Conformations of Cyclic Peptides. II. Side-Chain Conformation and Ring Shape in Cyclic Dipeptides[1]

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Abstract: Continued investigation of the proton magnetic resonance spectra of cyclic dipeptides has indicated that the preferred conformation of the arylmethyl side chains of histidine and tryptophan, like those of tyrosine and phenylalanine, is one in which the aromatic ring faces the dipeptide piperazinedione ring. The nature of the aromatic–piperazinedione interaction has been examined further, and its short-range, specific nature confirmed. From the spin–spin coupling of α- and amide protons it has been determined that several of the substituted 2,5-piperazinedione rings examined must be nonplanar in dimethyl sulfoxide solution, although they may become planar in trifluoroacetic acid.

Knowledge of the conformations of individual amino acid side chains is important to problems of protein secondary and tertiary structure. Side-chain conformations in the solid state of amino acids and simple peptides have been determined by X-ray crystallography,[2] and information about conformations of amino acids in solution has been obtained by nuclear magnetic resonance studies.[3] However, amino acids, and most of the derivatives examined, have electrical charges that may effect relative stabilities of the various conformations. We have felt that cyclic dipeptides, which lack this perturbing electrical influence but are equally simple structurally, may provide more general models for side-chain conformations in proteins.

Previous work[4] has demonstrated that the preferred conformation about the α–β bond of a tyrosyl residue in cyclic dipeptides is the one bringing the aromatic ring into closest proximity to the 2,5-piperazinedione ring constituting the cyclic peptide backbone. In continuing proton magnetic resonance studies of peptide conformation in solution, we have examined the interaction of histidyl and tryptophyl side chains with the piperazinedione ring, and have sought examples of competition between two different side chains for the space over the piperazinedione ring.

Using the enhanced sensitivity and resolution of a 100-MHz spectrometer, we have also examined spin–spin coupling of α- and β-protons of amino acid residues in cyclic dipeptides, and have investigated small chemical-shift differences, not caused by nearby aromatic rings, between geminal α- or β-proton pairs. In the course of this work we have determined that the piperazinedione ring, in some circumstances, departs from planarity.

Experimental Section

Cyclic Dipeptides. The cyclic dipeptides used were prepared according to published procedures.[5] They were recrystallized to constant melting point and were homogeneous according to thin-layer chromatographic and nmr criteria. With the exception of 2-phenylglycyl and homophenylalanyl residues, all of the α-substituted amino acid residues were of the L optical series.

Preparation of a previously unreported cyclic dipeptide is described below.

3-Phenyl-2,5-piperazinedione. This substance was prepared, starting from DL-2-phenylglycine (Eastman White Label), *via* N-chloroacetylation, ammonolysis, and fusion of the resulting glycyl-2-phenylglycine in phenol. The procedures used have been described in a previous paper.[5] Glycyl-DL-2-phenylglycine, crystallized from water, had mp 238–240° dec.

Anal. Calcd for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.43; H, 5.84; N, 13.24.

3-Phenyl-2,5-piperazinedione, crystallized from water, had mp 241–243° without decomposition.

Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.13; H, 5.32; N, 14.69.

Proton Magnetic Resonance Spectra. Most nmr spectra were obtained with a Varian HA-100 spectrometer, using an internal lock and frequency sweep. A few measurements were also made at 60 MHz, using a Varian A-60 instrument, but the HA-100 system was used for all variable-temperature studies. Probe temperature was determined with Varian samples of methanol, for temperatures below 40°, and ethylene glycol, for temperatures above 40°. The ambient-probe temperature was approximately 36° for both instruments.

Samples in organic solvents and dimethyl sulfoxide–water mixtures were degassed and sealed under vacuum. To avoid bumping at elevated temperatures solutions in D_2O were not sealed under vacuum. Peptide concentrations in all cases were close to 5% by weight, approximately 0.25 *M*.

Reference for the nonaqueous solutions was internal tetramethylsilane. For solutions containing water, a capillary of hexamethyldisiloxane or cyclohexane was used to provide both lock signal and reference. In studies in which two peptides were compared at varying temperatures, the same reference compound was used for each.

Calculations. Chemical shifts and coupling constants of the α-protons of glycyl residues were obtained by treating them as AB systems. The β-methylene proton patterns of other residues formed the AB part of ABX patterns; usually the X part (α-proton) was not sufficiently resolved so that only the AB part was analyzed. Only the absolute values of the coupling constants were determined. The geminal coupling constants are presumably negative, however.

Thermodynamic parameters for stabilization of the folded forms

(1) This work was supported by research grants from the National Science Foundation, GB 4514, and the National Institute of General Medical Sciences, U. S. Public Health Service, GM 14069.

(2) A. V. Lakshminarayanan, V. Sasisekharan, and G. N. Ramachandran in "Conformation of Bipolymers," Vol. 1, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, p 61.

(3) See, for example: (a) K. G. R. Pachler, *Spectrochim. Acta*, **20**, 581 (1964); (b) R. B. Martin and R. Mathur, *J. Am. Chem. Soc.*, **87**, 1065 (1965); (c) F. Taddei and L. Pratt, *J. Chem. Soc.*, 1553 (1964).

(4) K. D. Kopple and D. H. Marr, *J. Am. Chem. Soc.*, **89**, 6193 (1967).

(5) K. D. Kopple and H. G. Ghazarian, *J. Org. Chem.*, **33**, 862 (1968).

Table I. α-Proton Chemical Shifts and Coupling Constants of Glycine Residues in Cyclic Dipeptides, 35–42°

Dipeptide, *cyclo*-	Solvent[c]	Higher field		Lower field		
		δ_α[a]	$J_{\alpha NH}$[b]	δ_α[a]	$J_{\alpha NH}$[b]	$-J_{\alpha\alpha}$
Gly-Gly	DMSO	3.70	2.2	3.70	2.2	
	DMSO–20% D_2O	4.08		4.08		
	18% TFA–$CDCl_3$	4.28		4.28		
	TFA	4.45		4.45		
Gly-His	DMSO	3.33	1.5	3.57	2.5	17.4
	D_2O	3.58		4.13		18.5
	D_2O–D_3O^+	4.02		4.25		18.5
	TFA	4.38		4.38		
Gly-homoPhe[d]	DMSO	3.78		3.78		
	TFA	4.28		4.28		
	18% TDA–$CDCl_3$	4.19		4.19		
Gly-Leu	DMSO	3.63	3	3.83	1.5	17.5
Gly-Phe	DMSO	2.79	≤0.5	3.37	3	17.3
	TFA	3.04		3.96		18.8
Gly-PhGly[d]	DMSO	3.73	3	3.93	≤0.5	17.5
	TFA	4.54		4.54		
Gly-Trp	DMSO	2.86	≤0.5	3.35	3	17.5
	DMSO–20% D_2O	2.98		3.63		17.5
Gly-Tyr	DMSO	2.72	≤0.5	3.32	3	17.5
	TFA	3.35		4.10		18.8
Gly-Val	DMSO	3.63	3.0	3.81	~1	17.5
	DMSO–20% D_2O	4.11		4.28		18
	D_2O	4.58		4.74		18.5
	TFA	4.41		4.41		

[a] Chemical shifts in ppm below internal Me_4Si, except for solutions containing D_2O. For solution containing D_2O reference is capillary $Me_3SiOSiMe_3$. [b] Coupling constant between α-proton and vicinal N–H proton; only observed in DMSO solutions. [c] DMSO = dimethyl sulfoxide; TFA = trifluoroacetic acid. [d] PhGly = 2-phenylglycyl; homoPhe = homophenylalanyl.

