J in cyclic dipeptides [16] in $CDCl_3$ solution (0—1.3 Hz) to the analogous cyclohexa-1,4-diene derivatives [24] where it was found that $^5J_{cis}$ (9.63 Hz) is greater than $^5J_{trans}$ (8.04 Hz) and, for *cis*- and *trans*-1,4-dihydro-4-tritylbiphenyl, ^5J is 11 and 7.5 Hz respectively.[25] Similar behaviour in *cis*- and *trans*-derivatives was observed for cyclic dipeptides though the magnitudes of ^5J were markedly lower than those found in the cyclohexadiene derivatives.[16]

From accurate measurements of ^3J(HNCH) and ^5J(HC$^\alpha$C′NC$^\alpha$H) for a number of cyclic dipeptides in [$^2H_6$]DMSO solution, it is shown that ^5J(HH) can be rationalised in terms of homoallylic coupling according to relation (1) where ϕ' and ψ' correspond to torsional

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

angles for N–C$^\alpha$ and C$^\alpha$–C′ bonds, respectively. The positions of appropriate C$^\alpha$ protons define the torsional angles with respect to the planar peptide bond. A^s is a constant for groups in the *syn*-conformation and n equals the number of equivalent coupling paths, *i.e.* $n = 2$ for α-CH groups of cyclic dipeptides. In accordance with previous nomenclature [14] the terms *syn* and *anti* indicate relative positions of groups across peptide bonds whereas *cis* and *trans* are used to describe the conformational isomers of amides and peptides. For cyclic dipeptides the peptide bond is in the *cis*-conformation. From measurements of dimethylacetamide in D_2O solution [14] it is expected that $A^s(trans) = A^s(cis)$ and $A^a(trans) = A^a(cis)$ and that $A^a > A^s$. The derivation of equation (1) depends on a number of assumptions which are shown, in this work, to be approximately correct by analysis of the crystal structure data of cyclic dipeptides; the data were also utilised to investigate the likely errors involved in determining conformations from ^5J(HH).

The use of ^5J(HH) in determining conformations of cyclic dipeptides is examined. It is found that the magnitude of A^s depends on the solvent. The limited $CDCl_3$ solution data of cyclic dipeptides containing one cyclic imino-acid (Pip, Pro, or Aze) can also be analysed in terms of homoallylic coupling and the value of A^s in equation (1) is the same as for DMSO solutions. Different values of A^s were determined for D_2O and CD_3OD solutions using cyclo-Gly-L-Pro as reference compound. It is found that ^5J for *cis*-substituted DKP rings (LL or DD) can be used to determine the conformation of the ring, though, for *trans*-substituted DKP rings (LD) the correct conformation can only be determined if ^5J(HH) is supplemented by some other parameter, *e.g.* ^3J(HNCH). It is shown that homoallylic coupling as summarised in equation (1) can be used to explain the five-bond coupling between α-CH and NCH_3 groups in *N*-substituted cyclic peptides; this observation also applies to δ-CH_2 of proline and other cyclic imino-acid derivatives and changes in ^5J can be predicted for different conformations.

EXPERIMENTAL

(a) *Preparation of Cyclodipeptides.*—Both unsubstituted and *N*-substituted cyclodipeptides were prepared *via* the *N*-carbonylbenzoyloxy (CBZ)-dipeptide esters. The latter were made by reacting *N*-CBZ-amino-acids with amino-acid methyl esters according to the mixed anhydride method [26] in which one component (*N*-CBZ-amino-acids, in this case) was used in excess to obtain a higher yield.[27] The *N*-CBZ blocking group was removed either by hydrogenolysis in methanol over palladium (10%) on activated charcoal or by adding 35% hydrogen bromide in acetic acid solution. Cyclisation was accomplished by dissolving the dipeptide methyl ester in dry methanol and saturating with ammonia.[28] On leaving for a day the piperazinediones separated directly from the reaction mixture in most cases. Otherwise the reaction mixture was extracted with benzene, filtered, evaporated, and separated with preparative t.l.c. For mono-*N*-methylated cyclodipeptides the *N*-CBZ-amino-acids were methylated individually using CH_3I–Ag_2O in dry dimethylformamide solution [29] prior to preparation of the *N*-CBZ-dipeptide esters. The di-*N*-methyl cyclodipeptides were prepared by methylation of the appropriate cyclodipeptides.[30] The purity of each compound was checked by t.l.c., m.p., and n.m.r. analysis. Characterisation of each compound was made by optical rotation in water, methanol, or chloroform and 100 MHz ^{1}H n.m.r. in [$^2H_6$]DMSO solution and the composition of new compounds was confirmed by elemental analysis; these parameters are summarised in Table 1 for the different cyclic dipeptides. ^{1}H N.m.r. spectra of some cyclic dipeptides were also measured in D_2O solutions and the relevant spin coupling constants and chemical shifts are listed in Table 2.

(b) *N.m.r. Spectroscopy.*—The 100 MHz ^{1}H n.m.r. spectra of the cyclodipeptides were measured using a JEOL MH100 n.m.r. spectrometer operating at a probe temperature of 35° unless otherwise stated. For greatest accuracy the appropriate α-CH or NCH_3 signals were observed in the internal lock mode at 54 Hz sweep width and calibrated with a Racal 801R frequency counter. Double resonance experiments were performed in the internal lock mode using the JEOL SD100 spin decoupler whereas triple resonance experiments were performed in the external lock mode. ^3J(HNCH) Magnitudes were obtained from the multiplet patterns of the α-CH signals and confirmed by irradiation of the appropriate NH signal. The larger values of long-range coupling (>1 Hz, DMSO; >0.5 Hz, D_2O) were observed directly from the splitting patterns of the α-CH or NCH_3 multiplets but, generally, ^5J(HH) was determined from at least five measurements of the linewidths of coupled and decoupled signals. In some cases overlap of α-CH and/or Gly-CH_2 proton signals precluded complete analysis at

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TABLE 1

Physical properties of cyclic dipeptides.

