Conformations of Cyclic Peptides. VI. Factors Influencing Mono-, 1,4-Di-, and 1,2,4-Trisubstituted Cyclic Hexapeptide Backbones[1a]

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Abstract: Proton magnetic resonance spectra of the cyclic hexapeptides cyclo-(Gly-L-Leu-Gly)$_2$, cyclo-(Gly-d_2-L-Tyr-Gly)$_2$, cyclo-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly), and cyclo(Gly-L-His-Gly-L-Tyr-L-Ala-Gly) were measured, largely at 220 MHz, and compared with the spectra of three previously studied cyclic hexapeptides, cyclo-(Gly-Gly-Gly-d_2-Gly-d_2-L-Tyr-Gly), cyclo-(Gly-Gly-Gly-Gly-L-Leu-Gly), and cyclo-(Gly-L-His-Gly-d_2-Gly-d_2-L-Tyr-Gly). The principal solvents used were methanol and dimethyl sulfoxide. Peptide proton data, chiefly H–N–C_α–H coupling constants and chemical shift temperature coefficients, were used in constructing skeletal models having likely conformations of the seven peptides. From these models, which used standard, planar peptide units, suggested values for the conformational angles ϕ and ψ were obtained. The mono- and 1,4-disubstituted peptides have a backbone of the kind already described, with two internal, solvent-shielded peptide protons. The principal determinant for this backbone appears to be a requirement that the substituted amino acid residues take up conformations in the most stable region of the ϕ–ψ surface. Glycine residues occur in positions with extended conformations. Transannular hydrogen bonds of the 1–4 type are not important, according to the models. The 1,2,4-trisubstituted peptides have only one internal peptide proton. In these, the substituted residue that would be extended, were the backbone of the disubstituted peptides retained, adopts a less extended conformation instead. In the peptides studied, leucine, tyrosine, and histidine residues adopt values of ϕ near $-80°$ but alanine takes ϕ near $-60°$. In the circumstances, this indicates for certain side-chain rotamers interference between the backbone and groups in the side-chain δ position.

This work extends an exploration of solution conformations of peptides using cyclic peptides synthesized specifically for proton magnetic resonance studies. Previous studies of three cyclic hexapeptide backbones, two monosubstituted (III, IV) and one 1,4-disubstituted (VIII), have indicated a backbone conformation like that shown schematically in Figure 1.[2,3] In this conformation the residues numbered 1 and 4 are in almost extended conformations, with their peptide protons directed inward in the approximate plane of the ring and thereby shielded from solvent. The peptide links between residues 2 and 3 and between 5 and 6 are approximately perpendicular to the overall ring plane. In conformations like this there can be transannular hydrogen bonds between residues 1 and 4. The earlier work showed that the substituted residues are not in positions 1 or 4 of this conformation, but are most probably in positions 2 or 5. In the present work, ambiguity about the location of the substituted residues is removed and the conformational analysis is extended to some 1,2,4-trisubstituted cyclic hexapeptide backbones. The peptides studied for the present report, I, II, V, and VI (Table I), are closely related to those examined previously, and analogies and differences among the seven peptides are noted and interpreted. Cyclohexaglycine has also been examined, but without conclusive results.

Table I. Cyclic Hexapeptides Treated in This Paper[a]

Peptide	Position 1	2	3	4	5	6
I	Gly-d_2	L-Tyr	Gly	Gly-d_2	L-Tyr	Gly
II	Gly	L-Leu	Gly	Gly	L-Leu	Gly
III	Gly-d_2	L-Tyr	Gly	Gly	Gly	Gly
IV	Gly	L-Leu	Gly	Gly	Gly	Gly
V	Gly	L-His	Gly	L-Ala	L-Tyr	Gly
VI	Gly	L-His	Gly	L-Tyr	L-Ala	Gly
VII	Gly	L-His	Gly-d_2	Gly-d_2	L-Tyr	Gly

[a] Numbering corresponds to Figure 1.

The principal operation of our previous cyclic hexapeptide proton resonance work has been identification of certain residues as having solvent-shielded peptide (NH) protons. Peptide protons with chemical shifts that are relatively insensitive to temperature in hydrogen bond accepting solvents are considered solvent shielded. This criterion has since been tested or used in several naturally occurring cyclic peptides, including gramicidin S-A,[4] oxytocin,[5] ferrichrome,[6] and evolidine.[7] (In each of these there is at least one turn of the peptide chain similar to half the cyclic hexapeptide backbone shown in Figure 1, with a solvent-shielded peptide proton inside that turn.) In the present work we use in addition a recently developed correlation between H–N–C_α–H dihedral angle and coupling constant to establish details of conformation. The dihedral angle data are then used in constructing molecular models from which values of the backbone conformational angles ϕ and ψ are estimated.

(1) (a) This work was supported by a U. S. Public Health Service Grant, GM-14069, from the Institute of General Medical Sciences, National Institute of Health, and by a Public Health Service Research Career Development Award (to K. D. K.), GM-47357, from NIGMS. (b) National Science Foundation College Teacher Research Participant, 1969, 1970. (c) C. N. R. S., Institute de Biochimie, Faculte des Sciences, 91-Orsay, France. On leave 1970–71.

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Table II. Peptide Proton Resonances of Mono- and Disubstituted Cyclohexapeptides (I–IV)

Peptide	Residue[a] / Solvent	1(4) δ[b]	1(4) dδ/dT[c]	1(4) J[d]	2(5) δ[b]	2(5) dδ/dT[c]	2(5) J[d]	3(6) δ[b]	3(6) dδ/dT[c]	3(6) J[d]
I			Gly-d_2			Tyr			Gly	
	DMSO	7.55	0.001	*e*	8.36	0.0062	6.2	8.40	0.0056	5.6, 6.2[g]
	TFA	7.90		*e*	7.75		6	7.68		Σ = 11
II			Gly			Leu			Gly	
	DMSO	7.51	<0.001	Σ = 9	8.38	0.007	6.0	8.47	0.005	Σ = 11.2
	MeOH–MeCN–H_2O (4:3:3)	7.71	0.0013	Σ = 9.5	8.35	0.0075	5.5	8.39	0.0047	Σ = 11
	TFA	7.97		Σ = 10	7.68		6.5	7.97		Σ = 10
III[i]			Gly-d_2			Tyr			Gly	
	DMSO	7.70	0.0017	*e*	8.22	*f*	7.0	8.29	*f*	5.0, 6.5[g]
	DMSO–H_2O (4:1)	8.05	0.0018	*e*	8.67	0.0058	7.0	8.55	0.005	Σ = 11.5
			Gly(4)			Gly(5)			Gly-d_2 (6)	
	DMSO	7.78	0.002	Σ = 9.5	8.43	0.005	Σ = 10	8.17	*f*	*e*
	DMSO–H_2O (4:1)	8.14	0.0023	Σ = 9.5	8.79	0.005	Σ = 10	8.45	0.0038	*e*
IV			Gly			Leu			Gly	
	DMSO	(7.84		9.5)[h]	8.23		6	(8.26		6.5, ?[g])[i]
	MeOH–MeCN–H_2O (4:3:3)	(7.77	0.0015	*f*)[h]	8.50	0.0072	5.7	(8.35	0.0042	Σ = 12)[i]
			Gly(4)			Gly(5)			Gly(6)	
	DMSO	(7.74		Σ = 9.5)[h]	8.20		*f*	(8.45		5, ?[g])[i]
	MeOH–MeCN–H_2O (4:3:3)	(7.77	0.0015	*f*)[h]	8.65	0.0061	Σ = 10	(8.28	0.0042	Σ = 12)[i]
Cyclohexaglycine			Gly							
	DMSO	8.04	0.0024	11						
	TFA	7.93		10						
	H_2O	8.33		11.6						