Table II. Temperature Dependence of Glycyl α Resonances[a] in *c*-Gly-Trp, *c*-Gly-Val, and *c*-Gly-Gly

Solvent	T, °C	*c*-Gly-Trp		*c*-Gly-Val		*c*-Gly-Gly
DMSO[b]	18	2.79	3.34	3.64	3.83	3.70
	42	2.86	3.35	3.63	3.81	3.70
	62	2.92	3.37	3.63	3.79	3.70
	94	3.01	3.41	3.64	3.79	3.71
	126	3.11	3.43	3.64	3.78	3.71
	150	3.16	3.47	3.65	3.77	3.71
DMSO–20% D_2O[c]	42	2.98	3.63			4.08
	51	3.03	3.65			4.08
	62	3.08	3.67			4.09
	72	3.12	3.69			4.09
	81	3.16	3.70			4.10
	94	3.22	3.73			4.10
	106	3.28	3.76			4.11

[a] In parts per million. [b] DMSO = dimethyl-d_6 sulfoxide. Referred to internal tetramethylsilane. [c] Referred to capillary hexamethyldisiloxane.

Table III. Temperature Dependence of Glycyl α-Proton Resonances[a] in *c*-Gly-His

Solvent	T, °C	*c*-Gly-His		*c*-Gly-Gly
D_2O[b]	1.8	3.32	3.98	
	12.0	3.41	4.03	4.38
	23.8	3.48	4.08	
	34.0	3.54	4.11	4.44
	42.0	3.58	4.13	
	52.1	3.64	4.17	4.48
	62.7	3.69	4.19	
	73.4	3.74	4.22	4.49
	82.6	3.79	4.24	
	93.3	3.83	4.26	4.49
D_2O–CO_3^{2-}[c]	43.0	3.51	4.06	4.43
D_2O–D_3O^+[c]	43.0	4.02	4.25	4.39
	92.0	4.13	4.32	
DMSO[d]	17.5	3.30	3.56	3.71
	42.0	3.34	3.57	3.70
	61.7	3.37	3.59	3.70
	94.0	3.43	3.62	3.69
	125.5	3.47	3.64	3.69
	150.0	3.49	3.65	3.68
TFA[e]	63.0	4.24	4.35	4.40

[a] In parts per million. [b] Capillary reference cyclohexane actually used for these measurements; data are reported as referred to capillary hexamethyldisiloxane using measured difference at 35° of 1.58 ppm. [c] Capillary reference hexamethyldisiloxane used. [d] Dimethyl sulfoxide; internal tetramethylsilane reference. [e] Trifluoroacetic acid; internal tetramethylsilane reference.

of *c*-Gly-His, *c*-Gly-Trp, *c*-L-Tyr-L-Ala, and *c*-L-Tyr-L-Val were calculated as before,[4] from the temperature dependence of the difference between the chemical shift of the most affected glycyl, alanyl, or valyl proton in them and the chemical shift of the analogous proton in reference peptides lacking the aromatic residue. In the present work a computer was programmed to choose the only adjustable parameter, the value of the maximum chemical-shift difference, so as to give van't Hoff plots with minimum standard deviation of their slopes.

Results

Conformational information was derived from three aspects of the proton magnetic resonance spectra of 16 cyclic dipeptides: (1) chemical shifts of glycyl α-protons and coupling of those protons to adjacent amide protons, (2) aromatic ring-current effects observed as a function of temperature, and (3) coupling between side chain α- and β-protons.

Data of the first category are given in Table I and Figure 1. A number of 3-alkyl or aralkyl-2,5-piperazinediones (cyclic dipeptides *c*-Gly-X), when observed in dimethyl sulfoxide solution, exhibit unequal coupling of the two 6-protons (glycyl α-protons) to the 1-proton (amide proton). The piperazinedione ring must be nonplanar where this is true. In cases where there is no magnetically perturbing aromatic side chain, magnetic nonequivalence of the 6-protons disappears in tri-

fluoroacetic acid solution, suggesting that the piperazinedione ring may become planar in the latter solvent.

Ring-current effects were examined at varying temperature; the positions of side-chain proton resonances in those cyclic dipeptides bearing an aromatic side chain were compared to those of analogous resonances in nonaromatic cyclic dipeptides. Comparisons were made between *c*-Gly-Trp and *c*-Gly-Gly or *c*-Gly-Val, Table II, between *c*-Gly-His and *c*-Gly-Gly, Table III, and between *c*-L-Tyr-L-Val and *c*-Gly-Val, Table IV. In each case the measurements were made using dimethyl sulfoxide solutions of the peptides. The *c*-Gly-Trp, *c*-Gly-Gly and *c*-L-Tyr-L-Val, *c*-Gly-Val comparisons were also carried out using dimethyl sulfoxide–water mixtures, the valine-containing peptides were additionally examined in trifluoroacetic acid, and the *c*-Gly-His, *c*-Gly-Gly case was also studied in aqueous solutions. The data indicate that for the peptides bearing aromatic groups, except *c*-L-Tyr-L-Val in trifluoroacetic acid, the favored conformation about the aromatic side chain is a folded one in which the aromatic ring is in proximity to the α- or side-chain protons of the second residue (conformation F, Figure 1). This same conclusion was reached in the previous study of cyclic peptides containing the tyrosyl residue. The extent to which this folded conformation is stabilized was calculated in each case

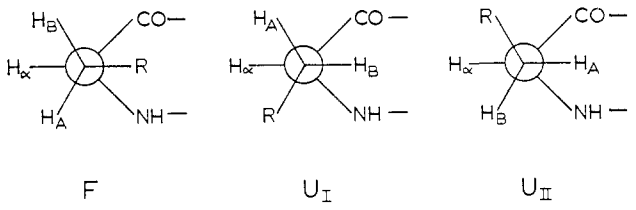


Figure 1. Conformations about the α–β bond of a β-substituted amino acid residue. Rotamer F is referred to in the text as the folded form.

