6a). Electron transfer could then result in ring formation with the concomitant release of a chloride ion (Figure 6b). A subsequent rotation about the C–N bond would then bring the product into the conformation found by Abraham, *et al.*[8] We note that the related inactive compound shown in Figure 4d, which differs by having a pyrazole ring in place of the imidazole ring, adopts a nonplanar conformation with no internal hydrogen bond and the amide group rotated by 180°;[9] nevertheless, a similar mechanism can be proposed for the cyclization of the active parent compound.

Supplementary Material Available. A complete list of observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-2593.

Conformation of Cyclic Peptides. VIII. Cyclic Hexapeptides Containing the L-Pro-D-Phe Sequence[1]

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Abstract: The cyclic peptides *cyclo*(L-xxx-L-Pro-D-Phe)$_2$, where xxx is Ala, Orn, or His, were prepared and their conformations were studied by proton magnetic resonance and model building. The data show that these peptides exist in two conformations with average C_2 symmetry. One of these (conformation A) has trans xxx-Pro peptide bonds and is composed of two β turns, with the amide protons of the xxx residues solvent shielded *and* transannularly hydrogen bonded. The other conformation (B) probably has cis xxx-Pro peptide bonds; a likely conformation is proposed. Only conformation A occurs in chloroform or hexafluoro-2-propanol. Conformation B is favored, but not exclusively, by solvents of high dielectric constant that can serve as good hydrogen bond proton acceptors (water, dimethyl sulfoxide). Conformation B is somewhat more favored in the histidine case than in the ornithine or alanine analogs, and potassium ion slightly increases its stability (alanine peptide in dimethyl sulfoxide). From the data collected on the internal peptide protons of conformation A, it is argued that the magnetic anisotropy of the 2–3 peptide bond of a β turn has no important influence on the chemical shift of the internal proton, rather, that the principal factor is hydrogen bonding.

In continuation of studies of conformation determining sequences in oligopeptides we have prepared the cyclic hexapeptides *cyclo*(L-xxx-L-Pro-D-Phe)$_2$, where the variable residue is alanine, histidine, or ornithine, and carried out a conformational study based on proton magnetic resonance and model building techniques. This work, described below, complements a previous study of the retro isomers of these peptides, *cyclo*(L-xxx-D-Phe-L-Pro)$_2$.[2]

Experimental Section

Spectra. Proton magnetic resonance spectra were obtained using the Bruker HX 270 spectrometer of the University of Chicago and the 250-MHz spectrometer at the NMR Facility for Biomedical Research, Carnegie-Mellon University. Most of the spectra were obtained as single scans in the continuous wave mode, although in some cases signal enhancement with the Carnegie-Mellon spectrometer was obtained using the multiple rapid scan correlation technique developed by Bothner-by, Dadok, and Sprecher.[3] We are grateful to Drs. Joseph Dadok and Richard F. Sprecher for instruction in the use of the method.

Resonances were assigned to amino acid residues of the peptides using the usual spin-decoupling technique and, in some instances, homonuclear indor.[4]

Peptide Synthesis. The linear precursors of the cyclic peptides were prepared by stepwise synthesis of tripeptide fragments, which were coupled to form the open-chain hexapeptides. Intermediates were not thoroughly purified, but thin-layer chromatography and proton magnetic resonance were used to ensure that each intermediate had the required composition before the next step was undertaken. The cyclization steps, purifications, and characterizations of final products are described below. For use in the procedures below the *N*,*N*-dimethylformamide was freed of amines and water by storage over and distillation from an ethylene–maleic anhydride copolymer (Monsanto EMA 11), and the *N*-methylmorpholine was freed of secondary amine by distillation from phenyl isocyanate.

cyclo(D-Phe-L-Orn-L-Pro)$_2$. Crude Boc-(D-Phe-L-Orn(Z)-L-Pro)$_2$-$NHNH_2$, 2.65 g, was dissolved in 25 ml of anhydrous trifluoroacetic acid and stored at room temperature for 15 min. The trifluoroacetic acid was evaporated under vacuum and the residue was triturated with a large excess of anhydrous ether, washed with ether, and dried under vacuum over phosphorus pentoxide. An almost homogeneous, ninhydrin-positive product (2.8 g) was obtained.

The above product (2.25 mmol) was dissolved in 8 ml of di-

(1) This work was supported by U. S. Public Health Service Grant GM 14069 for the Institute of General Medical Sciences, National Institutes of Health, and by a PHS Career Development Award to K. D. K., GM 47357, from NIGMS. The proton magnetic resonance work reported was performed in large part at the NMR Facility for Biomedical Studies, Carnegie-Mellon University, which is supported by Grant RR-00292 from the National Institutes of Health. The 270-MHz nmr spectrometer at the University of Chicago, also used in this work, is supported by a grant (GP 33116) from the National Science Foundation.