[a] Numbering corresponds to cyclohexapeptide backbone of Figure 1. [b] 35°, relative to internal tetramethylsilane. [c] Parts per million upfield/degree. Range 20° to >100° in DMSO, −45° to +55° in methanol–acetonitrile–water. [d] From NH proton lines. For glycines sum (indicated by Σ) of α-NH couplings is given except where values are taken from analysis of α-proton pattern. Estimated error ±0.5 Hz for Gly, ± 0.3 Hz for Leu or Tyr. [e] α-Deuterated. [f] Overlap. [g] From α-proton lines, estimated error ± 0.2 ppm. [h] Ambiguous assignment, residue 1 or 4. [i] Ambiguous assignment, residue 3 or 6. [j] Data of reference 2.

The spectral information from which the conclusions of this paper are derived came almost entirely from the peptide proton resonances, and only these are reported in any detail in this paper. A discussion of side-chain conformation of these peptides will be included in a subsequent report.

Experimental Section

Cyclic Peptides. The peptides used in this work were cyclized by the procedures indicated below. Details of the syntheses of precursors may be obtained from the authors.

Cyclohexaglycyl.[8] Cyclohexaglycyl was prepared by treatment of a 0.03 *M* aqueous solution of triglycine (Fox Chemicals) with 3 equivalents of ethyl-γ-dimethylaminopropylcarbodiimide hydrochloride (EDC·HCl). After 40 hr precipitated polymer was separated by filtration, and the filtrate was passed through a mixed-bed, ion-exchange column (Bio-Rad AG 1 X4 (OH) plus AG 50W X4 (H), 50–100 mesh). The eluate was concentrated to force crystallization of chromatographically pure cyclohexaglycine, 14% of theory. The product was characterized by identification of fragments up to and including linear hexaglycine upon thin-layer chromatography of a partial hydrolysis mixture. It charred at 350–355° without melting.

Cyclo-Gly-L-Leu-Gly-Gly-L-Leu-Gly.[9] This peptide was prepared by treatment of a 0.3 *M* aqueous solution of glycyl-L-leucylglycine (Mann Research) with 3 equiv of EDC·HCl. The solution was allowed to stand at room temperature 50 hr, then diluted 10- to 20-fold with equal volumes of methanol and water, filtered, and passed through a mixed-bed, ion-exchange column. The effluent was concentrated to dryness to yield the cyclic peptide in 23% of theory.

An analytical sample, which charred at 330° without melting, was obtained by recrystallization from water. In the mass spectrometer (Varian MAT CH-7, direct sample inlet) this peptide showed the expected parent peak at 454 mass units, and exhibited a cracking pattern like that already reported.[10] *Anal.*[11] Calcd for $C_{20}H_{34}N_6O_6 \cdot 0.5H_2O$: C, 51.82; H, 7.62; N, 18.13. Found: C, 51.59; H, 7.53; N, 17.90.

Cyclo-Gly-L-Tyr-Gly-d_2-Gly-L-Tyr-Gly-d_2. L-Tyrosylglycylglycine-d_2 hydrazide (1.0 g, 0.0032 mole) was dissolved in 10 ml of dimethylformamide (DMF) and cooled to −40°. Hydrogen chloride, 2 *N* in tetrahydrofuran (8 ml, 0.016 mole), was added, and the mixture was stirred at −40° while 0.52 ml (0.0038 mole) of isoamyl nitrite was added. Stirring at −40° was continued for 30 min, then 50 ml of cold DMF containing 3 ml (0.0195 mole) of triethylamine was added. The reaction mixture was held at −20° for 2 days, concentrated under reduced pressure, and mixed with water. The water-insoluble product was crystallized twice from hot water. Ultimate purification to give a chromatographically homogeneous sample (R_f 0.75 in 3:1:1 1-butanol–acetic acid–water) was achieved by crystallization from acetic acid containing about 3% water; 0.42 g (48%) was obtained. An analytical sample was dried for 3 days at 100° and 0.05 Torr. *Anal.* Calcd for $C_{26}H_{28}D_4O_8N_8 \cdot 0.5H_2O$: C, 55.37; (H + D) as H, 5.62; N, 14.90. Found: C, 55.19; H, 5.35; N, 14.93.

Cyclo-Gly-L-His-Gly-L-Tyr-L-Ala-Gly. The open-chain hexapeptide, Gly-N^{im}-benzyl-L-His-Gly-L-Tyr-L-Ala-Gly dihydrobromide, was obtained by hydrogenolysis and hydrogen bromide in acetic acid cleavage of analytically pure BOC-Gly-N^{im}-benzyl-L-His-Gly-L-Tyr-L-Ala-Gly-*p*-nitrobenzyl ester. The terminally unblocked peptide (6.6 g, 0.008 mole) was dissolved in 400 g of molten phenol containing 2 equiv of triethylamine and about 3 equiv of dicyclohexylcarbodiimide. The mixture was stirred at 45° for 2 days, phenol was then removed by lyophilization, and the residue was taken up in ethanol–water containing a small amount of acetic acid to hydrolyze excess carbodiimide. The mixture was filtered, and the filtrate was treated with charcoal and concentrated to dryness. The cyclic peptide was isolated from the residue by 50-tube countercurrent distribution between 1-butanol and water. Crystallization of appropriate fractions from ethanol afforded 525 mg, 10% of theoretical, of chromatographically homogeneous, ninhydrin-negative product, R_f 0.6 in 5:2:1 1-butanol–pyridine–water.

Prolonged hydrogenolysis (2 weeks), using an equal weight of 10% palladium on carbon with acetic acid as solvent, was required to remove the *im*-benzyl group. The product, which could not be crystallized directly, was purified by chromatography on a cellulose powder column using 6:1 1-butanol– water and was then crystallized from 1-butanol; 245 mg of peptide was obtained from 380 mg of crude hydrogenolysis product. It was ninhydrin-negative, Pauly-positive, R_f 0.4 in 5:2:1 1-butanol–pyridine–water. Integration of its nmr spectrum corresponded exactly to the expected amino

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Table III. Peptide Proton Resonances of Histidine, Tyrosine-Containing Cyclohexapeptides (V, VI, VII)