Table IV. Temperature Dependence of Valyl β- and γ-Proton Resonances in *c*-L-Tyr-L-Val and *c*-Gly-Val[a]

Solvent[b]	T, °C	*c*-L-Tyr-L-Val			*c*-Gly-Val		
		β	γ	γ	β	γ	γ
DMSO	17.5	1.75	0.33	0.69	2.12	0.86	0.93
	42.0	1.75	0.41	0.71	2.12	0.86	0.93
	62.0	1.77	0.49	0.74	2.12	0.87	0.93
	94.0	1.79	0.56	0.77	2.13	0.90	0.96
	126.0	1.84	0.64	0.81	2.14	0.91	0.96
	150.0	1.87	0.68	0.83	2.14	0.91	0.96
DMSO–30% D_2O	42	1.91	0.68	1.03	2.48	1.22	1.29
	62	1.95	0.75	1.06	2.50	1.22	1.30
	81	2.00	0.82	1.09	2.50	1.24	1.30
	94	2.03	0.87	1.10	2.50	1.26	1.32
	106	2.06	0.90	1.12	2.52	1.26	1.32
TFA	−7	2.19	0.83	1.12	2.56	1.10	1.20
	4	2.22	0.83	1.12	2.55	1.10	1.19
	15	2.18	0.83	1.11	2.55	1.10	1.19
	25	2.20	0.83	1.10	2.55	1.10	1.19
	36	2.19	0.82	1.09	2.53	1.10	1.19
	41	2.20	0.83	1.09	2.54	1.10	1.19
	51	2.20	0.84	1.09	2.53	1.10	1.19
	62	2.19	0.84	1.08	2.52	1.09	1.18
	72	2.19	0.85	1.09	2.52	1.09	1.18

[a] Chemical shifts in parts per million from internal tetramethylsilane, except for the dimethyl sulfoxide–water mixture, for which reference is capillary hexamethyldisiloxane. [b] DMSO = dimethyl-d_6 sulfoxide; TFA = trifluoroacetic acid.

Table V. Stability of Folded Forms of Aromatic-Containing Cyclic Dipeptides[b]

Compound	Reference	Solvent[a]	K_{25}[d]	ΔH, kcal/mol	SD,[e] %	ΔS, eu	$\Delta\delta_{max}$,[g] ppm
c-L-Ala-L-Tyr	*c*-L-Ala_2	D_2O–DCO_3^- [c]	2.4	−2.4		−6.4	1.45
		DMSO[c]	4.5	−3.1		−7.6	0.85
		TFA[c]	3.8	−3.1		−7.8	0.95
		DMF	4.55	−3.5	2.6	−8.6	0.77
c-Gly-His	*c*-Gly_2	D_2O	9.2	−5.4	2.0	−13.5	1.03
		DMSO	2.7	−3.3	2.6	−9.0	0.54
c-Gly-Trp	*c*-Gly_2	DMSO	5.9	−3.5	5.4	−8.1	1.05
		DMSO–20% D_2O	4.1	−3.1	3.5	−7.7	1.46
	c-Gly-Val[f]	DMSO	3.3	−2.7	2.1	−6.6	1.32
c-Gly-Tyr	*c*-Gly_2	DMSO[c]	3.3	−2.8	1.8	−7.0	1.31
	c-Gly-Val[f]		3.2	−2.6	1.1	−6.5	1.45
c-L-Tyr-L-Val	Gly-Val	DMSO	1.8	−2.9	4.2	−8.6	0.79
		DMSO–30% D_2O	0.8	−2.1	2.2	−7.7	1.38

[a] DMSO = dimethyl-d_6 sulfoxide; TFA = trifluoroacetic acid; DMF = dimethylformamide. [b] Calculations described in Experimental Section. [c] Reported in ref 4. Measurement in water is of the O-carboxymethyltyrosyl compound. Values of the entropy change given in ref 4 are one-half the correct values, and are here given correctly. [d] [Folded conformation]/[all unfolded conformations]. [e] Standard deviation of slope of computer-fitted van't Hoff plot. [f] Less-shielded glycyl proton, presumably *cis*, used as reference. [g] Calculated maximum upfield shift of alanyl methyl or high-field glycyl α-proton relative to analogous group in reference compound.

making the assumption that shielding of the protons of the nonaromatic side chain by the aromatic ring is nil in the nonfolded conformations. The results of these calculations are given in Table V. To a first approximation, it appears that the hydroxyphenyl of tyrosine, the indolyl group of tryptophane, and the neutral imidazole ring of histidine all interact similarly with the piperazinedione ring of the cyclic dipeptides. The chemical shift data for *c*-Gly-His indicate that shielding by the ring current of imidazole is comparable to that produced by a benzenoid ring.

c-Gly-homoPhe, in which an aromatic ring is attached to the piperazinedione ring by an ethylene, rather than methylene, link, was also examined. Neither the glycyl α-proton resonances nor the amide N–H proton resonances gave any evidence that the aromatic ring spends an appreciable amount of time near the piperazinedione ring (see Tables I and VI).

Table VI. Chemical Shifts of Amide Protons in Cyclic Dipeptides, 35–42°

Dipeptide, *cyclo*-	Chemical shift, ppm[a] DMSO	TFA
Gly-Gly	7.93	8.26
L-Ala-L-Ala	8.03	8.39
Gly-L-Val	7.89, 8.07	8.30, 8.47
Gly-L-His	7.87, 7.95	
Gly-PhGly[b]	8.08, 8.57	8.32, 8.49
Gly-L-Phe	7.83, 8.08	8.31, 8.47
Gly-homoPhe	8.02, 8.28	8.18, 8.41
Gly-L-Tyr	7.82, 8.05	8.03, 8.30
L-Ala-L-Tyr	7.97	8.25, 8.38
L-Val-L-Tyr	7.86, 7.95	8.25
L-Leu-L-Tyr	7.98, 8.00	8.35

[a] Internal tetramethylsilane reference; DMSO = dimethyl sulfoxide; TFA = trifluoroacetic acid. [b] PhGly = 2-phenylglycyl; homoPhe = 2-phenylethylglycyl.

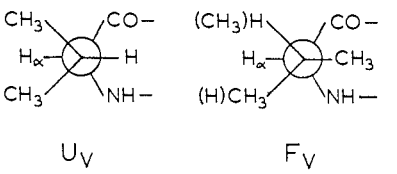
Comparison of *c*-L-Tyr-L-Ala with *c*-L-Ala-L-Ala, which was previously carried out in water, dimethyl sulfoxide, and trifluoroacetic acid, was extended to dimethylformamide (Table VII). Results of the dimethylformamide study (Table V) show that interaction of the aromatic group with the amide solvent, if any does occur, does not appear to reduce that ring's interaction with the piperazinedione ring to which it is attached.