Cyclic dipeptides	M.p. (°C) (lit. values)	$[\alpha]_D^{25}$		Elemental analysis (%) C	H	N	δ ([$^2H_6$]DMSO) (*J*/Hz)
Gly-Sar	145—146		Req.	46.85	6.3	21.85	Gly: NH 8.10 (2.0), α 3.850 (2.0)
			Found	46.75	6.25	21.85	Sar: NCH_3 2.845, α 3.957
Gly-L-Ala	208—210 (209—210) [a]	+36.6 (H_2O, *c* 2)					Gly: NH 8.20 (2.2), α 3.73 (2.2) Ala: NH 8.20 (2.0), α 3.845 (2.0, 6.8), β 1.66 (6.8)
Gly-L-val	261—263 (264—265) [b]	+15.1 (H_2O, *c* 1.5)					Gly: NH 8.03 (2.9, 1.0), α_L 3.614 (2.9), α_D 3.805 (1.0) [*J*(αα) −17.8] Val: NH 8.214 (3.0), α 3.518 (3.0, 4.0), β 2.10 (4.0, 6.8), γ 0.818 (6.8), 0.918 (6.8)
Gly-L-Leu	254—256 (254—255) [a]	−14.8 (H_2O, *c* 2)					Gly: NH 8.02 (3.2, 1.0), α_L 3.597 (3.2), α_D 3.818 (1.0), [*J*(αα) −17.5] Leu: NH 8.36 (3.0), α 3.653 (3.0, 4.9), β 1.521 (4.9), γ 1.764 (4.9, 6.4); δ 0.891 (6.4), 0.870 (6.4)
Gly-L-Phe	267—269 (268—269) [c]	+97.6 (MeOH, *c* 1)					Gly: NH 7.88 (2.8, 0.7), α_L 3.357 (2.8), α_D 2.777 (0.7) [*J*(αα) −17.4] Phe: NH 8.14 (2.6), α 4.079 (2.6, 4.3, 5.2), β_A 3.108 (4.3), β_B 2.919 (5.2), [*J*(ββ) −13.5], phenyl 7.30
Gly-L-Pro	214—216 (216—218) [d]	+25.6 (MeOH, *c* 3)					Gly: NH 8.12 (4.4, 0.2), α_L 4.071 (0.2), α_D 3.591 (4.4) [*J*(αα) −17.2] Pro: α 4.18, β and γ 1.70—2.30 and δ ~ 3.5
Sar-L-Val	136—138	−8.3 ($CHCl_3$ *c* 2)	Req.	56.45	8.3	16.45	Sar: NCH_3 2.908, α_L 3.88, α_D 4.064 [*J*(αα) −18.0] Val: NH 8.30 (2.6), α 3.704 (2.6, 3.8), β 2.19 (3.8, 6.8, 7.0), γ 0.888 (6.8), 0.989 (7.0)
			Found	56.4	8.25	16.45	
Sar-L-Phe	183—185	+79.2 ($CHCl_3$, *c* 1)	Req.	66.05	6.45	12.85	Sar: NCH_3 2.63, α_L 3.409, α_D 2.487 [*J*(αα) −16.7] Phe: NH 8.30 (2.8), α 4.22 (2.8, 3.8, 4.6), β_A 3.096 (3.8), β_B 2.867 (4.6), [*J*(ββ) −13.1], phenyl 7.26
			Found	66.1	6.45	12.8	
NMe-L-Ala-L-Ala	116—117	+63.7 ($CHCl_3$, *c* 3)	Req.	50.0	8.35	19.45	MeAla: NCH_3 2.923, α 3.948 (7.0), β 1.41 (7.0) Ala: NH 8.30 (3.0), α 3.961 (3.0, 7.0), β 1.49 (7.0)
			Found	50.05	8.3	19.45	
L-Pro-L-Phe	133—136 (135—136) [e]	−80.0 (MeOH, *c* 1)					Pro: α 4.06, β and γ 1.70—2.30, δ 3.274 Phe: NH 7.94 (0.3), α_L 4.345 (0.3, 4.4, 5.0), β_A 3.396 (4.4), β_B 3.014 (5.0), [*J*(ββ) −11.5], phenyl 7.26
L-Pro-D-Phe	152—155 (152—154) [e]	−98.6 (MeOH, *c* 1)					Pro: α,[f] β, and γ 1.20—2.10, δ 3.02 Phe: NH 8.17 (3.7), α_D 4.064 (3.7), β,[f] phenyl 7.30
D-Ala-L-Phe	260—262	+73.1 (MeOH, *c* 1)	Req.	66.05	6.4	12.85	Ala: NH 8.18(0.56), α_D 2.985 (0.56, 7.0), β 1.175 (7.0) Phe: NH 8.18 (2.6), α 4.177 (2.6, 4.3, 5.0), β_A 3.20 (4.3), β_B 2.996 (5.0) [*J*(ββ) −13.75], phenyl 7.36
			Found	66.1	6.5	12.9	

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100 MHz so measurements were made at 220 MHz (PCMU, Harwell) or 270 MHz (by kind permission of Drs. G. Chapman and M. Bradbury, Portsmouth Polytechnic). The chemical shifts and spin coupling constants of cyclic dipeptides in [$^2H_6$]DMSO and D_2O (α-CH only) are summarised in Tables 1 and 2 respectively.

The analysis of the crystal structure data of cyclic dipeptides was performed using the MOLGOM programme developed by the Department of Crystallography, Birkbeck College, and we acknowledge the advice and help of Dr. P. Timmins. Peptide conformational angles (ω, ϕ, and ψ) are listed in Table 3 and the corresponding homoallylic torsional angles (ϕ' and ψ') determined from hydrogen atom positions are summarised in Table 4.

DISCUSSION

It can be seen from Table 2 that the coupling constant data of cyclic dipeptides in DMSO solutions indicate a correlation between ^5J and the conformation of the diketopiperazine ring as expressed by ^3J(HNCH). A similar variation of ^5J is observed in DMSO and D_2O solutions though the magnitude of the coupling for the same molecule in each solvent is quite different. Previous work [16] on a number of cyclic dipeptides containing cyclic imino-acids (Pip, Pro, or Aze) has shown a similar correlation between ^3J(HNCH) and ^5J(α-CH,α-CH). There are two classes of molecules in which the conformation of the diketopiperazine ring is expected to be

TABLE 2

Chemical shifts and spin coupling constants of cyclic dipeptides

		DMSO solutions								D₂O solutions				
		δ			J/Hz					δ			J/Hz	
Cyclic dipeptide		α-CH₁	α-CH₂	NR	$^3J_1$ [a]	$^3J_2$ [a]	^5J(HH)	^5J(α-CH, NR)	$(B+C-{}^3J_1)$ / $(B+C-{}^3J_2)$ [b]	α-CH₁	α-CH₂	NR	^5J(HH)	^5J(α-CH, NR)
1 Gly-L-Phe	LL	3.357	4.079		2.8	2.6	0.46 (±0.08)		11.6					
2 Gly-L-Phe	DL	2.777	4.079		0.7	2.6	0.74 (±0.14)		18.9					
3 Gly-L-Val	DL	3.805	3.518		1.0	3.0	0.8 (±0.05)		9.9	4.369	4.104		1.3	
4 Gly-L-Val	LL	3.614	3.518		2.9	3.0	0.4 (±0.05)		15.8	4.203	4.104		1.0	
5 Sar-L-Phe	LL	3.409	4.22	2.63		2.8	0.37 (±0.013)	[c]	10.9 [c]	3.166	4.08	2.418	0.8	0.17 (±0.11)
Sar-L-Phe	DL	2.487	4.22	2.63		2.8	0.9 (±0.05)			2.226	4.08	2.418	1.2	
Sar-L-Val	DL	4.064	3.707	2.908		2.6	0.8 (±0.1)	0.11 (±0.04)		4.363	4.054	2.87	1.4	0.2 (±0.1)
6 Sar-L-Val	LL	3.880	3.707	2.908		2.6	0.5 (±0.1)		12.3 [c]	4.099	4.054	2.87	1.0	
7 Gly-L-Leu	DL	3.818	3.653		1.0	3.0	0.7 (±0.05)		15.8	4.186 [f]	4.141		1.1	
Gly-L-leu	LL	3.597	3.653		3.2	3.0	≤0.03		9.0	4.037 [f]	4.141		1.3	
8 *N*-Me-L-Ala-L-Ala	LL	3.948	3.961	2.923		3.0 [g]	0.26 (±0.05) [g]	0.16 [g]	9.6 [c]	4.05	4.13	2.99	0.5 [h]	0.23 (±0.07)
9 D-Ala-L-Phe	DL	2.985	4.177		0.56	2.6	0.8 (±0.1)		19.3					
10 L-Phe-L-Pro	LL	4.345	4.06	3.274	0.3		1.3 (±0.15)	[c]	33.6 [c]					
D-Phe-L-Pro	DL	4.064		3.02	3.7		0.15 (±0.1)	[c]						
11 Gly-L-Pro	LL	4.071	4.18	3.43	0.2		1.65 (±0.05)	0.75 (±0.05)	34.8 [c]	4.221	4.32	3.60	2.6	0.9 (±0.1)
Gly-L-Pro	DL	3.591	4.18	3.43	4.4		~0	~0		3.931	4.32	3.60	0.4	0.1 (±0.05)
Gly-Sar	LL/DD	3.850	3.957	2.845	2.0 [d]		1.1 (±0.1) [d]	0.25 (±0.1)	16.8 [d]	4.014	4.075	2.98	1.6 (±0.1)	0.37 (±0.06)
Gly-L-Ala	LL	3.73	3.845		2.2 [d]	2.0 [d]	0.9 (±0.1) [d]		16.0 [d]	4.084 [f]	4.164		1.2	
Gly-L-Ala	DL	3.73	3.845		2.2 [d]	2.0 [d]	0.9 (±0.1) [d]		16.0 [d]	4.022 [f]	4.164		1.7	