Peptide[a]	Solvent	δ[b]	dδ/dT[c]	J[d]	δ[b]	dδ/dT[c]	J[d]	δ[d]	δd/dT[c]	J[d]
V (CF_3COOH)			Gly(1)			His(2)			(Gly(3 or 6)	
	DMSO	7.94	0.0011	9.5 ± 0.5	8.57	0.0057	8.2	8.48	0.0052	10 ± 0.5
	MeOH	8.20	0.0002	8.5 ± 0.5	8.44	0.0053	8.5	8.55	0.0053	10 ± 0.5
	H_2O	8.30	0.0055	10 ± 0.3	8.35	0.0055	8.5	8.46	0.0064	11 ± 1
			Ala(4)			Tyr(5)			Gly(3 or 6)	
	DMSO	8.18	0.0038	5.7	8.05	0.0049	7.0	8.26	0.0042	10.5 ± 0.5
	MeOH	8.44	0.0034	5.0	7.95	0.0065	7.0	8.33	0.0036	10 ± 0.5
	H_2O	8.39	0.0055	5.0	8.13	0.0061	7.3	8.46	0.0055	11 ± 1
VI[e] (HBr)			Gly(1)			His(2)			Gly(3)	
	MeOH	(8.13	0.0004	9 ± 1)	(8.42	0.009	*f*)	(8.36	0.0042	10 ± 0.5
			Tyr(4)			Ala(5)			Gly(6)	
	MeOH	(7.85	0.0023	6.8)	(8.42	0.009	4.5[i])	(8.23	0.0033	9.5 ± 0.5)
VII (CF_3COOH)			Gly(1)			His(2)			Gly-d_2(3)	
	DMSO[i]	7.59	0.0012	10 ± 1	8.48	0.006	*f*	8.40	0.006	*g*
	MeOH	7.95		*h*	8.60		7.0 ± 0.3	8.51		*g*
	H_2O[i]	7.83		10.5 ± 0.5	8.30		8 ± 1	8.45		*g*
			Gly-d_2(4)			Tyr(5)			Gly(6)	
	DMSO[i]	7.53	0.0012	*g*	8.40	0.006	*e*	8.34	0.006	*e*
	MeOH	7.86		*g*	8.47		*e*	8.56		*e*
	H_2O[i]	7.75		*g*	8.10		6.2 ± 0.2	8.30		*e*

[a] Residue numbering as in Figure 1. All imidazolium salts of indicated acids. [b] At 20° for V, VI, and 30° for VII; reference internal TMS or DSS. [c] Parts per million upfield/degree; range −40 to +20° in methanol, 20–80° in dimethyl sulfoxide and water. [d] For glycines sum of α-NH couplings is given. [e] Overlaps preclude assignments to specific residues. Doublet at 7.85 is not alanine and is assigned to tyrosine to fit model analogous to V. [f] Overlap. [g] α-Deuterated. [h] Unresolved because of line width. [i] Data of reference 3. [j] From α-proton by decoupling β-CH_3, DMSO solution.

acid composition once associated butanol had been removed by evaporation (0.01 Torr) of a dimethyl sulfoxide solution.

An analytical sample was prepared by heating at 100° at 0.05 Torr for 48 hr. It decomposed above 300°. *Anal.* Calcd for $C_{24}H_{30}N_8O_7 \cdot HBr \cdot 0.5C_4H_9OH$: C, 47.28; H, 5.49; N, 16.97. Found: C, 47.43; H, 5.45; N, 17.25.

Cyclo-Gly-L-His-Gly-L-Ala-L-Tyr-Gly. An impure sample of the dihydrobromide of Gly-N^{im}-benzyl-His-Gly-Ala-Tyr-Gly, containing some debenzylated hexapeptide (4.3 g, 0.0053 mol), was dissolved in 250 ml of dimethylformamide. Two equivalents of triethylamine was added, followed by 4 g of dicyclohexylcarbodiimide in 50 ml of DMF. After 72 hr aqueous acetic acid was added to destroy unreacted carbodiimide, and precipitated dicyclohexylurea was removed by filtration. The solution was concentrated *in vacuo* and the residue was made alkaline with sodium bicarbonate. Cyclic peptide was isolated by countercurrent distribution between 1-butanol and water. The tubes containing cyclic peptides were concentrated and the residue was taken up in ethanol, from which 440 mg of *im*-benzyl cyclic peptide crystallized. The mother liquors from this crystallization were subjected to chromatography on cellulose powder using the 1-butanol–water system. An additional 380 mg of cyclic benzyl product was thus obtained, and 150 mg of debenzylated cyclic peptide was also obtained from the column.

The blocked cyclic peptide was hydrogenolyzed in glacial acetic acid using an equal weight of 10% palladium on charcoal. Complete hydrogenolysis required 3 weeks at 25° and 1 atm. The product did not crystallize readily; it was purified by precipitation from ethanol with ether. It was chromatographically homogenous, ninhydrin-negative and Pauly-positive. It was insufficiently volatile to afford a mass spectrum (direct inlet, 250°). An analytical sample was dried at 100° and 0.01 Torr for 48 hr. *Anal.* Calcd for $C_{24}H_{30}N_8O_7 \cdot H_2O$: C, 51.42; H, 5.75; N, 20.00. Found: C, 51.70; H, 5.88; N, 19.39.

Nmr Spectra. Spectra were obtained at 60 MHz on Varian A-60 or HA-60 instruments and at 220 MHz on a Varian HR-220 instrument. The techniques of measurement have been described in previous publications.[2,3,7] Representative 220-MHz spectra are shown for cyclo-Gly-L-His-Gly-L-Ala-L-Tyr-Gly in Figures 4 and 5.

Spectral assignments were made, as in previous work, by homonuclear spin decoupling of peptide (NH) and α-protons, and identification of α-protons of nonglycine residues by spin decoupling from β-protons after replacement of exchangable protons by deuterons. Histidine and tyrosine β-protons, which give overlapping patterns near 3 ppm, were distinguished on the basis of their geminal coupling constants; the histidine β-protons have higher geminal coupling constants. In the deuterated compounds I and III assignments are predicated on the synthetically known positions of deuterated glycine residues. Assignments in the analogous undeuterated leucine peptides, II and IV, were made on the assumption of similar backbone conformations for leucine and tyrosine analogs.

Results

The peptide proton (NH) resonances of the cyclic hexapeptides I–IV are described in Table II and those of V–VII are given in Table III. The Tables give assignments of resonances to the positions on the backbone indicated in Figure 1. These assignments have been made on the bases of internal evidence for each peptide and the numerous similarities among the seven peptides.

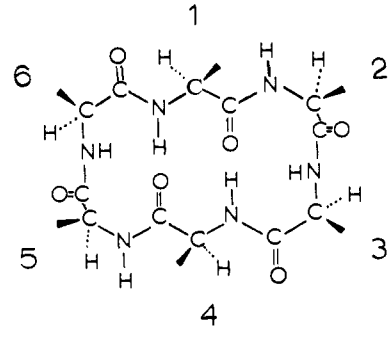

Figure 1. General cyclic hexapeptide backbone of type described in this and earlier papers. The numbering of residues is different from that of previous papers in the series and corresponds to the notation used in references 21 and 22 to describe sequences in which the peptide chain reverses direction.

The spectra of I, cyclo-(Gly-d_2-L-Tyr-Gly)$_2$, and II, cyclo-(Gly-L-Leu-Gly)$_2$, are similar. Both indicate molecules of C_2 symmetry, at least in the nmr time average. In dimethyl sulfoxide (or methanol) solutions, each shows two glycyl peptide protons at higher field with weakly temperature-dependent chemical shifts, and the remaining four peptide protons, including those of the nonglycine residues, are at lower

field with strong temperature dependences. This pattern has already been described[3] for an unsymmetrically 1,4-substituted compound, cyclo-Gly-L-His-Gly-d_2-Gly-d_2-L-Tyr-Gly, VII (see Table III). From this it seems likely that there is a common backbone conformation for 1,4-disubstituted hexapeptide rings.