Table VII. Temperature Dependence of β-CH_3 Resonance in *c*-L-Ala-L-Tyr and *c*-L-Ala_2 in Dimethylformamide[a]

T, °C	Chemical shift, ppm *c*-Ala-Tyr	*c*-Ala_2
42	0.795	1.38
52	0.815	1.38
63	0.845	1.385
72	0.865	1.385
92.5	0.923	1.385
94	0.93	1.385
150	1.045	1.39

[a] Below Me_4Si as internal reference.

Studies made at ambient temperatures only indicate that folding of the aromatic side chain into proximity with the other residue also occurs in the peptides *c*-L-Tyr-L-Ser and *c*-L-Tyr-L-Met. The β protons of the nonaromatic side chains in these peptides are also strongly affected by the anisotropy of the aromatic ring.



U_V F_V

Figure 2. Conformations about the α–β bond of a valyl residue.

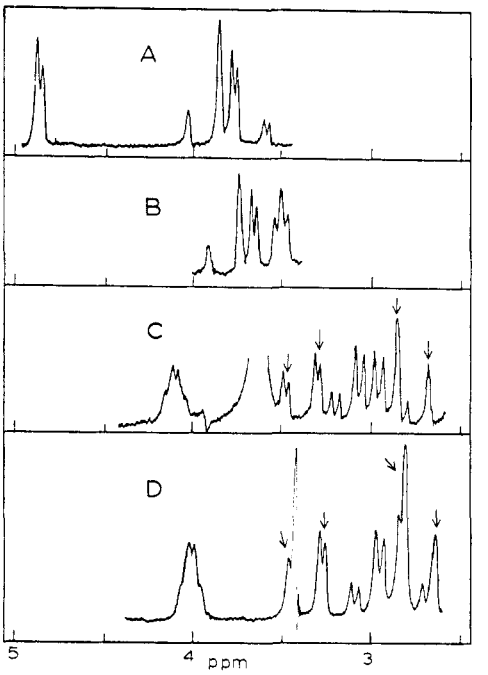


Figure 3. Spectra of the α- and β-protons of cyclic dipeptides in dimethyl sulfoxide: A, 3-phenyl-2,5-piperazinedione (*c*-Gly-PhGly); B, 3-isopropyl-2,5-piperazinedione (*c*-Gly-Val); C, 3-benzyl-2,5-piperazinedione (*c*-Gly-Phe) (the truncated peak is water); D, 3-*p*-hydroxybenzyl-2,5-piperazinedione (*c*-Gly-Tyr) (the sharp line is a trace of water). In C and D the arrows point to the lines from the glycyl α-protons.

α–β coupling constant data are summarized in Table VIII. The β protons of a cyclic dipeptide side chain were usually observably nonequivalent at 100 MHz, and two coupling constants to the adjacent α proton could be determined. The magnitude of the couplings correlated well with the side-chain conformations deduced from chemical-shift data. For those side chains that are not most stable in the folded conformation two unequal coupling constants were generally observed, indicating that a particular one of the unfolded conformations (U_I or U_{II}, Figure 1) was more heavily populated. The small α–β coupling observed for the valyl side chain indicates that, in all solvents examined, one of the conformations, F_V (Figure 2), is preferred.

Discussion

Conformation of the Piperazinedione Ring. In dimethyl sulfoxide solutions dipeptides of the *c*-Gly-X variety exhibit unequal coupling constants between the

Table VIII. β-Proton Chemical Shifts and Coupling Constants in Cyclic Dipeptides, 35–42°

Dipeptide[a] *cyclo-*	Solvent[b]	Chemical shift, ppm[c] High	Low	Coupling constants, Hz[d] $J_{\alpha h\beta}$	$J_{\alpha l\beta}$	$-J_{\beta\beta}$
Gly-His	DMSO	2.93	2.93			
	DMSO (150°)	2.90	3.05	7.4	4.6	15
	TFA	3.59	3.59			
	D_2O	3.45	3.59	5.0[f]	4.3[f]	15
Gly-Phe	DMSO	2.90	3.10	5.3	4.7	13.5
	TFA	3.27	3.39	4.5	4.5	14.3
Gly-Trp	DMSO	3.06	3.26	4.8	4.8	14.5
	DMSO–20% D_2O	3.36	3.57	5.0	4.6	14.5
Gly-Tyr	DMSO	2.89	3.11	4.5	4.5	13.5
	TFA	3.27	3.37	5.2	4.3	14.5
Gly-Val	DMSO	2.12	...	4.2	...	...
	DMSO–30% D_2O	2.48	...	4.5	...	...
	D_2O	2.84	...	3.7	...	...
	TFA	2.54	...	3.5	...	...
L-Ala-L-Tyr	DMSO	2.77	3.04	4.4	4.4	14.4
	TFA	3.24	3.37	5.0	4.5	14.5
L-His-L-Tyr[g] (H)	D_2O–D_3O^+	2.27	2.86	7.7[j]	4.8[j]	15.6
(H)	TFA	2.42	2.70	6.5	5.4	15.6
L-Leu-L-Tyr (T)	DMSO	2.73	3.04	4.8	3.7	13.8
(L)[h]		0.20	0.80	9.0	4.5	
(T)	TFA	3.20	3.40	5.0	4.5	14.8
(L)[h]		0.50	1.25	10.5	3	13.5
L-Leu-L-CMTyr[e] (T)	D_2O–DCO_3^-	3.26	3.55	4.9	3.6	14.1
(L)		0.35	1.34	10	4	13
L-Met-L-Tyr (T)	DMSO	2.76	3.06	5.4	3.8	13.8
(M)[h]		1.11	1.36	5.9	3.6	13.3
(T)	TFA	3.24	3.44	4.8	4.8	14.6
(M)[h]		1.00	1.81			
L-Ser-L-Tyr (T)	DMSO–10% D_2O	2.93	3.04			14
(S)		2.85	3.32	6.0	3.5	10.8
O-CF_3CO-L-Ser-L-Tyr (T)[i]	DMSO	~2.9	~2.9			
(S)		~2.9	3.6			11.5
(T)	TFA	3.30	3.30			
(S)		3.55	4.42	7.1	3.5	10.6
L-Val-L-Tyr (T)	DMSO	2.80	3.01	5.0	5.0	13.5
(V)		1.74	...	4.0	...	...
(T)	DMSO–30% D_2O	3.17	3.40	4.8	4.8	14
(V)		1.90	...	4.3	...	...
(T)	TFA	3.22	3.41	8.0	4.1	14
(V)		2.19	...	4.3	...	...
L-Val-L-CMTyr[e] (T)	D_2O–DCO_3^-	3.35	3.45	5	5	14
(V)		1.86	...	5	...	...