[a] Error in *J ca.* ±0.1 Hz. [b] Calculations made using *B* 6.1 and *C* 0 Hz as described in text. [c] *N*-Substituted amino-acid precludes observation of ^3J. For symmetric molecules $\phi_1' \sim \phi_2'$ *i.e.* $^3J_1 \sim {}^3J_2$. [d] Deceptively simple spectra observed at 220 MHz; only average values of ^3J(HNCH) and ^5J(HH) are listed. [e] Not observed due to overlap of signals. [f] Assignment not unambiguous. [g] Observed in 25% benzene–DMSO solution to separate α-CH signals. [h] 270 MHz measurement.

TABLE 3

Conformational parameters of cyclic dipeptides from crystal structures [a]

Cyclic dipeptide	ω_1	ϕ_1	ψ_1	ω_2	ϕ_2	ψ_2	Ref.
L-DHPro-L-DHPro [b]	1.1	−39.5	37.0	1.1	−39.5	37.0	37
L-Pro-L-Leu	6.4	−41.6	33.7	6.3	−41.6	33.8	36
L-Pro-Gly	0.4	−44.0	38.5	7.4	−37.3	32.7	35
L-Ala-L-Ala	8.2	−25.6	20.6	0.8	−32.4	26.8	34
Sar-L-Val [c]	−0.5	20.9	−14.3	−12.5	31.9	−24.4	39
Gly-L-Tyr	−4.0	19.3	−13.1	−7.1	16.2	−10.2	40
L-Ser-L-Tyr	4.6	−11.7	6.1	5.9	−10.4	5.0	40
L-Ala-D-Ala	3.2	3.0	−2.7	−3.2	−3.0	2.7	34
Gly-Gly	−1.3	−1.3	1.2	1.3	1.3	−1.2	32
Sar-Sar [c]	−7.1	−7.0	6.5	7.1	7.0	−6.5	33

[a] Designation of angles shown in structure (III). [b] DHPro = 3,4-didehydroproline. [c] Sar = *N*-methylglycine.

TABLE 4

Homoallylic torsional angles of cyclic dipeptides calculated from crystal structures [a]

Cyclic dipeptide		R_1	R_1'	R_2	R_2'	ϕ_1'	ψ_1'	ϕ_2'	ψ_2'	$N = \sin^2\phi_1' \times \sin^2\psi_2'$	$D = \sin^2\phi_2' \times \sin^2\psi_1'$	Ratio N/D	Angle between planes (°)	Ref.
L-DHPro-L-Pro [b]	LL	CH_2	CH_2	CH_2	CH_2	281.6	277.6	281.6	277.6	0.9428	0.9428	1.00	139	36
L-Pro-L-Leu	LL	CH_2	CH_2	CH_2	H	286.7	280.2	283.4	268.1	0.9164	0.9166	0.99	143	35
L-Pro-Gly [c]	LL	CH_2	CH_2	H	H	281.3	276.2	278.6	273.5	0.9580	0.9662	0.99	142	34
L-Pro-Gly [c]	LD	CH_2	CH_2	H	H	281.3	276.2	165.1	159.2	0.1212	0.0653	1.85	142	34
L-Ala-L-Ala	LL	CH_3	H	CH_3	H	266.7	261.8	276.9	270.3	0.9966	0.9655	1.03	151	33
Sar-L-Val	LL	H	CH_3	CH	H	211.4	221.4	209.8	219.9	0.1117	0.1080	1.03	158	38
Sar-L-Val	DL	H	CH_3	CH	H	98.4	111.6	209.8	219.9	0.4027	0.2135	1.88	158	38
Gly-L-Tyr	LL [d]	H	H	CH_2	H	214.7	215.2	221.9	217.4	0.1195	0.1482	0.81	167	39
Gly-L-Tyr	LL [e]	H	H	CH_2	H	221.0	227.0	221.1	227.1	0.2310	0.2311	1.00	167	39
Gly-L-Tyr	DL [d]	H	H	CH_2	H	98.5	106.5	221.9	217.4	0.3608	0.4100	0.88	167	39
Gly-L-Tyr	DL [e]	H	H	CH_2	H	102.5	108.9	221.1	227.1	0.5115	0.3868	1.32	167	39
L-Ser-L-Tyr	LL [d]	CH_2	H	CH_2	H	249.0	258.7	242.4	232.4	0.5471	0.7550	0.72	173	39
L-Ser-L-Tyr	LL [e]	CH_2	H	CH_2	H	249.2	244.0	248.7	242.7	0.6900	0.7012	0.98	173	39
L-Ala-D-Ala	LD	CH_3	H	CH_3	H	238.4	238.4	121.6	121.6	0.5263	0.5263	1.00	180	33
Gly-Gly	LL	H	H	H	H	241.4	240.6	239.9	234.6	0.5122	0.5681	0.90	180	31
Gly-Gly	DD	H	H	H	H	120.1	125.4	118.6	119.4	0.5681	0.5122	1.11	180	31
Gly-Gly	LD	H	H	H	H	241.4	240.6	118.6	119.4	0.5851	0.5851	1.00	180	31
Gly-Gly	DL	H	H	H	H	120.1	125.4	239.9	234.6	0.4973	0.4973	1.00	180	31
Sar-Sar	LL	H	CH_3	H	CH_3	245.4	244.8	231.8	232.0	0.5133	0.5056	1.02	180	32
Sar-Sar	DD	H	CH_3	H	CH_3	128.2	128.0	114.6	115.2	0.5056	0.5133	0.98	180	32
Sar-Sar	LD	H	CH_3	H	CH_3	245.4	244.8	114.6	115.2	0.6768	0.6768	1.00	180	32
Sar-Sar	DL	H	CH_3	H	CH_3	128.2	128.0	231.8	232.0	0.3835	0.3835	1.00	180	32

[a] Designation of angles shown in structure (III). [b] HDPro = 3,4-didehydroproline. [c] Calculations made using fractional co-ordinates derived from high-angle refinement given in ref. 34*b*. [d] Calculations made using fractional co-ordinates given in ref. 39. [e] Calculations made using nuclear co-ordinates given in ref. 39.