Dimethyl sulfoxide spectra of the deuterium-substituted peptide I, considered together with spectra of VII, which is differently deuterium substituted with respect to the nonglycine residues, unequivocally establish the position of the side chain in the 1,4-substituted cases to be that shown in Figure 1. The deuterated residues in compound I are, by synthesis, on the amino side of tyrosine residues, and the peptide protons that are internal by the chemical-shift temperature-dependence criterion are those belonging to these deuterated residues. The glycine α-protons on the carboxyl side of the tyrosine have a chemical shift nonequivalence of 0.35 ppm, which is in accord with the suggestion made earlier[2,3] that the glycines with similar large nonequivalences in III and VII are on the carboxyl side of tyrosine in those peptides.

The peptide proton spectra of the monosubstituted cyclohexapeptides cyclo-Gly-d_2-Gly-d_2-L-Tyr-Gly_3, III, and cyclo-Gly_5-Leu, IV, in dimethyl sulfoxide (or methanol) solution have the same four-and-two pattern described above.

The spectra of the C_2 peptides I and II were also measured in trifluoroacetic acid. In this solvent, the peptide proton resonance pattern (Table II) differs markedly from that of dimethyl sulfoxide or methanol solutions. The resonances are more closely grouped, and their relative positions for each kind of residue are changed. One interpretation of these observations is that the backbone structure of Figure 1, which has not only solvent-shielded NH groups but solvent-shielded carbonyls as well, is no longer predominant because of strong solvation of the amide carbonyl groups. In a strongly proton-donating solvent, structures with internal, solvent-shielded, carbonyl groups would become relatively destabilized, and conformations with all carbonyl oxygens exposed should become most important. On the other hand, if the backbone is unchanged in trifluoracetic acid, the relative upfield shift of the exposed protons could be ascribed to weaker hydrogen bonding.[3]

One intention of this work was to compare conformational stabilities of mono- and 1,4-disubstituted cyclohexapeptide backbones in a solvent in which the Figure 1 conformation is favored. The NH temperature dependence of the leucine-containing peptides, II and IV, was determined over the range $-45°$ to $+55°$ in a solvent mixture of methanol, water, and acetronitrile (chosen to dissolve the peptides sufficiently, yet freeze at a low temperature). Given the assignments of residues to positions, one can see from Table II that for the two peptides there is precise parallelism of temperature coefficients as a function of position on the backbone. Over the 100° range, the chemical shifts are described by linear temperature dependence to within a standard deviation of 3% of the slope, and the plots have no detectable trend to curvature. This suggests that both peptides retain their favored conformations over this temperature range. Since temperature dependence depends on the position of an NH in a particular backbone conformation, nonlinear dependence should occur at temperatures sufficiently higher so that different conformation states become appreciably populated. In the high temperature limit, there would be convergence of temperature coefficients to a narrow range; averaging of chemical shifts would, of course, also occur.

Cyclohexaglycine yields only single α- and peptide-proton resonances. It is insufficiently soluble in methanol to allow low temperature measurements. Thus we could not attempt to slow conformational exchange by cooling to the point where differing glycine residues could be detected, as they would be if the most stable conformation is other than sixfold symmetric. Though we cannot, therefore, comment on the preferred solution conformation of cyclohexaglycine, it is noteworthy that the temperature coefficient of the single glycine peptide proton resonance is smaller than the average of the three resonances in the C_2 peptides. It is a speculation of dubious value to suggest that if there are two internal protons in the favored conformation their environment may be more rigid than in the substituted rings.

The isomeric trisubstituted cyclohexapeptides, V and VI, differ by the addition of one methyl side chain from cyclo-Gly-L-His-Gly-Gly-L-Tyr-Gly (*c*-GHGGTG, VII), for which the Figure 1 backbone has already been suggested. Data for the three compounds V–VII, obtained from methanol solutions of the imidazole-protonated form, are presented in Table III. For V (*c*-GHGATG) and VII the Table also gives data taken from dimethyl sulfoxide and aqueous solutions. The NH resonances of the nonglycine residues of V were assigned by spin decoupling in dimethyl sulfoxide solution; these assignments were extended to the methanolic and aqueous solutions on the basis of similarities in $J_{HC_\alpha NH}$. There are enough additional similarities in the total set of data to support the underlying assumption that the conformation of V is approximately the same in methanol and dimethyl sulfoxide. Absorption overlaps in the peptide and α-proton regions of VI (*c*-GHGTAG) precluded complete identification of its resonances, and therefore this peptide was less extensively studied.

The distinct four-and-two chemical shift pattern of peptide proton resonances common to the mono- and disubstituted cyclohexapeptides previously discussed is not apparent for *c*-GHGTAG and *c*-GHGATG, as Figure 2 shows. Further, the trisubstituted peptides have at most only one weakly temperature-dependent proton ($dS/dT < 0.002$ ppm/deg). Although this belongs to a glycine residue, as in all of the other peptides examined, it is not consistently at the high-field end of the NH pattern. These differences suggest that the trisubstituted peptides depart in some way from the backbone conformation of the earlier cases.

For all of the cyclic peptides reported on, values of $H–C_\alpha–N–H$ coupling constants for the nonglycine residues were obtained from the doublet peptide proton resonances. In one case, VI, the coupling constant of the alanine residue was obtained from the α-proton lines on decoupling the β-methyl. The glycine peptide protons are X nuclei of ABX systems; their resonances appear as poorly resolved triplets. The frequency differences between the outer peaks of these triplets are