[a] Letters in parentheses indicate residue for which β-proton data are given. [b] DMSO = dimethyl-d_6 sulfoxide; TFA = trifluoroacetic acid. [c] Internal tetramethylsilane reference, except for all solutions containing D_2O, where reference is capillary hexamethyldisiloxane. [d] h and l in subscripts indicate couplings to high- and low-field β-protons, respectively. Values ±0.3 Hz. [e] CMTyr = O-carboxymethyltyrosyl. [f] These coupling constants ±0.1 Hz; taken from least-squares plot of J *vs.* temperature, 16 separate measurements. At 0°, 5.1 and 3.7 Hz. [g] Data of ref 4. [h] Analyzed by spin decoupling. [i] Estimated; overlaps serine β-proton pattern. [j] At 5°, 8.2 and 4.3 Hz.

two glycyl α-protons and the (presumably) vicinal amide proton. Examples are shown in Figure 3. The observed splittings disappear when deuterium oxide is added to the solution, and thereby can be distinguished from long-range couplings between protons on different α-carbon atoms. (The long-range couplings, which are occasionally observed, are of the order of 0.8–1.5 Hz, the *cis* coupling generally being slightly smaller than the *trans*.)

In the aromatic-containing dipeptides, *c*-Gly-Tyr (Figure 3D), *c*-Gly-Phe (Figure 3C) and *c*-Gly-Trp, the lower field glycine α proton, which is *trans* to the aralkyl side chain, is coupled to the amide proton to the extent of 3.0 Hz. The *cis*-α-proton, which is the more diamagnetically shielded by the nearby aromatic ring, has no clearly resolvable coupling, *i.e.*, $J \leq 0.5$ Hz. Similarly, in the nonaromatic cyclic dipeptides with bulky alkyl side chains, *c*-Gly-Val (Figure 3B) and *c*-Gly-Leu, the glycyl α protons are also magnetically different, and again two unequal coupling constants can be distinguished. In 3-alkyl-2,5-piperazinediones we assign the 3.0-Hz coupling to the *trans*-6-proton, by analogy with the 3-aralkyl derivatives, where the assignment is unambiguous. Thus in alkyl, as distinguished from aralkyl, piperazinediones the *trans* proton is the more shielded, usually by about 0.2 ppm.

The spectrum of 3-phenyl-2,5-piperazinedione in dimethyl sulfoxide (Figure 3A) shows the couplings to amide protons for all three α-protons. The benzylic proton has J = 3.0 Hz, and the glycyl α-protons have J's of 3.0 and ≤0.5 Hz. If the *trans*-6-proton again has the 3.0-Hz coupling, then both protons on the face of the ring opposite the substituent have the same coupling, 3.0 Hz, to their respective vicinal amide protons. As in the alkyl cases it is the *trans*-glycyl proton that is the more shielded by 0.2 ppm; this is consistent with estimates indicating negligible magnetic effect of the phenyl group on the glycyl α-protons. (Distances taken from

Dreiding models and shieldings taken from the benzene ring-current calculations of Johnson and Bovey[6] indicate that only in conformations in which the aromatic ring plane is perpendicular to the piperazinedione ring would there be significant shielding, the *cis*-proton shifted downfield by less than 0.2 ppm and the *trans* by less than 0.1. In all other rotations of the phenyl ring the shielding and the difference between *cis*- and *trans*-protons approaches zero. If rotation of the phenyl ring is not restricted, we can expect the two glycyl protons of a planar piperazinedione ring to be virtually identical.)

Chemical shift values and coupling constants for glycyl α protons in cyclic dipeptides are given in Table I.

Nonequivalence of the H–N–C_α–H coupling constants requires that the two H–N–C_α–H dihedral angles be unequal, or unequal in time average, and therefore that there be a nonplanar conformation for the piperazinedione ring at its energy minimum. This is also consistent with the chemical-shift difference between the glycyl α-protons in *c*-Gly-Val and *c*-Gly-Leu, since the alkyl side chains in these peptides would not be expected to have much direct shielding effect on the protons across the ring. From the coupling constant values it is impossible to put figures to the dihedral angles, because the magnitudes of the σ and π contributions to the three-bond coupling in this kind of system have not been estimated. Two models, shown in Figure 4, fit the logic of the coupling constant assignment we have just given. One of these, A, is a simple boat form, which retains the planarity of the amide groups. The other, B, is a twist boat, obtained by twisting the amide links out of planarity, both in the same screw sense. (Only a small amount of twist, say 10–20°, is suggested.) We prefer conformation B; in the boat conformation, A, if the substituent side chain is to be in the *quasi* equatorial position, the dihedral angle closer to 90° would have to correspond to the coupling constant of larger magnitude. If the σ contribution to this coupling constant dominates the π contribution, as appears to be the case in three-bond couplings between vinyl and allyl protons,[7] the coupling constant for the angle closer to 90° should be the smaller, not the larger, of the two observed. This condition is met in conformation B. A definitive assignment, however, will have to await experimental determination of the angular dependence of proton–proton coupling in the –C(=O)-NHCH< system.

c-Gly-Gly itself, which in dimethyl sulfoxide solution shows only one coupling constant between its α- and amide protons, is either planar, as it is in the crystal,[8,9] or is exchanging rapidly between two equally populated enantiomeric boat or twist-boat states. In dimethyl sulfoxide the observed coupling is 2.2 Hz; the half-height width of the two lines is 0.7 Hz at about 30°. In 3-(2-phenylethyl)-2,5-piperazinedione (*c*-Gly-homoPhe) there is apparently also no significant difference between the glycyl α-protons, which suggests that the 2-phenylethyl side chain exerts a negligible effect in distorting the piperazinedione ring. *c*-Gly-homoPhe is further discussed below.