similar to that observed in the solid state by *X*-ray crystallography. In the first case a planar ring has been found for such molecules as cyclo-Gly-Gly,[31] cyclo-Sar-Sar,[32] and cyclo-D-Ala-L-Ala [33] which possess a centre of symmetry. In Table 2 it can be seen for such molecules that ^3J(HNCH) varies between 2.0 and 2.2 Hz, ^5J(α-CH,α-CH) between 0.9 and 1.1 Hz and, where appropriate, ^5J(α-CH,NCH_3) is *ca.* 0.25 Hz. Similar behaviour is observed for cyclic dipeptides [16] containing D-pipecolic acid in $CDCl_3$ solutions. The second case consists of cyclic-Pro-X derivatives in which three crystal structure conformation of the DKP ring. It is shown that five-bond proton coupling in cyclic peptides can be rationalised in terms of homoallylic coupling and that the coupling can be used in the determination of the conformational properties of peptide N–Cα and Cα–C′ bonds. It is also found that the magnitude of other five-bond coupling in peptides ^5J(α-CH,NR) (where R = CH_3 or δ-CH_2 of proline) can be explained using the same analysis. In this work the assumptions used in the analysis of ^5J in terms of homoallylic coupling are investigated using the crystal structure data of cyclic dipeptides and,

TABLE 5

Homoallylic coupling (Hz) for cyclic dipeptides in $CDCl_3$ solution

Cyclic dipeptide [a]	Configuration	^5J(HH)	^3J(HNCH) $^3J_1$	^3J(HNCH) $^3J_2$	$(B + C\cos^2{}^3J_1) \times (B + C\cos^2{}^3J_2)$ [b]	Ref.
D-Pip-Gly	DD	0.8	2.2 [c]	2.2	15.2	16
D-Pip-D-Phe	DD	0.7	2.2 [c]	2.2	15.2	16
D-Pip-L-Phe	DL	0.5	1.8 [c]	1.8	18.5	16
L-Pro-D-Phe	LD	0.6	3.9 [c]	3.9	4.8	16
L-Pro-Gly	LL	1.68	~0 [c]	~0	~36	*d*
L-Pro-Gly	LD	~0	4.15 [c]	4.15	3.8	*d*
D-Aze-Gly	DD	1.3	~0 [c]	~0	~36	16
D-Aze-Gly	DL	~0	~0 [e]	5	1.2	16
D-Aze-L-Leu	DL	~0	~0 [e]	4.8	1.7	16
D-Aze-D-Leu	DD	~1	≪1	≪1	~26	16
Gly-Sar	LL/DD	1.1	2.0	2.0 [c]	16.8	*d*

[a] Abbreviations: Pip = pipecolic acid, Pro = proline, Aze = azetidine-2-carboxylic acid. [b] Calculations made using *B* 6.1 and *C* 0 Hz as described in text. [c] *N*-Substituted amino-acid precludes observation of $^3J_1$. For symmetrical molecules $\phi_1' \sim \phi_2'$ *i.e.* $^3J_1 \sim {}^3J_2$. [d] This work; spectra were simplified by adding $Eu(fod)_3$. [e] Approximate value determined by reference to cyclo-D-Aze-Gly.

determinations [34-36] indicate very similar boat conformations of the diketopiperazine ring with the dihedral angle of the fold being *ca.* 141(±2°). In these molecules the α-CH bonds take up pseudo-axial and -equatorial positions which produce corresponding differences in the appropriate coupling constants. The variation in behaviour is shown by cyclo-Gly-L-Pro in DMSO solution where α-CH(Pro) is pseudoaxial and the two α-CH(Gly) are either pseudo-axial or -equatorial. The pseudo-axial Gly proton is indicated by ^3J(HNCH) 0.2 Hz and the same proton exhibits ^5J(α-CH,α-CH) 1.65 Hz with α-CH(Pro) (pseudo-axial). On the other hand, the pseudo-equatorial Gly proton, which is indicated by ^3J(HNCH) 4.4 Hz, exhibits ^5J(α-CH,αCH) < 0.2 Hz. A similar difference is observed in ^3J and ^5J of other proline derivatives in DMSO solutions in Table 2 and $CDCl_3$ solutions [16] in Table 5.

The magnitude of ^3J(HNCH) is a measure of the buckled conformation of the diketopiperazine ring assuming a Karplus type dependence with θ(HNCH) and a planar peptide bond. A correlation is observed between ^3J(HNCH) and ^5J(α-CH,α-CH) of a number of cyclodipeptides which indicates that ^5J(HH) depends on the subsequently, the use of homoallylic coupling in determining peptide conformations is discussed.

Homoallylic Coupling

(i) *Definition of Torsional Angles.*—Homoallylic coupling constants in molecular fragment (II) are found to vary with the conformation about both C–C single bonds.[24,25] It can be seen that ϕ and ϕ' correspond to the same bonds as the peptide conformational angles ϕ(N–Cα) and ψ(Cα–C′) in fragment (I). Following the nomenclature for homoallylic coupling in the molecular fragment H–C(1)–C(2)–C(3), ϕ is zero for H–C(1) *cis*-planar with respect to C(2)–C(3) and positive angles are generated by clockwise rotation of the C–H bond.[38] Similarly ϕ' is zero for C(2)–C(3) *cis*-planar with respect to C(4)–H and positive angles are generated by clockwise rotation of the C–H bond. It was shown [20-23] by valence bond theory that ^5J(HH) is related by ϕ and ϕ' according to equation (2) where *A*, a constant, = 4.99 for the but-2-ene system.*

$$^5J(\mathrm{HH}) = A\sin^2\phi\sin^2\phi' \qquad (2)$$

For peptides in fragment (I) the angles ϕ' and ψ' correspond to the N–Cα and Cα–C′ bonds respectively; the

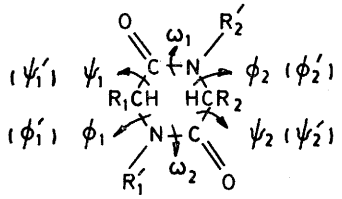
* It was shown that INDO-MO theory is better than valence bond theory in accounting for the details of homoallylic coupling.[37] In this work, the conclusions from valence bond theory are used [equation (2)] to describe the general features of ^5J(HH) in peptides as the results are easier to apply than MO theory which is needed to account for the fine details in behaviour.

[31] R. Degeilh and R. E. Marsh, *Acta Cryst.*, 1959, **12**, 1007.
[32] P. Groth, *Acta Chem. Scand.*, 1969, **23**, 3155.
[33] E. Sletten, *J. Amer. Chem. Soc.*, 1970, **92**, 172.
[34] (*a*) G. R. Pettit, R. B. von Dreele, G. Bollinger, P. M. Traxler, and P. Brown, *Experientia*, 1973, **29**, 521; (*b*) R. B. Von Dreele, *Acta Cryst.*, 1975, **B31**, 3887.
[35] I. L. Karle, *J. Amer. Chem. Soc.*, 1972, **94**, 81.
[36] I. L. Karle, H. C. J. Ottenheym, and B. Witkof, *J. Amer. Chem. Soc.*, 1974, **96**, 539.
[37] M. Barfield and S. Sternhell, *J. Amer. Chem. Soc.*, 1972, **94**, 1905.
[38] P. Timmins, personal communication.

primed superscript refers to torsional angles used for homoallylic coupling which involve α-hydrogen atoms. By analogy with the previous definition, ϕ' (or ψ') which refers to the molecular fragment H–C_1^α–N–C′ (or N–C′–C_2^α–H) is zero for H–C_1^α *cis*-planar with respect to N–C′ (or N–C′ *cis*-planar to C_2^α–H). Positive ϕ' and ψ' are generated by clockwise rotation of the C–H bond. It should be noted that directions of bond sequences used for determining peptide homoallylic torsional angles (ϕ' and ψ') differ from those used for peptide conformational angles (ϕ and ψ).