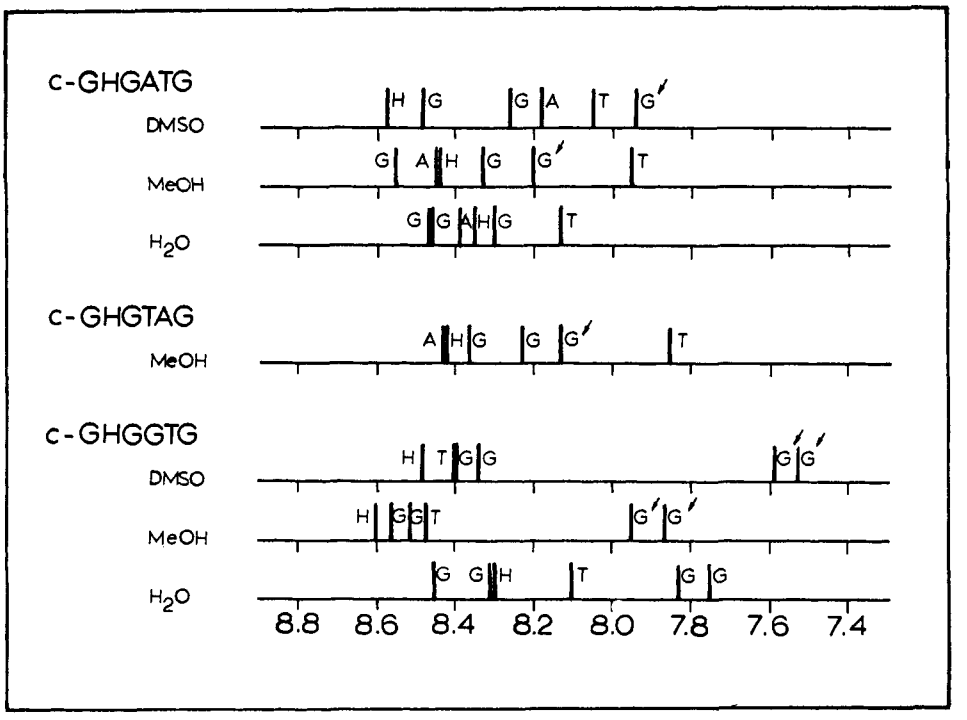

Figure 2. Schematic representation of peptide proton chemical shifts and assignments for the cyclic peptides containing histidine and tyrosine residues, V, *c*-GHGATG, VI, *c*-GHGTAG, and III, *c*-GHGGTG. The arrows indicate resonances with small, <0.002 ppm/degree, temperature sensitivity.

the sums of the two H–C_α–N–H coupling constants, and this is what is reported for glycine in most cases in Tables II and III. In certain instances, however, individual coupling constants were obtained from the α-proton patterns measured at 220 MHz. The coupling constants have been used as important input in estimating the conformations of the cyclic peptides, as described in the Discussion, below.

Infrared spectra have been reported to indicate that cyclic hexapeptides with three or four residues of the same optical series contain cis peptide bonds.[12,13] The infrared spectra of V and VII were compared in both KBr pellet and Nujol mull and showed no indication of any difference in absorption near 1340 cm^{-1}, the region ascribed to cis amide III absorption. If the 1,4-disubstituted peptide VII does not contain cis bonds, it may thus be presumed that V also does not.

Discussion

Preferred backbone conformations for cyclic oligopeptides can be estimated by use of nmr data in construction of molecular models, if planar peptide units with the usual bond angles and distances can be assumed. The structures so obtained, while probably not absolutely rigid, are likely to represent an average over a continuum of closely related, relatively stable conformations. Peptide bond planarity is probably a good approximation, since X-ray crystallographic analysis of cyclo-Gly-Gly-Gly-Gly-D-Ala-D-Ala shows a deviation from planarity as large as 8° for only one residue; all other residues deviate by less than 4°.[14] The peptide bonds of cyclohexaglycine are also very close to planarity.[15]

Proton magnetic resonances provide two kinds of clues for this model building. Dihedral angles are obtained from coupling between α- and peptide protons, and an indication of folding is obtained from temperature coefficients of the peptide-proton resonances. Additional information helpful in choosing a model is, of course, obtained from non-nmr sources: in the present cases circular dichroism results and dipeptide conformational energy maps have been of aid.

Temperature Coefficients. A shift upfield of peptide-proton resonances with increasing temperature may result from a shift in equilibrium between hydrogen-bonded and nonhydrogen-bonded states. However, even in systems where equilibrium strongly favors a hydrogen bond, temperature variation may still result, but rather from population shifts among the closely spaced levels of the hydrogen bond stretching vibration.[16] The stretching vibration can be expected to be strongly anharmonic for a bond so weak as the hydrogen bond, so that an increased population of higher levels will result in an increase average bond length. Naturally, neither equilibrium shifts nor vibrational excitation will produce temperature dependence for a buried peptide proton that is not solvated and not bonded intermolecularly to another functional group. There will also be little temperature variation for a very strongly intramolecularly bonded proton, since the fraction bonded will remain effectively unity, and the effect of vibrational excitation will be less because of greater separation between levels and reduced anharmonicity. A proton internal by the temperature coefficient criterion may therefore be involved in either

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(13) L. Mladenova-Orlinova, K. Bláha, and J. Rudinger, *ibid.*, 32, 4070 (1967).
(14) I. L. Karle, J. W. Gibson, and J. Karle, *J. Amer. Chem. Soc.*, 92, 3755 (1970).
(15) I. L. Karle and J. Karle, *Acta Crystallogr.*, 16, 969 (1963).
(16) N. Muller and R. C. Reiter, *J. Chem. Phys.*, 42, 3265 (1965).

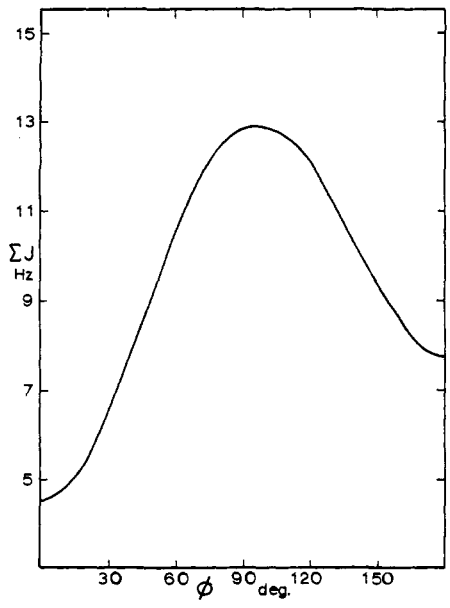

Figure 3. Relationship between sum of α-NH proton coupling constants and dihedral angle ϕ for a glycine residue.

a strong hydrogen bond or no hydrogen bond at all. On the other hand, the two factors can generate a wide range of temperature coefficients for peptide protons forming intramolecular hydrogen bonds of intermediate strength between less than rigid segments. Therefore, one may conclude that a peptide proton with a low temperature coefficient is shielded from solvent, but one may not assume the converse. A proton shielded from the solvent need not have a low temperature coefficient.

We assume, in working out the conformations described below, that protons with temperature coefficients < 0.002 ppm/degree (upfield) are internal. Readily solvated peptide protons, *e.g.*, in *N*-methylacetamide, have coefficients of 0.006–7 ppm/deg in dimethyl sulfoxide, methanol, and water (relative to internal TMS or DSS).

H–N–C_α–H Coupling Constants. The relation between dihedral angle, θ, and coupling constant, J, for the H–N–C_α–H system of peptides has been reported recently to be that given in eq 1.[17] The observed

$$J = 7.9\cos^2\theta - 1.55\cos\theta + 1.35\sin^2\theta \quad (1)$$

couplings can be interpreted in terms of the conformational angle ϕ, since $\theta = |\phi - 60°|$ for an L residue.[18] For $J < 6.3$ Hz, eq 1 leads to two values of θ, one of which may sometimes be excluded by model building or examination of a dipeptide energy map.

For glycine residues, the NH resonances give only the sum of the two N–H–C_α–H coupling constants. The two individual values will not usually be available from the α-protons, which may have the same chemical shift or may overlap other α-protons. (Separate values were obtained in this work in two instances, however.) Figure 3 shows the relation based on eq 1 (including an electronegativity correction to J) between the summed coupling constants and α for a glycine residue. The ambiguity in ϕ is greater than for an optically active residue, in that both positive and negative values of ϕ correspond to each dihedral angle.