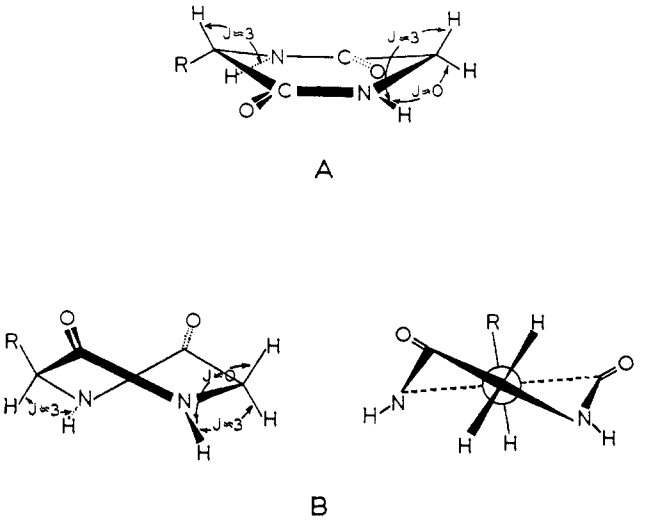


Figure 4. Boat (A) and twist-boat (B) conformations for the 2,5-piperazinedione rings. The structures show the coupling constants that would have to be assigned in each case.

In trifluoroacetic acid solutions of the cyclic dipeptides the α-proton–amide-proton couplings, though detectable, are not resolved because exchange of the latter with solvent broadens the α-proton lines slightly. For *c*-Gly-Gly, at about 30°, the single, unresolved α-proton line is 2.0 Hz wide at half-height, while in trifluoroacetic acid-*d* the line is only 1.0 Hz wide. On the other hand, the chemical-shift difference (0.2 ppm) that the glycyl protons of *c*-Gly-Val, *c*-Gly-Leu, and *c*-Gly-Ph-Gly do show in dimethyl sulfoxide should be resolved if it were present in trifluoroacetic acid solution; as Table I indicates, this difference is not observed. We suggest that the piperazinedione ring of these peptides is planar in trifluoroacetic acid solution although it may have a preferred nonplanar configuration in dimethyl sulfoxide. In trifluoroacetic acid, whether the amide groups are protonated[10] or merely strongly hydrogen bonded,[11] there would be an increase in the double bond character of the amide C–N bonds. This would make a departure of the amide group from planarity less favorable and could drive the twist-boat conformation to a fully planar one. A boat conformation, if it were preferred in dimethyl sulfoxide, could be flattened out in trifluoroacetic acid by coulombic repulsion between the positive charges developed by complete or partial (hydrogen bond) proton transfer to the amide systems.[12] A further example of this conformation change is taken

(6) C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958). The calculations are tabulated in J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, Ltd., Oxford, 1965, pp 595–604.

(7) E. W. Garbisch, Jr., *J. Am. Chem. Soc.*, **86**, 5561 (1964).

(8) R. Degeilh and R. E. Marsh, *Acta Cryst.*, **12**, 1007 (1959).

(9) R. B. Corey, *J. Am. Chem. Soc.*, **60**, 1598 (1938).

(10) I. M. Klotz, S. F. Russo, S. Hanlon, and M. A. Stake, *ibid.*, **86**, 4774 (1964).

(11) F. Quadrifoglio and D. W. Urry, *J. Phys. Chem.*, **71**, 2364 (1967).

(12) Dr. Frank A. Bovey has pointed out to us that large values (17–19 Hz) of the geminal glycyl proton couplings are consistent with a planar conformation of the piperazinedione ring. Molecular orbital calculations (M. Barfield and D. M. Grant, *J. Am. Chem. Soc.*, **85**, 1899 (1963), and earlier papers) predict that when a π-electron system, the carbonyl in this case, is adjacent to a methylene group, the geminal coupling will be most strongly negative when the geminal internuclear axis is perpendicular to the nodal plane of the π system. We note that more negative coupling constants, near 18.5 Hz, are observed in trifluoroacetic acid solutions, in which the ring may be planar, than in dimethyl sulfoxide (near 17.5 Hz) where it may depart from planarity.

up below in the discussion of the spectra of *c*-L-Val-L-Tyr.

The results just cited suggest that in a given solvent, piperazinedione ring shape may not be temperature independent. If this is so, the quantities we report for stabilization of the folded form of aromatic cyclic dipeptides do not refer only to rotation about the α–β bond of the aromatic amino acid residue; they must also contain contributions from changes in piperazinedione ring shape, although it is likely that these contributions are minor.

Aromatic Ring–Piperazinedione Interaction. An intramolecular attraction exists between the aromatic ring of a tyrosyl (or phenylalanyl) residue and the piperazinedione ring in cyclic dipeptides.[4] This interaction results in a conformational preference for that rotation about the α–β bond of the tyrosyl residue that brings the two rings into proximity (F, Figure 1). Table V presents the results of some additional measurements (and earlier data for comparison) on the extent to which this folded form is stabilized, and includes results for the indole ring of tryptophyl and the imidazole ring of histidyl. These results are discussed below.

c-L-**Ala**-L-**Tyr.** The enthalpy of stabilization of the folded form of aromatic cyclic dipeptides was shown previously to be close to 3 kcal/mol in three different solvents, trifluoroacetic acid, dimethyl sulfoxide, and water. This independence of solvent can be taken as evidence of the short-range nature and directionality of the force involved, which is probably of a dipole-induced dipole nature, amide groups providing the dipole and the aromatic ring providing a polarizable π-electron cloud. We have now examined the temperature dependence of the proton resonance spectrum of *c*-L-Ala-L-Tyr in dimethylformamide, an amide solvent that should also interact with the aromatic ring of the dipeptide. The observed upfield shifts of the alanine β-methyl proton resonance, relative to the corresponding resonance of L-alanine anhydride, are reported in Table VII. From these data we have extracted the stabilization parameters for the folded conformation, which are compared in Table V with the earlier data for *c*-L-Ala-L-Tyr in the other solvents. It is seen that dimethylformamide, although an amide, and known to associate weakly with aromatic rings,[3a,13] does not reduce the relative stability of the folded form. This reinforces the suggestions that the attractive force is a short-range one, and may be rationalized as follows. The electric quadrupole of the diketopiperazine ring can be resolved into the dipoles shown in Figure 5. In the folded form of *c*-Tyr-X the dipole component lying across the tyrosyl residue is very close to the aromatic ring, and may provide an interaction with which dipolar solvents cannot compete.

c-**Gly-Trp.** Considering the results already obtained with the tyrosine-containing dipeptides, it is not particularly surprising to find that *c*-Gly-Trp, like *c*-Gly-Tyr and *c*-Gly-Phe, appears to be more stable in a conformation in which the aromatic ring current diamagnetically shields the glycyl *cis*-α proton by 1 ppm or more. The stabilization of the folded form is indicated in Table V to be similar to that in *c*-Gly-Tyr, at least in dimethyl sulfoxide and a dimethyl sulfoxide–water mixture. (We performed no studies in water because of peptide insolubility, and none in trifluoroacetic acetic acid because of peptide instability.) The results shown in Table V were obtained by comparing, as previously, the chemical shift of protons in *c*-Gly-Gly. A better reference compound than *c*-Gly-Gly, which may be planar, would be nonplanar *c*-Gly-Val, using its less shielded glycyl proton, taken to be the *cis* proton. Using the chemical-shift data given in Table II, calculations were made both ways for the dimethyl sulfoxide solution, with no difference, except in $\Delta\delta_{max}$, that we consider significant. Recalculation of the parameters given previously for *c*-Gly-L-Tyr in dimethyl sulfoxide, using the *c*-Gly-Val reference, also results in changes of doubtful significance.