That homoallylic coupling behaviour accounts for the observed ^5J(HH) in cyclic peptides is demonstrated by showing that the predicted correlation between ^5J(HH) and ^3J(HNCH) is observed for cyclic dipeptides in [$^2H_6$]DMSO solution. The analysis depends on a number of assumptions which are tested using the crystal structure data of cyclic dipeptides.

(ii) *Crystal Conformations and Homoallylic Torsional*



(III)

Angles.—The properties of cyclic dipeptides (III) in the crystal state are summarised in Table 3 with subscripts 1 and 2 referring to residues 1 and 2 respectively. Calculations of ω, ϕ, and ψ have been made for those cases not previously reported. It was shown [36] that the angle between peptide planes varies with buckling of the diketopiperazine ring from *ca.* 141° for the most buckled conformations (cyclo-Pro-X derivatives) to 180° for planar molecules (cyclo-L-Ala-D-Ala, cyclo-Gly-Gly, and cyclo-Sar-Sar). It can be seen in Table 3 that the peptide bond is only approximately planar with ω varying from 0.4 (cyclo-L-Pro-Gly) to 12.5 (cyclo-Sar-L-Val). The average deviation from planarity of the *cis*-peptide bond of the cyclic dipeptides listed in Table 3 is 4.5. As a planar peptide bond is assumed for the definition of homoallylic torsional angles in this work, an error would result in the analysis if peptide bonds maintain the same conformation in solution as well as the solid state. The error involved would be considerable for buckled diketopiperazine rings which are also twisted, *i.e.* cyclo-L-Ala-L-Ala (ω_1 8.2, ω_2 0.8) and cyclo-Sar-L-Val (−0.5 and −12.5). The analysis also assumed that the diketopiperazine ring is buckled symmetrically (*i.e.* $|\phi_1| = |\psi_1|$ and $|\phi_2| = |\psi_2|$). The effect of possible twisted ring conformations on ϕ and ψ is demonstrated by the results for cyclic dipeptides in Table 3. It can be seen that $|\phi_1| \sim |\psi_1|$ and $|\phi_2| \sim |\psi_2|$ and that the assumption used subsequently in the n.m.r. analysis is approximately correct. For symmetrically buckled conformations of the diketopiperazine ring the sums of ϕ and ψ should be zero whereas the data in Table 3 indicate the magnitude of the possible error involved in that assumption, *i.e.* $\Sigma|(\phi_1 + \psi_1)|/n = \Sigma|(\phi_2 + \psi_2)|/n = 4.0$.

A similar analysis can be made for the homoallylic torsional angles ϕ' and ψ'. Using the published co-ordinates of hydrogen atom positions for crystal structures of cyclic dipeptides the separate homoallylic torsional angles (ϕ_1', ψ_1', ϕ_2', ψ_2') were calculated. In Table 4 it can be seen that $\phi_1' \sim \psi_1'$ and $\phi_2' \sim \psi_2'$ for both D- and L-amino-acid derivatives. It is also shown for each cyclic dipeptide that $\sin^2\phi_1' \times \sin^2\psi_2' \sim \sin^2\phi_2' \times \sin^2\psi_1'$ which allows equation (1) to be written in the form

$$^5J = nA^s\sin^2\phi_1'\sin^2\phi_2' \qquad (3)$$

(3). The ratio of $\sin^2\phi_1'\sin^2\psi_2' : \sin^2\phi_2'\sin^2\psi_1'$ is close to unity for the coupling paths of most cyclic dipeptides though certain molecules, *i.e.* cyclo-Sar-L-Val, cyclo-Gly-L-Tyr, and cyclo-L-Ser-L-Tyr, show particularly large deviations from unity.* An indication of the magnitude of the error involved is made by calculating ^5J for different conformations of the diketopiperazine ring using the average observed error for homoallylic torsional angles. It is found from Table 3 that $\Sigma|(\phi_1' - \psi_1')|/n = \Sigma|(\phi_2' - \psi_2')|/n$ *ca.* 5. Variations of ±5 for each angle in equation (3) leads to variations of ^5J(HH) 1.7 (±0.1) Hz for cyclo-Pro-X derivatives ($\phi' \sim 280$), ^5J(HH) 1.0 (±0.2) Hz for planar molecules ($\phi' = 240$) and ^5J(HH) 0.3 (±0.1) Hz for buckled molecules such as cyclo-Sar-L-Val and cyclo-Gly-L-Tyr ($\phi' \sim 220$).† The expected variations in ^5J(HH) are about the same as the observed errors. Analysis of the crystal structure data indicates that, within the accuracy of determining ^5J, the assumptions used in deriving equation (3) are correct. However it will be seen that observation of ^5J(HH) alone cannot distinguish unequivocally between buckled conformations of the diketopiperazine ring and those conformations that are slightly twisted.

(iii) *Determination of A^s in Different Solvents*.—Using the results from crystal structure analyses of cyclic dipeptides it is shown that ^5J is related to the torsional angles ϕ' and ψ' according to equations (1) or (3). Hence ^5J can be used in peptide conformational analysis assuming A^s is calibrated for different solvent systems. The Karplus relation [5] between ^3J(HNCH) and θ(HNCH) is approximately represented by (4) for variation in θ

$$^3J(\text{HNCH}) = B\cos^2\theta + C = B + C - B\sin^2\theta \qquad (4)$$

over a small range of angles found in diketopiperazine rings, *i.e.* $0 < \theta < 90°$. To a first approximation the Karplus relation proposed by Thong *et al.*[9] for six-

* It can be seen for cyclo-Gly-L-Tyr [39] and cyclo-L-Ser-L-Tyr [39] that the ratio is closer to unity for homoallylic angles derived from hydrogen positions calculated from nuclear co-ordinates compared to observed positions (fractional co-ordinates). The two methods of analysis indicate that the difficulty in location of hydrogen atoms by *X*-ray crystallography will also lead to significant error in the derived homoallylic torsional angles.

† Calculations were made using equation (3) with $n = 2$ and A^s(DMSO) = 0.87 Hz.

[39] Chi-Fan Lin and L. E. Webb, *J. Amer. Chem. Soc.*, 1973, **95**, 6803.

membered heterocyclic compounds with *cis*-amide bonds (as modified by Bystrov *et al.*[1] taking account of electronegativity effects) was adapted to give values of B 6.1 and C 0 Hz for $0 < \theta < 90°$. It is found that $\sin^2\theta = \sin^2\phi'$ as $\phi' = (180 \pm \theta)$ for either L- or D-amino acids; hence eliminating $\sin^2\phi'$ and $\sin^2\theta$ for equations (3) and (4) leads to the general relation (5) where $^3J_1$ and $^3J_2$ correspond to

$$^5J = nA^s\,(B + C - {}^3J_1) \times (B + C - {}^3J_2)/B^2 \quad (5)$$

^3J(HNCH) for amino-acids 1 and 2 respectively. The data for cyclic dipeptides in DMSO solutions (Table 2) which is plotted according to equation (5) in Figure 1 shows the same linear correlation for both LL and LD coupling paths. The key to compounds plotted in Figure 1 is given in Table 2. The intercept is close to the