Discussion of individual cyclic hexapeptide conformations (in solvents other than trifluoroacetic acid) will begin with the simplest case, the peptides with C_2 symmetry.

1,4-Substituted Cyclohexapeptides. The two peptides in this classification are I, cyclo-(Gly-d_2-L-Tyr-Gly)$_2$ (GTGGTG), and II, cyclo-(Gly-L-Leu-Gly)$_2$ (GLGGLG). We assume a close analogy, supported by similarities in their spectra, between them. There are only two kinds of glycine resonances and one set of tyrosine or leucine lines. As described in the Results section, it is certain that the glycine residues on the amino side of the substituted residues have the two internal peptide protons.

In the 220-MHz spectra of I and II almost all of the ABX pattern of the glycines with the nonequivalent α-protons (the residues on the carboxyl side of the substituted residue) may be identified. The H–N–C_α–H coupling constants are about 5 and 6 Hz, which limits the possible values of ϕ for this residue to ± 60–$70°$. HN–C_αH couplings are not available for the other glycine in I since all the α-protons have been replaced. In II and other peptides the corresponding α-protons are not split sufficiently to allow analysis of the α proton pattern; use of Figure 3 on the NH resonance data limits ϕ to $\pm 150°$ or $\pm 50°$. Model building shows that the peptide protons would be solvent exposed for $\phi = \pm 50°$.

ϕ is best known for the substituted residue. In I and II, the observed H–N–C_αH coupling is 6 Hz, corresponding to a dihedral angle near 0° or 140°, $\phi = +60$ or $-80°$. $\theta = 0°$ ($\phi = 60°$), which corresponds to a maximum of eq 1, may probably be ruled out because residues situated in analogous positions in other cyclic hexapeptides show $J > 6$ Hz. In addition, peptide maps[19] indicate that L residues have a deeper potential well near $\phi = -80°$.

The best fits of conformational angles to these data and to the constraints of both framework and space-filling models are given in Table IV. Values of ϕ are

Table IV. Likely Backbone Conformation of 1,4-L,L-Disubstituted Cyclohexapeptides with C_2 Symmetry

Residue[a]	Gly(1)	Leu or Tyr(2)	Gly(3)
ϕ[b]	−150	−80	+70
ψ[b]	−170	+120	0
θ	90,150	140	10,130
J, calcd, Hz[c]	1.4, 7.8	6.5	6.3, 5.4
J, obsd, Hz[d]	$\Sigma J = 9$[e]	6.0[f]	6.2, 5.2
	Ferrichrome A[g]		
Residue	Ser(1)	Ser(2)	Gly(3)
ϕ	−163	−57	+82
ψ	+174	+132	−1

[a] Numbering given in Figure 1 for all Tables. [b] Convention of reference 18 used. Angles rounded to nearest 10°. [c] Corresponding to dihedral angles of model. [d] Representative values, complete set in Table II. [e] Error estimated +1 Hz −0.5 Hz, corresponding to $77° > \theta > 98°$ and $137° > \theta > 158°$. [f] Corresponds to $\theta = 137°$. Range of J's observed corresponds to $135° > \theta > 145°$. [g] Data of ref 20.

(17) G. N. Ramachandran, R. Chandrasekaran, and K. D. Kopple, *Biopolymers*, in press. An earlier but less precise correlation has been given by V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, *Tetrahedron*, **25**, 493 (1969).

(18) This is in terms of the latest convention; IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970).

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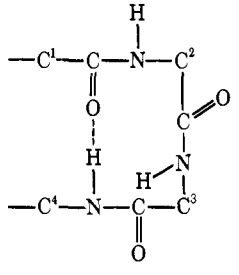
Table V. Likely Backbone Conformations for Monosubstituted Cyclohexapeptides[a]

Residue	Gly(1)	Leu or Tyr(2)	Gly(3)	Gly(4)	Gly(5)	Gly(6)	Est N—H...O distance, Å[c] 4→1	1→4
J_{obsd}[b]	Σ = 9–10	5.7–7.0	5.0, 6.5	Σ = 9–10	Σ = 9–10	Σ = 11–12		
θ	90,150	140	10,130	90,150	10,110	10,130		
(a) ϕ	−150	−80	+70	−150	−50	+70	3.5	2.9
ψ	180	+120	0	+150	+120	+20		
(b) ϕ	−150	−80	+70	+150	+50	−70	3.6	3.2
ψ	180	+120	−30	−150	−150	+40		

[a] Numbering according to Figure 1. Conformation angles estimated to nearest 10°. [b] Range of observed values from both peptides, all solutions, various instruments, including error estimates. [c] From Kendrew Skeletal Models.

input and values of ψ, the second backbone angle, are output from the Kendrew framework models used. (All angles are rounded to 10° in this and subsequent tables.) The proposed conformation turns out to be very similar to the turn formed by the three residue sequence L-Ser-L-Ser-Gly in the cyclic hexapeptide ferrichome A, the structure of which has been determined by X-ray crystallographic analysis.[20] The corresponding angles from the X-ray structure are also shown in Table IV. Although encouraging, this similarity need not signify a minimum energy conformation. The backbone conformation of ferrichrome A is determined in part by unusual constraints on the side chains of the three ornithine residues that complete its sequence.

Venkatachalam[21] has listed a number of possible hydrogen-bonded conformations (A) of three linked peptide units in which the peptide chain turns through 180°. Two such turns make a cyclic hexapeptide with



A

C_2 symmetry. Our proposed conformation for the C_2 peptides corresponds to his estimate of a favorable turn in which residue 2 is a member of the L series and residue 3 is a glycine, but with a significant exception. It also corresponds to a conformation calculated by Ramakrishnan and Sarathy[22] as a likely stable arrangement for a cyclic hexapeptide with twofold symmetry and two internal hydrogen bonds, with the same significant exception. In order that there be the hydrogen bond shown in A above between residues 1 and 4 (N—H···O distance <3 Å), ϕ_2 must be near −60° according to these calculations and models constructed from them. ϕ_2 is definitely not −60° in peptides I and II, according to the observed couplings and eq 1; ϕ = −60° corresponds to a coupling constant of 4 Hz, well outside any experimental uncertainty. For ϕ_2 near −80°, our experimental value, the N—H···O distance is near 3.5 Å. Neither space-filling models nor published dipeptide maps offer any obvious reason for occurrence of this dihedral angle at the expense of a potential hydrogen bond.

A model constructed with the parameters of Table IV maintains internal peptide NH's for residues 1 and 4. The carbonyls of these residues are moved slightly out of the interior of the ring, toward the face opposite the side chains of L residues, by the departure from −60°.

The α-protons of the corner glycine residues, positions 3 or 6, Figure 1, differ in chemical shift by 0.2 ppm in *c*-GLGGLG (II) and 0.35 ppm in *c*-GTGGTG (I), while the protons of the extended (ϕ, ψ near 180°) glycine residues, positions 1 or 4, have coincident chemical shifts. Part of this difference may arise because the corner α-protons are less symmetrically disposed relative to the planes of the flanking, magnetically anisotropic, peptide bonds. A second factor may be the relative rigidity of the two regions. Experience with molecular models suggests that there is likely to be more flexibility about $C_\alpha{}^1$ than $C_\alpha{}^3$, so that differences in local environment are more likely to be averaged about $C_\alpha{}^1$. Averaging might occur, for example, by oscillation of the plane of the peptide bond between the two glycine residues. (A model with ϕ_1 = +150°, ψ_3 = +60° can also fit the nmr data, at the expense of increasing the internal NH···O distance to over 4 Å.)