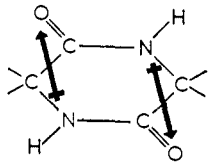


Figure 5. Local dipoles in a piperazinedione ring.

c-**Gly-His.** Previously we reported that *c*-L-His-L-Tyr, in the acidic aqueous solutions in which it is soluble, favors a folded conformation in which the tyrosyl hydroxyphenyl lies over the piperazinedione ring and the histidyl imidazole lies out in the solvent. Without protonation, *c*-Gly-His is sufficiently soluble in water to permit study, and its examination at varying temperature led to the data given in Table III. These data indicate, first, that the imidazole ring exerts magnetic shielding effects comparable to those of a benzene ring, and second, they lead to the conclusion reported in Table V, that the neutral imidazole ring, given the opportunity, also associates with the piperazinedione ring or the amide groups in it.

(Variable-temperature measurements were not made in alkaline solution, which would have suppressed ionization of the imidazole ring, because the relevant protons were quickly exchanged for deuterium, but results for 42° are included in Table III.) Although the imidazole ring must be strongly solvated by water, it appears that this solvation is not hindered in the folded conformation. Solvation may even help to stabilize the folded form, judging from the fact that both ΔH and ΔS are more negative in water than in dimethyl sulfoxide.

In neutral aqueous solution at, for example, 42°, the higher field glycyl proton of *c*-Gly-His lies upfield of the protons in *c*-Gly-Gly by about 0.9 ppm, and the two glycyl protons differ by about 0.55 ppm. In acidic solution, however, the upfield shift is only about 0.3 ppm, and the nonequivalence is about 0.2 ppm. Although these latter differences do decrease with increasing temperature (Table III), the changes are not large enough relative to uncertainties in the measurements to permit trustworthy analysis of variable-temperature data. This is unfortunate, because it would be of interest to know how much of the reduced shielding of glycyl protons in imidazole-protonated *c*-Gly-His results from reduced stability of the folded conformation and how much from reduced shielding by protonated imidazole. An esti-

(13) (a) J. V. Hatton and W. G. Schneider, *Can. J. Chem.*, **40**, 1285 (1962); (b) J. V. Hatton and R. E. Richards, *Mol. Phys.*, **5** 139 (1962).

mate from the available data gives $\Delta H = -4.5 + 1$ kcal/mol, not much changed from the neutral form, but $\Delta\delta_{max} = -0.45 \pm 0.1$ ppm, indicating that the second effect probably dominates the observations.

Measurements in dimethyl sulfoxide solution (Table III) can also be analyzed in terms of folded and unfolded conformations, with the results shown in Table V. In this solvent the folded conformation is somewhat less stable than in water.

c-Gly-homoPhe. As a means of exploring the geometry of the attraction between aromatic rings and the piperazinedione ring, we have examined the spectra of 3-(2-phenylethyl)-2,5-piperazinedione. With the side-chain methylenes of this compound retained in fully staggered arrangements, parallel planar arrangements of the rings are possible in which the C_1–C_2 bond of the phenyl is directly over the C–N bond of one or the other amide. If there can be some departure from a strictly staggered side-chain conformation, a parallel planar arrangement in which the aromatic ring is centered over the nitrogen of the glycyl residue is also possible. Any of these conformations would result in diamagnetic shielding, of about 2 ppm, for one amide proton, and would have only a small effect on the other. (The effects on the glycyl α-protons in these conformations would be negligible in the first cases, but would amount to 1–2 ppm diamagnetic shielding in the second.) It can be seen from Table VI, in which are listed representative chemical-shift data for amide protons in the cyclic dipeptides, that the *c*-Gly-homoPhe N–H resonances do not differ significantly from those of cyclic dipeptides lacking an aromatic ring. Also, as recorded in Table I, we did not detect significant differences in the chemical shifts of the two glycyl protons. Even at approximately −60° in chloroform–trifluoroacetic acid solution, these protons appear to be magnetically alike. We take, as the simplest interpretation, these observations to indicate that the diketopiperazine ring in this cyclic peptide is planar (or rapidly oscillating between nonplanar forms) and that side-chain conformations in which the two rings come into proximity are not significantly populated. This indicates again the rather specific structural requirements for the aromatic–amide interaction; in *c*-Gly-homoPhe, optimum alignment of amide and aromatic ring might only be achieved at the expense of unfavorable side-chain conformations, and the strength of the amide–aromatic attraction will not compensate for much in the way of strain energy. In addition, the population of any folded state will be influenced unfavorably by the necessity of freezing out one more C–C bond rotation than in, say, *c*-Gly-Phe.

Conformations about α–β Bonds from Coupling Constants. To this point we have been drawing conclusions about side-chain conformations from the influence of an aromatic ring on distant protons. Additional sources of information, for aromatic or nonaromatic side chains, are the coupling constants between α and β protons of a given residue. We have analyzed the spectral patterns produced by the β-methylene protons in a number of cyclic dipeptides and given the results in Table VIII. The α–β coupling constants reported in Table VIII are good only to ±0.3 Hz.

The relationship between α–β coupling constants and α–β conformer populations in amino acids has been discussed by Pachler,[3a] who suggests the values of 13.6 and 2.6 Hz for the coupling constants between *trans*- and *gauche*-proton pairs, respectively, in these substances. Taking these values for the cyclic dipeptides, one will expect for the folded arrangement (F, Figure 1) equal couplings near 3 Hz, and for a single unfolded conformation (U_I or U_{II}, Figure 1) one large (14 Hz) and one small (3 Hz) coupling. If the two unfolded conformations are equally populated, and the folded form is negligibly so, the two α–β couplings should be equal and about 8 Hz, and if all three conformations are equally populated, the couplings should be equal and near 6.5 Hz. (In *c*-L-Ala-L-Ala and *c*-L-Ala-L-Tyr, the observed couplings to the methyl protons are, in fact, 7 Hz.)

For all of the cases in which we have established from chemical-shift data that the folded form of an aromatic side-chain predominates, Table VIII shows that the α–β couplings of that side chain are about equal and cluster near 4.5 Hz. Not much weight can be put on the small differences between individual values, in view of their uncertainties, but a mean near 4.5 Hz is consistent with a predominance of the conformation F for the aromatic side chains. (If one uses the 13.6 and 2.6 Hz values for *trans* and *gauche* couplings, a value of 4 Hz is calculated for the population distribution $3F + 0.5U_I + 0.5U_{II}$.)