(2) K. D. Kopple, A. Go, T. J. Schamper, and C. S. Wilcox, *J. Amer. Chem. Soc.*, **95**, 6090 (1973).

(3) (a) J. Dadok, R. F. Sprecher, and A. A. Bothner-by, 13th Experimental NMR Conference, Asilomar, Calif., 1972, paper 15-4; (b) J. Dadok and R. F. Sprecher, 14th Experimental NME Conference, Boulder, Colorado, 1973, Section 2C.

(4) W. A. Gibbons, H. Alms, R. S. Bockman, and H. R. Wyssbrod, *Biochemistry*, **11**, 1721 (1972).

methylformamide. To this solution, held at −35°, was added 6.8 ml of freshly prepared 1.65 *N* hydrogen chloride in tetrahydrofuran (11.25 mmol) followed by 0.40 ml (3 mmol) of isoamyl nitrite. After 30 min at −30° the reaction mixture gave a negative spot test for residual hydrazide. The mixture was then chilled to −60° and diluted with 1200 ml of dimethylformamide also at −60°, and to the diluted solution was added 1.60 g (15.75 mmol) of *N*-methylmorpholine. The cyclization mixture was held at −10° for 2 days.

The solvent was removed by distillation under vacuum and the residue was triturated with 200 ml of water, taken up in ethyl acetate and washed successively with 5% aqueous sodium bicarbonate, 0.5 *N* hydrochloric acid, and water. The ethyl acetate solution was evaporated and the residue was crystallized from methanol; in two crops 1.45 g (65% overall) of cyclic peptide was obtained. The product was ninhydrin negative, chromatographically homogeneous, and had the proper composition as determined by integration of its proton magnetic resonance spectrum. An analytical sample was dried at 100°, 0.02 mm, for 30 hr.

Anal. Calcd for (Phe-Orn(Z)-Pro)$_2$, $C_{54}H_{64}N_8O_{10}$: C, 65.84; H, 6.55; N, 11.37. Found: C, 65.89; H, 6.60; N, 11.58.

***cyclo*(D-Phe-L-Orn-L-Pro)$_2$ Hydrochloride.** *cyclo*(D-Phe-L-Orn-(Z)-L-Pro)$_2$, 1 g, in 300 ml of 95% ethanol containing 0.3 ml of 6 *N* hydrochloric acid, was hydrogenated over 150 mg of 10% palladium on carbon. The product was recrystallized from absolute ethanol; 0.68 g (85%) was obtained and dried under vacuum at 100°. The chromatographically homogeneous product melted with decomposition at 260°; its pmr spectrum, upon integration, indicated the proper composition.

Anal. Calcd for (Phe-Orn-Pro)$_2$·2HCl·H$_2$O, $C_{38}H_{56}N_8O_7Cl_2$: C, 56.60; H, 6.99; N, 13.87; Cl, 8.78. Found: C, 56.57; H, 6.97; N, 13.78; Cl, 8.17.

The hydrochloride was sufficiently volatile (direct inlet at 225°) to provide a mass spectrum. The spectrum exhibited the parent ion ($C_{38}H_{52}N_8O_6$) at *m/e* 716, strong peaks at M − H$_2$O and M − 2H$_2$O, and as the expected fragments derived from these by loss of amino acid residues, the corresponding imines, and C_7H_7.

Z-(D-Phe-L-Ala-L-Pro)$_2$NHNH$_2$. To 5.3 g (*ca.* 11 mmol) of crude Z-D-Phe-L-Ala-L-Pro-NHNH$_2$ in 50 ml of dimethylformamide at −35° were added, in order, 20.6 ml of freshly prepared and standardized 2.67 *N* hydrogen chloride in tetrahydrofuran (55 mmol) and 1.93 ml of isoamyl nitrite (14.3 mmol, 1.3 equiv). After 30 min at −30° a negative test for hydrazide was obtained. The reaction mixture was chilled to −60°. *N*-Methylmorpholine (5.57 g, 55 mmol) was added, followed by 4.02 g (11.6 mmol) of crude H-D-Phe-L-Ala-L-Pro-OMe in 7 ml of dimethylformamide also at −60°. The reaction mixture was stirred 2 days at −10°.

The solvent was removed under vacuum and the residue was distributed between chloroform and water. The chloroform solution was washed successively with 5% bicarbonate, *N*-hydrochloric acid, and water and then it was dried over magnesium sulfate. On evaporation of the solvent 7.9 g was obtained of a product showing on thin-layer chromatographic analysis only faint traces of minor components. This was converted to the hydrazide by 5 hr of heating at reflux with 8 ml of 95% hydrazine in 100 ml of absolute methanol. The hydrazide crystallized from the reaction solution on cooling. It was recrystallized from 250 ml of methanol. An analytical sample, mp 177–178° dec, was obtained by a second recrystallization.