Monosubstituted Cyclic Hexapeptide Backbones. Spectra (100 MHz) of cyclo-Gly-d_2-L-Tyr-Gly-Gly-Gly-Gly-d_2 (III, GTGGGG) and cyclo-Gly$_5$-L-Leu (IV, GLGGG) have been reported,[2,3] and additional data were collected at 220 MHz in this work. These spectra show many points of similarity to each other and to those (Figures 4 and 5) of the C_2 peptides. The assignments of Table II for the deuterated peptide III are definite, and the assignments for IV were made by analogy. Similarities include the four-and-two arrangement of the peptide proton resonances, with the high-field pair consisting of weakly temperature-dependent glycine protons, and the nonequivalence of the residue 3 glycine α-protons, 0.4 ppm in III and 0.2 ppm in IV. Those individual H–N–C_α–H coupling constants that are apparent also match in the four spectra. The simplest hypothesis is that *c*-GLGGGG and *c*-GTGGGG have about the same backbone as *c*-GLGGLG and *c*-GTGGTG.

Table V describes two possible backbones for the monosubstituted cases. In the first of these (a) the backbone is approximately the same as that proposed for the C_2 peptides, except that the value of ϕ for the glycine in position 5, where there would be a substituted residue in the strictly C_2 cases, is closer to −50° than −80°. This estimate of ϕ_5 is made from the observed

(20) A. Zalkin, J. D. Forrester, and D. H. Templeton, *J. Amer. Chem. Soc.*, 88, 1810 (1966).
(21) C. M. Venkatachalam, *Biopolymers*, 6, 1425 (1968).
(22) C. Ramakrishnan and K. P. Sarathy, *Int. J. Protein Res.*, 1, 103 (1969).

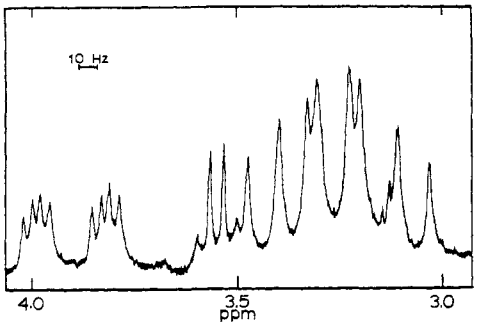


Figure 4. α-Proton region of 220-MHz spectrum of compound V, *c*-GHGATG, in dimethyl sulfoxide, ND form, 70 mg/ml.

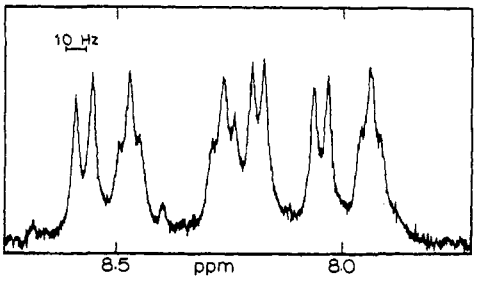


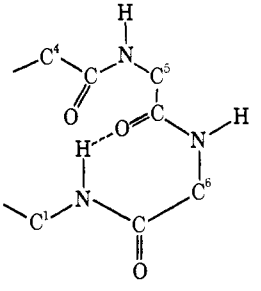
Figure 5. Peptide proton resonance of compound V, *c*-GHGATG, in dimethyl sulfoxide, 220 MHz, 20°, 70 mg/ml.

$\Sigma J_{H-N-C\alpha-H}$ of about 10 Hz, using Figure 3; if ϕ_5 had been −80°, $J_{H-N-C\alpha-H}$ would have been near 12.5 Hz, an easily discernible difference. ϕ_5 near −50° allows a transannular hydrogen bond at the all-glycine turn, with an N–H···O distance of about 2.9 Å. If model a does describe the conformation of these peptides, it emphasizes the effect of a side chain in position 2 in destroying hydrogen bond stabilization of the peptide turn, which has already been alluded to in the discussion of the C_2 peptides.

Ziegler and Bush[23] have reported some of the optical properties of peptides II, III, IV, and VII. They suggest that the circular dichroism of the n–π* peptide bond absorptions indicate differences in placement of peptide bonds between the monosubstituted and 1,4 disubstituted cases. We therefore also considered models of centrosymmetric cyclic hexapeptide backbones. X-Ray crystallographic analyses have shown more or less centrosymmetric peptide backbones for cyclohexaglycine[15] and cyclo-Gly$_4$-D-Ala$_2$.[14] Because $J_{H-N-C\alpha-H}$ for the substituted residue (position 2) and for the glycine on its carboxyl side (position 3) are the same in both mono- and disubstituted cases, the simplest hypothesis is that any non-C_2 backbone will retain at the substituted corner the same conformation as occurs at the two identical corners of the C_2 peptides. This proposition is also supported by yet unpublished optical results of Bush and Gibbs.[24] Their studies of the circular dichroism of the tyrosine side-chain absorptions indicate that in peptides I, III, V, and VII these aromatic chromophores are in similar environments relative to other nearby chromophores.

One of a number of alternative backbones is described as case b of Table V. This model retains the conformation of the substituted turn, but has the plane of the peptide bond joining residues 5 and 6 rotated by about 120°. In this case there can be a hydrogen bond between the NH of residue 1 and the carbonyl of residue 5, as shown schematically in B. Infrared studies have shown this kind of seven-membered hydrogen bonded ring to occur in noncyclic dipeptide models, *N*-acetylamino acid *N*-methylamides.[25,26]

1,2,4-Trisubstituted Cyclic Hexapeptides. Because of its close spectral analogies to the strictly C_2 peptides I and II, cyclo-Gly-L-His-Gly-Gly-L-Tyr-Gly (VII, *c*-GHGGTG) is likely to have a backbone conformation very much like that given in Table IV for *c*-GTGGTG and *c*-GLGGLG. However, replacement of a further glycine residue by alanine produces clear changes in the peptide proton region of the nmr spectra (see Figure 3). In methanol or dimethyl sulfoxide cyclo-



B

Gly-L-His-Gly-L-Ala-L-Tyr-Gly (V, *c*-GHGATG) has only one definitely internal peptide proton by the temperature-dependence criterion, but this internal proton does belong to a glycine residue, as it does in all of the other cases. The H–N–C_α–H dihedral angles of the histidine and tyrosine residues of *c*-GHGATG, estimated from the observed coupling constants, are close to those for the corresponding residues in *c*-GHGGTG. Infrared spectra of *c*-GHATG give no reason to suspect the presence of cis peptide bonds, and, as already mentioned, circular dichroism studies[24] indicate that the tyrosine aromatic chromophore is similarly situated in *c*-GTGGTG, *c*-GTGGGG, *c*-GHGGTG, and *c*-GHATG. The simplest hypothesis for V, therefore, is that its backbone conformation is related to the C_2 cases, with histidine and tyrosine residues at the corners of a structure like Figure 1 and the alanine residue in what would correspond to an extended position.