In *c*-L-Tyr-L-His and *c*-L-Tyr-L-Leu the tyrosyl side chain takes predominantly the folded conformation; the other side chain perforce must prefer an unfolded position. In these two cases there are observed not only the large chemical-shift differences between the protons of the non-tyrosyl methylene but also large coupling-constant differences, with coupling-constant values consistent with predominance of one of the folded arrangements, U_I or U_{II}, over the other. (The coupling constants for the β-methylene of the leucyl side chain of *c*-L-Tyr-L-Leu, to take the more striking example, can be approximately matched by assuming a conformer population of $5U_I + 2F + U_{II}$ (or $5U_{II} + 2F + U_I$).) Coupling constant and chemical shifts of the β-protons of the seryl residue in *c*-L-Tyr-L-Ser and its O-trifluoroacetyl derivative (formed on storage of the peptide in trifluoroacetic acid) indicate that the most favored form here too is one in which the tyrosyl side chain lies over the piperazinedione ring and the seryl hydroxyl lies away. (Because of the electronegative oxygen substituent on the β-carbon, the seryl α–β coupling constants are somewhat lower.[14])

In *c*-L-Tyr-L-Ser, *c*-L-Tyr-L-His, and *c*-L-Tyr-L-Leu, the larger coupling to the β-methylene of the second residue corresponds to the higher field methylene proton; *i.e.*, the proton closer to the aromatic ring is *trans* to its α-proton. In *c*-Gly-His in dimethyl sulfoxide and *c*-L-Tyr-L-Val in trifluoroacetic acid (further discussed below), in which one of the unfolded forms is preferred over the other, the α-proton that is *trans* in the preferred unfolded form is again the higher field one. The chemical-shift difference is much smaller since there is no nearby aromatic ring face. If it were possible to assign the high- and low-field environments in the absence of a perturbing aromatic group, one could say whether U_I or U_{II} is the more stable conformation. Martin and Mathur[3b] have tentatively extrapolated the evidence from β-deuterio-substituted malic acid of known (*erythro*) stereochemistry, in which the higher field methy-

(14) R. J. Abraham and K. G. R. Pachler, *Mol. Phys.*, 7, 165 (1964).

lene proton was established as *trans* to the vicinal proton in the preferred conformation,[15] to open-chain histidine and cysteine derivatives that exhibit analogous spectra, and they suggest that the conformation corresponding to U_I (Figure 1) is favored in these compounds. On the other hand, work of our own,[16] a study of *c*-Gly-d_2-Gly-d_2-Tyr-Gly-Gly-Gly, indicates that in this neutral peptide conformation U_{II} is preferred by the tyrosyl side chain. In this cyclic hexapeptide the higher field of the tyrosyl methylene protons also has the stronger coupling to the α-proton. We are inclined to believe that the hexapeptide model has the greater relevance, and would ourselves tentatively suggest U_{II} as the preferred unfolded conformation for tyrosyl and histidyl residues in the cyclic dipeptides.

***c*-Gly-Val and *c*-L-Tyr-L-Val.** Chemical-shift measurements of *c*-Gly-Val do not indicate the conformational preference of the isopropyl side chain, but on the basis of α–β coupling it is possible confidently to exclude one possibility. In both this peptide and *c*-L-Tyr-L-Val, the α–β coupling observed for the valyl side chain, in all our solvents, is close to 4 Hz (Table VIII), far from the (*trans*) value to be expected if the unfolded form U_V (Figure 2) were predominant, but close to the (*gauche*) coupling in a folded conformation, F_V (Figure 2). We therefore assign to the isopropyl group a preferred arrangement in which one methyl group projects over the piperazinedione ring. A possible reason for relative stability of the F_V conformations appears on examination of space-filling models. In the U_V conformation, the methyl groups are crowded by the carbonyl oxygen and amide proton flanking them; this crowding is reduced in the F_V conformations.

A conformation represented by F_V is also preferred by valine itself, since in aqueous solution at all pH's the α–β coupling, too, is near 4 Hz.[3c]

Judged from its own α–β coupling constants (Table VIII), the tyrosyl side chain in *c*-L-Tyr-L-Val prefers the folded conformation in water and in dimethyl sulfoxide. In trifluoroacetic acid, in contrast, the tyrosyl side chain is chiefly in an unfolded position. *c*-L-Tyr-L-Val in trifluoroacetic acid is so far the only case in which we have observed a tyrosyl side chain to be displaced from over the piperazinedione ring. The chemical-shift data tabulated in Table IV indicate, in addition, that there is no *temperature-dependent* shielding of the valyl side-chain protons by the aromatic ring in trifluoroacetic acid solution.

If the piperazinedione ring is planar in trifluoroacetic acid solution, as we have already suggested it may be, the tyrosyl and valyl side chains of *c*-L-Tyr-L-Val cannot, for steric reasons, simultaneously maintain folded conformations. Steric interference aside, if both side chains were folded there would be a very large (several parts per million) upfield shift of one of the valyl methyl resonances, a shift that is not, in fact, present. The spectral data for trifluoroacetic acid solution seem therefore best accommodated by postulating a planar piperazinedione with the valyl side chain strongly favoring the F_V conformations. In the other solvents, where both side chains appear to be in folded conformations, the piperazinedione ring cannot be planar. A boat form, with the two substituents in *quasi* equatorial positions, does account approximately for the chemical-shift differences (Table IV) between this peptide and *c*-Gly-Val. The twist boat, suggested above for other peptides, is probably excluded, since it would lead to much larger chemical-shift differences for at least one of the methyl resonances.

It is possible to use the temperature dependence in Table IV to fit van't Hoff plots; from the chemical-shift differences between the higher field methyl resonances of the two peptides one obtains the numbers given in Table V for dimethyl sulfoxide and dimethyl sulfoxide–water solutions. These presumably refer to stabilization of the folded form of the tyrosyl side chain, but they do so only if the piperazinedione ring remains in one conformation and the conformer population of the valyl side chain does not change with temperature. Nonetheless, the values calculated for *c*-L-Tyr-L-Val are similar to those that more certainly refer to rotation of the tyrosyl side chain in other peptides; this suggests that they, too, refer to the conformational preference of that group.

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(15) O. Gawron and T. P. Fondy, *J. Am. Chem. Soc.*, **81**, 6333 (1959); F. A. L. Anet, *ibid.*, **82**, 994 (1960).
(16) K. D. Kopple, M. Ohnishi, and A. Go, paper in preparation.