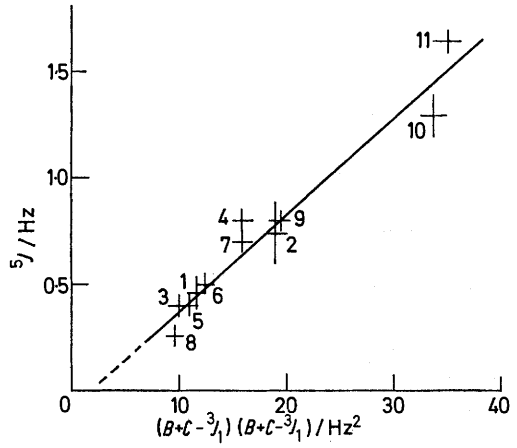


FIGURE 1 Linear correlation between homoallylic coupling, ^5J(HH), and $(B + C - {}^3J_1) \times (B + C - {}^3J_2)$ for cyclic dipeptides in [$^2H_6$]DMSO solution; B 6.1 and C 0 Hz. Key to compounds in Table 2

predicted value of zero which indicates that ^5J(HH) can be interpreted in terms of homoallylic coupling. For DMSO solutions A^s is determined from the slope to be 0.85 Hz. The value is similar to that calculated from equations (1) or (3) using the observed ^5J(HH) of 1.65 Hz for cyclo-Gly-L-Pro and assuming that the structure of two interlocking rings observed in the solid state is maintained in solution. It was shown that each cyclic dipeptide containing a proline residue exhibits very similar buckled conformations of the DKP ring.[36] This behaviour is reflected in the similarity of calculated ϕ_1', ψ_1', ϕ_2', and ψ_2' for these molecules listed in Table 4. Average values of ϕ_1' ($\sim\psi_1'$) and ϕ_2' ($\sim\psi_2'$) are 280.6 ($\pm$2.6) and 277.1 ($\pm$4.2) respectively, which lead to determination of A^s(DMSO) as 0.87 ($\pm$0.04); this value is similar to that calculated from the relation between ^5J(HH) and ^3J-(HNCH). The fact that, for DMSO solutions of cyclic dipeptides, A^s is the same magnitude determined either from crystal structure data using equation (3) or from solution data using the predicted relation between ^5J(HH) and ^3J(HNCH) indicates that the former method for determining A^s in different solvents is valid irrespective of whether or not ^3J(HNCH) can be observed. This conclusion is particularly important for peptides in solutions (*e.g.* D_2O and CD_3OD) where ^3J(HNCH) cannot be observed, *i.e.* for cyclo-Gly-L-Pro the observed $^5J(D_2O)$ of 2.6 Hz leads to $A^s(D_2O)$ 1.37 Hz and $^5J(CD_3OD)$ 2.1 Hz gives $A^s(CD_3OD)$ 1.10 Hz.

Previous measurements on cyclic dipeptides containing a cyclic imino-acid (Pip, Pro, or Aze) by Bláha *et al.*[16] are analysed in Table 5 together with a number of measurements of cyclic dipeptides in $CDCl_3$ measured in this work. The results show a linear correlation between ^5J(HH) and ^3J(HNCH) according to equation (5) similar to that observed for DMSO solutions in Figure 1, *i.e.* ^5J(HH) of cyclic dipeptides in $CDCl_3$ can be interpreted in terms of homoallylic coupling. As the variation of ^5J and ^3J for the cyclic peptides measured previously[16] is greater than that observed for cyclic dipeptides in DMSO solutions shown in Figure 1, A^s is determined from the present measurements of ^5J(HH) of 1.68 ($\pm$0.08) Hz for cyclo-Gly-L-Pro assuming the conformations of cyclic proline derivatives observed in the solid state are maintained in solution, *i.e.* $A^s(CDCl_3) = 0.88$ ($\pm$0.04) Hz. It can be seen that A^s varies with solvent decreasing in the order D_2O (1.37) > CD_3OD (1.10) > $CDCl_3$ (0.88) ~ DMSO (0.87 Hz).

A check can be made on the reliability of the magnitudes of A^s by comparison of predicted ^5J(HH) values for a planar molecule [*i.e.* $\phi' = \psi' = 240°$ using equation (1)] with those observed for a molecule expected to be planar; predicted values of ^5J(HH) are $CDCl_3$ (0.99), DMSO (0.98), CD_3OD (1.24), and D_2O (1.54 Hz) and observed values of ^5J(HH) for cyclo-Gly-Sar are $CDCl_3$ (1.1), DMSO (1.1), CD_3OD (1.3), and D_2O (1.6 Hz). The close correspondence of ^5J(calc) and ^5J(obs) for cyclo-Gly-Sar in different solvents confirms the method of analysis and the magnitudes of A^s. The rationalisation of ^5J(HH) in peptides in terms of homoallylic coupling is relevant to the determination of peptide conformations. It can be seen from the previous discussion that for comparison of conformations in different solvents using ^5J(HH) the appropriate A^s must be used.

Peptide Conformations

(i) *Cyclic Dipeptides, n = 2.*—The observation of ^5J(HC$^\alpha$NC'C$^\alpha$H) for cyclic dipeptides permits the determination of the conformation of substituted diketopiperazine rings provided that homoallylic coupling has been calibrated for the relevant solvent. The scope of the information determined from ^5J(HH) observations depends on the substitution (*cis* or *trans*) of the DKP ring.

For *cis*-substituted cyclodipeptides (LL or DD) the results of crystal structure determinations summarised in Table 4 indicate that for each molecule $\phi_1' \sim \psi_1' \sim \phi_2' \sim \psi_2'$. Hence equations (1) or (3) reduce to the form (6).

$$^5J = nA^s\sin^4\phi' = nA^s\sin^4\psi' \quad (6)$$

The dependence of ^5J(HH) on ϕ' (or ψ') for cyclic dipeptides is plotted in Figure 2 for two of the solvents measured in this work (DMSO, A = 0.87 Hz; D_2O, A = 1.37 Hz). It can be seen from Figure 2 that ^5J(HH) is an extremely sensitive parameter for approximately planar conformations, ϕ' *ca.* 240°(LL) and ϕ' *ca.* 120°(DD);

^5J(HH) is less sensitive at the extreme ranges of permitted angles with both hydrogen atoms either pseudo-axial or pseudo-equatorial. Equation (6) can be used to determine the extent of buckling of the DKP ring from observed ^5J(HH) assuming planar peptide bonds, tetrahedral α-carbon atoms, and symmetrical buckled forms. Further evidence on the symmetry of buckling of the ring can be derived from observation of both ^3J(HNCH) in the appropriate solvent. The similarity of $^3J_1$ and $^3J_2$ for cyclo-Gly-L-Phe, cyclo-Gly-L-Val, cyclo-Gly-L-Leu, and cyclo-Gly-L-Ala in Table 2 indicates that within the accuracy of the measurements (±0.2 Hz corresponds to a maximum variation of ±2° according to the Karplus relation used in this work) the DKP ring exists in a symmetrical buckled form.