A model of *c*-GHGATG very like that given in Table IV for the C_2 peptides can be constructed consistent with the H–N–C_α–H coupling constants. Its parameters are given in Table VI, case b. However, examination of this model indicates that if the C_2 peptides and the related *c*-GHGGTG have two internal glycine protons, *c*-GHGATG should have one internal glycine proton and one internal alanine proton. Since *c*-GHGATG does not show the internal alanine proton, an alternative conformation about the alanine residue is likely.

The alternative is case a of Table VI. In b the alanine is almost fully extended, with $\phi = -170°$, but in a $\phi_{Ala} = -70°$; both of these values correspond to the H–N–C_α–H dihedral angle of 130° indicated by the 5-Hz H–N–C_α–H coupling. Conformation a puts the alanine residue in a somewhat more favorable region

(23) S. M. Ziegler and C. A. Bush, *Biochemistry*, **10**, 1330 (1971).
(24) C. A. Bush, private communication.
(25) M. Marraud, J. Neel, M. Avignon, and P. V. Huong, *J. Chim. Phys.*, 67, 959 (1970).
(26) M. Avignon, P. V. Huong, J. Lascombe, M. Marraud, and J. Neel, *Biopolymers*, **8**, 69 (1969).

Table VI. Suggested Conformation of *c*-Gly-L-His-Gly-L-Ala-L-Tyr-Gly (V)

Residue[a]	Gly(1)	His(2)	Gly(3)	Ala(4)	Tyr(5)	Gly(6)
J	$\Sigma = 9.2$	$\Sigma = 8.5$	$\Sigma = 10.5$	5.0	7.0	$\Sigma = 10.5$
θ[c]	90,150	160	0.120	130	145	0, 120
(a) ϕ[b]	−150	−100	+60	−70	−85	+60
ψ[b]	−150	+130	−50	180	+140	+20
(b) ϕ[b]	−150	−100	+60	−170	−85	+60
ψ[b]	−150	+120	+30	−170	+120	+30

[a] Numbering as in Figure 1. [b] Rounded to nearest 10°. [c] Corresponding to ϕ. [d] Corresponding to θ, for correlation, to be compared to observations reported in Table III.

of the dipeptide conformational map, and it also brings the glycine on its amino side, residue 3, into the conformation $\phi = +60°$, $\psi = +30°$, which allows a seven-membered 1–3 hydrogen bond of the type shown in B above. In this hydrogen bond the peptide proton of the alanine residue is associated with the carbonyl of the histidine residue, but it cannot be said to be shielded from the solvent and should not have a small temperature dependence. In conformation a the peptide proton of the extended glycine residue, residue 1, is still shielded from the solvent by the ring backbone, and should be temperature insensitive.

A conformation such as that just described for the Gly-Ala sequence of *c*-GHGATG ($\phi_3 = 60°$, $\psi_3 = -50,°$ $\phi_4 = -70°$) might have occurred in its Gly-Gly sequence as well, and, for that matter, in the Gly-Gly sequences of all of the 1,4 disubstituted hexapeptides. It does have the seven-membered, hydrogen-bonded ring B, while there is no seriously competing 1–4 hydrogen bond in the $\phi_3 = 70°$, $\psi_3 = 0°$, $\phi_3 = -150°$ conformation (Table IV). However, in forming the seven-membered ring hydrogen bond, the peptide protons of residues 1 and 4 would become solvent exposed, which is contrary to observation. This suggests there may be a stabilization specific to glycine residues in the region of $\phi = \psi = 180 \pm 30°$.

The spectra of peptide VI, cyclo-Gly-L-His-Gly-L-Ala-Gly (*c*-GHGTAG), in which the tyrosine and alanine residues are transposed, have overlaps that limit analysis. In methanol, *c*-GHGTAG does show one temperature-independent glycine peptide proton. The peptide resonance of tyrosine has a coupling constant corresponding to a dihedral angle of 140°, probably ϕ −80°. The alanine and histidine peptide resonances coincide, but for the alanine $J_{\text{H-N-C}\alpha\text{-H}}$ was determined to be 4.5 Hz (ϕ most probably −65°) on decoupling the α-proton resonance from the β-methyl. On the limited evidence at hand, this peptide might have the same backbone as peptide V, with minor changes in angles. In support of this is the report[24] that the circular dichroism of the tyrosine chromophore in this peptide, *c*-GHGTAG, is different in sign and magnitude from that of peptides *c*-GTGGTG, *c*-GTGGGG, *c*-GHGGTG, and *c*-GHGATG. If the backbone is indeed about the same, with only the alanine and tyrosine residues transposed, *c*-GHGTAG would be the one case in which a different environment for the tyrosine occurs.

Temperature-dependence studies in water were done only for peptide V. Although the coupling constants of the substituted residues remain the same in water as in methanol or dimethyl sulfoxide, there is no temperature-independent glycine resonance in water. That all of the peptide protons are exposed to water may have more to do with the size of the solvent molecule than with major change in conformation.

Conclusions

The conformations taken by cyclic hexapeptides containing one, two or three primary amino acids other than glycine seem to be determined mainly by a requirement that as many as possible of the substituted residues take values of ϕ and ψ that put them in the most favorable region of the conformational energy map for a substituted peptide unit, near −60°, +120°. Transannular hydrogen bonds do not seem to be important conformational determinants. As a matter of fact, the unimportance of transannular hydrogen bonds was indicated some time ago by Schwyzer's observation that derivatives of H-Gly-O-CH_2-CO-Gly-OH dimerize readily even though they cannot associate in a head-to-tail manner through intermolecular hydrogen bonding.[27]

There is also an indication that the Schwyzer type of structure, that indicated in Figure 1, is favored by a relative stability of glycine residues in the extended form ϕ, ψ near 180°, which region is less favorable for substituted residues.

In static models there is no very obvious reason why ϕ of leucine, tyrosine, or histidine residues in position 2 or 5 need be near −80°, rather than near −60°, in the peptides we have examined. The latter value gives a satisfactory transannular hydrogen bond of the 1,4 type, and probably does occur for the alanine in *c*-GHGTAG and for all-glycine turns of the monosubstituted peptides. $\phi_2 = -80°$ yields 1,4 N—H···O distances that are too large for effective hydrogen bonding. An explanation may lie in the observation from models that, although the three principal values of χ_1 (α–β rotation) are all allowed for leucine, tyrosine, and histidine residues in positions 2 or 5, χ_2 (β–γ rotation) is restricted at $\phi = -60°$ when χ_1 is 300°. It may be that the rotational entropy of the side chain is a factor in determining the backbone conformation.

Acknowledgment. We are grateful to Dr. J. J. Katz of the Argonne National Laboratory for use of the 220-MHz spectrometer, and we acknowledge helpful discussions with Professor G. N. Ramachandran of the University of Chicago and the Indian Institute of Science, Bangalore, and with Professor S. I. Miller of this department. Mr. Thomas Schamper assisted with some of the 60-MHz measurements.

(27) R. Schwyzer, J. P. Carrion, B. Gorup, H. Nolting, and T.-K. Aung, *Helv. Chim. Acta*, 47, 441 (1964).