Calculations of homoallylic torsional angles from observed ^5J(HH) for cyclic dipeptides according to

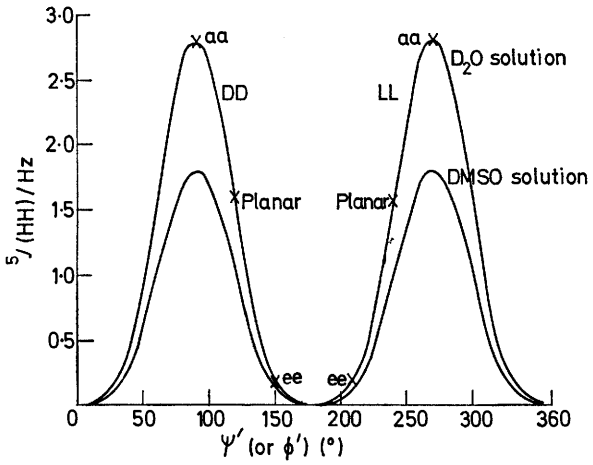


FIGURE 2 Variation between ^5J(HH) and ϕ' (or ψ') for groups in the *syn*-conformation across peptide bonds according to equation (6) with $n = 2$, A^s(DMSO) 0.87 Hz, and A^s(D$_2$O) 1.37 Hz

equation (6) yields $0 < \phi' < 90°$; these angles are converted to the appropriate values for L-($=180° + \phi'$) and D-($=180° - \phi'$) amino-acid residues. The magnitudes of ^5J(HH) in Tables 2 and 5 for cyclo-Gly-Sar ($CDCl_3$, DMSO, and D_2O) and cyclo-Gly-L-Ala (DMSO) show that these molecules are approximately planar though cyclo-Gly-L-Ala in D_2O is slightly buckled [*i.e.* ^5J(HH) 1.2 and 1.7 Hz]. It is found that cyclo-L-Pro derivatives (Gly, D-Phe, L-Phe) exhibit similar buckled conformations of the substituted diketopiparezine ring. On the other hand ^5J(LL) of 0.4 — 0.5 Hz for cyclo-Gly-L-Val and cyclo-Gly-L-Phe indicate a buckled conformation with $\phi' \sim 225°$ and the α-CH approaching the pseudo-equatorial position. In this conformation the Val or Phe side chain tends towards the pseudoaxial position; a similar conclusion is found from an analysis of ^3J(HNCH) of these molecules in DMSO solution.[15] The observation of ^5J(HH) for cyclic dipeptides in D_2O solution permits the determination of the conformation of the diketopiperazine ring, even though ^3J(HNCH) cannot be observed in these solutions. An indication of the similarity of the cyclic dipeptide ring conformation in different solvents (*e.g.* DMSO and D_2O) can be made by investigation of the ratio of ^5J(HH) for the same molecule. It is predicted from equation (6) that for the same ring conformation (ϕ' constant) equation (7) holds. The ratio of ^5J(HH) for

$$\frac{^5J(D_2O)}{^5J(DMSO)} = \frac{A^s(D_2O)}{A^s(DMSO)} = 1.57 \qquad (7)$$

cyclo-Pro-X derivatives is close to 1.57 as these molecules were used to calibrate A^s for different solvents. The ratio is also close to 1.57 for approximately planar molecules (*i.e.* 1.45 for cyclo-Gly-Sar) whereas greater variation is observed for buckled DKP ring conformations (*i.e.* 2.5 for cyclo-Gly-L-Val and 2.3 for cyclo-*N*-Me-L-Ala-L-Ala). The previous discussion shows that observation of ^5J(HH) can be used to determine detailed information about the conformation of symmetrical cyclic dipeptides (LL or DD), *i.e.* *cis*-substituted DKP rings.

For *trans*-substituted cyclic dipeptides (LD or DL) the results of crystal structure determinations summarised in Table 4 indicate that for each molecule $\phi_1' \sim \psi_2'$ and $\phi_2' \sim \psi_2'$ so that equations (1) or (3) can be applied to determine ring conformations of these molecules. As two possible combinations of ϕ' and ψ' lead to the same magnitude of ^5J(HH), unequivocal determination of the conformation of LD cyclic dipeptides can only be accomplished using supplementary evidence such as ^3J(HNCH). It was shown that ^5J(HH) for both *cis*- and *trans*-substituted DKP rings conform to homoallylic coupling (Figure 1). In those molecules that exhibit both ^5J(LL) and ^5J(LD), *e.g.* cyclo-Gly-X derivatives, ^5J(LD) can be predicted from ϕ_L' calculated from ^5J(LL) and ψ_D' determined from $\psi_D' = (\phi'_L - 120°)$. For example, cyclo-Sar-L-Val, cyclo-Gly-L-Val, cyclo-Sar-L-Phe, and cyclo-Gly-L-Phe in DMSO exhibit ^5J(LL) *ca.* 0.45 Hz which corresponds to ϕ_L' *ca.* 225°. Assuming ϕ_D' *ca.* 105° the calculated ^5J(LD) is 0.83 Hz which is close to the observed value (*ca.* 0.8 Hz) for each of these molecules. Equation (7) can also be applied to *trans*-substituted cyclic dipeptides in order to indicate the similarity of DKP ring conformations in different solvents.

The present analysis indicates that the conformation of both *cis*- and *trans*-substituted DKP rings can be determined from observed ^5J(HH). The analysis also rationalises previous observations of ^5J for cyclic dipeptides in $CDCl_3$ solutions containing at least one cyclic imino-acid[16] and invalidates the conclusion that ^5J for *cis*- is necessarily greater than for *trans*-substituted derivatives. The magnitudes of ^5J(HH) depend on the conformation of the cyclic dipeptide.

(ii) *Cyclic Dipeptides*, $n = 1$.—For *N*-substituted cyclic dipeptides it is found that ^5J (α-CH,NCH_3) varies for different molecules and that the variation depends on the conformation of the ring. These observations are accounted for using the results derived from homoallylic coupling ($n = 1$) by amending equation (1) to take account of the average ϕ_i' for rotation of the methyl group, *i.e.* equation (8) where p_i is the relative population

$$^5J(HH) = A^a\sin^2\psi' \times \Sigma_i p_i \sin^2\phi_i' = 0.5A^a\sin^2\psi' \quad (8)$$

of conformer i defined by the torsional angle ϕ_i'. A^a is a constant for peptide groups in *anti*-conformations. For cyclic dipeptides measured in this work, it is assumed that $A^a = A^s$ though from ^5J results for linear peptides [14] it is expected that $A^a > A^s$.

It is readily calculated that the contribution from a freely rotating methyl group (or any freely rotating group) to ^5J is a factor 0.5 assuming three conformations 120° apart, *i.e.* equation (9) applies. The reliability of

$$\Sigma_i p_i \sin^2\phi_i' = \tfrac{1}{3}[\sin^2\phi' + \sin^2(\phi' + 120) + \sin^2(\phi' + 240)] = 0.5 \quad (9)$$

such a calculation is checked for the two crystal structures of cyclic dipeptides containing *N*-methylated glycine in Table 6, *i.e.* for cyclo-Sar-Sar and cyclo-Sar-L-Val contributions of 0.49 and 0.59, respectively, are calculated. Hence an estimate of ^5J(α-CH,NCH_3) can be made for (6) ($n = 2$, $A = 0.87$) an observed ^5J(α-CH,α-CH) of 0.26 Hz (DMSO) corresponds to $\phi' \sim \psi' \sim 218°$ which on substitution in equation (8) predicts ^5J(α-CH,CH_3) 0.17 Hz. The value is close to that observed, *i.e.* 0.16 Hz. A similar correlation is observed for the same molecule in D_2O solutions where ^5J(α-CH,α-CH)$_{obs.}$ of 0.5 Hz corresponds to ψ' *ca.* 220.5°; ^5J(α-CH,NCH_3)$_{calc.}$ of 0.29 Hz is close to the observed value of 0.23 Hz. The previous examples show that ^5J(α-CH,NCH_3) has a useful role in conformational determinations of peptides containing *N*-methylated amino-acids.

It can be seen that equation (1) also relates to ^5J(HH) observed between α-CH protons and ω-CH_2 protons of cyclic imino-acids. For example, the DKP ring of cyclo-D-Pip-X (X = Gly, Leu, or Phe) has been shown to exist in an approximately planar conformation and the six-membered D-Pip ring in the chair conformation.[16] From

TABLE 6

Homoallylic torsional angles and ^5J(HH) for *N*-substituted peptides ($n = 1$)

Cyclic dipeptide	*N*-Substituted peptide ϕ_x'	ϕ_y'	ϕ_z'	$\Sigma p_i \sin^2\phi_i'$	α-CH ψ'	^5J(αCH,NR)/Hz Calc.	Obs.
						DMSO solutions [d]	
Sar-Sar [a]	118.7	235.3	350.6	0.49	244.8	0.35	0.25 (±0.1)
Sar-Sar [a]	118.7	235.3	350.6	0.49	232.0	0.26	0.25 (±0.1)
Sar-Sar				0.5 [c]	240 [b]	0.33	0.25 (±0.1)
Sar-L-Val [a]	104.6	237.3	336.9	0.59	219.9	0.21	0.20 (±0.1)
Sar-L-Val				0.5 [c]	219.9	0.18	0.2 (±0.1)
						D_2O solutions [e]	
L-DHPro-L-DHPro [a]	77.7	317.7		0.70 (±0.25)	277.6	0.94	
L-Pro-L-Leu [a]	57.3	276.6		0.84 (±0.14)	268.1	1.15	
L-Pro-Gly [a]	48.8	288.5		0.73 (±0.16)	273.5	1.00	0.9 (±0.1)
L-Pro-Gly [a]	48.8	288.5		0.73 (±0.16)	159.2	0.13	~0.1
Pro γ-CH_{2endo} [b]	90	330		0.625	160	0.10	
γ-CH_{2exo} [b]	30	270		0.625	280	0.83	

[a] Angles derived from crystal structures; appropriate references in Tables 2 and 3. [b] Angles derived from Dreiding models. [c] Theoretical value for freely rotating methyl groups (0.5, as described in text). [d] DMSO solution, A^s 0.87 Hz. [e] D_2O solution, A^s 1.37 Hz.

different conformations of the DKP ring; for a planar molecule [$\psi' = 120$(D) or 240°(L)] ^5J(α-CH,NCH_3) is calculated to vary with solvent, *e.g.* 0.32 (DMSO) and 0.51 Hz (D_2O) which can be compared with the values found for cyclo-Gly-Sar, *i.e.* 0.25 (DMSO) and 0.37 Hz (D_2O). Although the magnitudes of observed and calculated ^5J are slightly different the ratio of ^5J in both solvents (1.48) is close to the predicted value (1.57). For buckled conformations of cyclo-Sar-L-Val and cyclo-Sar-L-Phe in DMSO ψ' was previously calculated as *ca.* 225°; ^5J(α-CH,NCH_3) corresponding to this conformation is calculated from equation (8) to be 0.22 Hz (DMSO) which is close to the observed value of 0.2 Hz. Calculations of ^5J from crystal conformations of cyclo-Sar-X derivatives are compared with observed values for DMSO solutions in Table 6. Magnitudes calculated for cyclo-Sar-Sar and cyclo-Sar-L-Val are quite close to the observed values. For molecules whose conformations have not been determined by *X*-ray crystallography the magnitude of ^5J-(α-CH,NCH_3) can be rationalised with ^5J(α-CH,α-CH) according to equations (6) and (8). The data for *N*-Me-L-Ala-L-Ala in DMSO and D_2O solutions illustrate the reliability of the correlation. According to equation

the preceding discussion it is expected that five-bond long range proton coupling exists between α-CH of X and ω-CH_2 of D-Pip; the magnitude of ^5J can be predicted from equation (1) with $n = 1$ and A^s depending on solvent. For the chair form of D-Pip the ω-CH_2 protons exist in axial and equatorial positions and are markedly non-equivalent depending on their relation to the peptide carbonyl group; ω-CH_a (δ 4.69) and ω-CH_e (*ca.* δ 2.5). The corresponding ϕ' values are 120° (H_a) and 0 (H_e). The value of ψ' is either 120(D) and/or 240°(L) for α-CH of amino-acids and glycine. From equation (1) coupling of ω-CH_e ($\phi' = 0$) to either D- or L-amino-acids and both glycine methylene protons is predicted to be approximately zero. On the other hand for ω-CH_a (ϕ' *ca.* 120°) ^5J($CDCl_3$) is predicted to be 0.5 Hz for both D- (ψ' *ca.* 120°) and L-amino-acids (ψ' *ca.* 240°) and glycine in planar conformations. In general the presence of this coupling was not included in the n.m.r. parameters of cyclo-D-Pip-X derivatives though the present explanation may account for the magnitude of the unexplained coupling constant of $0 < {}^5J \leqslant 0.4$ Hz listed for cyclo-D-Pip-Gly.[16] Predictions of ^5J(α-CH,ω-CH) for cyclo-L-Pro-X and cyclo-D-Aze-X derivatives could also be made in a similar

manner. Magnitudes of such coupling are sensitive to the conformations of both ring systems.

In this work ^5J(α-CH, δ-CH_2) was observed in some cyclic dipeptides containing proline. Coupling in cyclo-L-Pro-Gly provides an example for all cyclo-Pro-X derivatives of D- and L-amino-acids. Due to near magnetic equivalence of the δ-CH_2 protons average values of ^5J(α-CH,δ-CH) are observed, *i.e.* ^5J(δ-CH, α-CH_L) = 0.9(±0.1) and ^5J(δ-CH,α-CH_D) = 0.1 Hz in D_2O solution. Assuming a fixed conformation of the proline ring similar to that observed in the solid state, ϕ' is calculated from crystal data of cyclo-Pro-X derivatives as summarised in Table 6. Each δ-CH_2 has a different ϕ' which gives a different $\sin^2\phi'$ contribution to ^5J. The mean $\sin^2\phi'$ contributions listed in Table 6 together with the corresponding ψ' lead to calculated ^5J values according to equation (8). It is found that ^5J(HH) values of 1.0 and 0.13 Hz calculated for cyclo-Gly-L-Pro are close to the observed values of 0.9 and 0.1 Hz which suggests that ^5J(α-CH,δ-CH) can be interpreted in terms of a unique conformation of the proline ring similar to that observed in the solid state. An alternative explanation is possible assuming that the proline ring oscillates rapidly between γ-CH_2 *endo*- and *exo*-positions as defined from crystal observations [40,41] and proposed for aqueous solutions of proline.[42] Assuming an oscillation between an equal mixture of γ-CH_2 *endo*- and *exo*-conformations that is rapid on the n.m.r. time scale, the ϕ' contribution is calculated to be *ca.* 0.625 using ϕ_i' derived from Dreiding models and listed in Table 6. This factor of 0.625 together with approximate ψ' values leads to determination of ^5J(δ-CH,α-CH) of 0.83 and 0.1 Hz for cyclo-Gly-L-Pro. The similarity of the observed and calculated ^5J(HH) shown in Table 6 indicates that five-bond long range coupling in Pro-X derivatives (δ-CH_2,α-CH of X) can also be interpreted in terms of interconverting puckered conformations of the proline ring. Further measurements would be needed to decide between these alternative explanations.

It has been shown that ^5J(α-CH,α-CH) of cyclic dipeptides and some *N*-substituted derivatives can be analysed in terms of homoallylic coupling according to equation (1) with $n = 2$. ^5J(α-CH, NR) of *N*-substituted cyclic dipeptides are compatible with ^5J(α-CH,α-CH) values for the same molecule according to equation (8). Magnitudes of ^5J(HH) depend on the conformation of the cyclic dipeptide and on the nature of the solvent.

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