Anal. Calcd for Z-(Phe-Ala-Pro)$_2$NHNH$_2$·$^1/_2$H$_2$O, $C_{42}H_{53}N_8O_{8.5}$: C, 62.59; H, 6.63; N, 13.91. Found: C, 62.69; H, 6.45; N, 14.03.

***cyclo*(D-Phe-L-Ala-L-Pro)$_2$.** The blocked hexapeptide hydrazide described above (3 g) was dissolved in 350 ml of methanol and hydrogenated at atmospheric pressure over 500 mg of 10% palladium on carbon. Thin-layer chromatography showed removal of the carbobenzyloxy group to be complete after 18 hr. The catalyst was removed by filtration, the solvent was evaporated, and the residue (2.5 g) was dried under vacuum.

The hexapeptide hydrazide, 2.5 g (3.77 mmol), in 10 ml of dimethylformamide was diazotized and cyclized exactly as described previously for the ornithine analog. The crude product, obtained on evaporation of solvent and trituration of the residue with water, weighed 1.8 g and was chromatographically homogeneous and ninhydrin negative. It was recrystallized from hot 95% ethanol. In three crops 1.51 g was obtained (67%), mp 300° dec.

An analytical sample was dried at 100° (0.02 mm) for 40 hr.

Anal. Calcd for (Phe-Ala-Pro)$_2$·H$_2$O, $C_{34}H_{44}N_6O_7$: C, 62.95; H, 6.84; N, 12.95. Found: C, 63.21; H, 6.60; N, 13.11.

The mass spectrum (direct inlet, 230°) of this peptide was identical with that of its retro isomer, *cyclo*(L-Ala-D-Phe-L-Pro)$_2$,[2] with only minor differences in some relative intensities. It showed a strong parent ion peak at *m/e* 630 and fragment ions corresponding to the loss of Phe, Ala, and Pro residues, the corresponding imines, and the C_7H_7 group.

***cyclo*(D-Phe-L-His-L-Pro)$_2$.** One portion of a preparation of Z-D-Phe-L-His-L-Pro-O-*t*-Bu was unblocked at the carboxyl using anhydrous trifluoroacetic acid, and another portion was unblocked at the amino end by hydrogenolysis over palladium on carbon. Equimolar quantities of the components (7.7 mmol) were combined in 40 ml of dimethylformamide at −10°. To this solution was added 2.5 g (9.2 mmol) of diphenylphosphoryl azide[5] in 5 ml of dimethylformamide, followed by 2.6 g (25 mmol) of *N*-methylmorpholine. The reaction mixture was stirred at −10° for 8 hr and then at room temperature overnight.

The solvent was removed under vacuum and the residue was distributed between ethyl acetate and 5% sodium bicarbonate. The ethyl acetate solution was then repeatedly washed with water to remove a ninhydrin-positive contaminant. The residue obtained on evaporation of the ethyl acetate was dried by repeated evaporation of added toluene and then completely unblocked by treatment in trifluoroacetic acid with anhydrous hydrogen bromide. After evaporation of the trifluoroacetic acid the residue was washed with anhydrous ether.

The resultant crude unblocked hexapeptide (tri)hydrobromide (4.9 g, 4.8 mmol) was dissolved in 40 ml of dimethylformamide at 0°. To this solution was added 1.58 g (5.7 mmol) of diphenylphosphoryl azide[5] in 10 ml of the same solvent. The solution was stirred for 10 min and diluted with 1 l. of purified dimethylformamide at 0°; *N*-methylmorpholine (5.5 g, 26.3 mmol) was added, and the reaction mixture was stored at 0°.

Thin-layer chromatography of a sample of the reaction mixture taken after 24 hr showed that cyclization was incomplete. An additional 1.58 g of diphenylphosphoryl azide and 1 g of *N*-methylmorpholine were added. After a further 48 hr at 0° the reaction mixture was evaporated at reduced pressure.

The residue was taken up in 400 ml of 50% ethanol and shaken several hours with AG 1 ×4 anion exchanger in the hydroxide form (*ca.* 80 g wet resin, 100 mequiv exchange capacity). The resin was washed with 50% ethanol. The solutions were combined and evaporated to dryness at reduced pressure, and the residue was stored under vacuum at room temperature overnight. The residue was then crystallized from water to yield 0.5 g (15%) of an almost homogeneous product that was ninhydrin negative and Pauly positive. This was crystallized from 95% ethanol containing sufficient hydrochloric acid to form the salt and was recrystallized from ethanol to afford the analytical sample.

The product was chromatographically homogeneous. Its mass spectrum (direct inlet at 240°) exhibited a parent ion peak of low intensity at 762. The integrated pmr spectrum was consistent with the composition (Phe, His, Pro).

Anal. Calcd for (His-Phe-Pro)$_2$·2HCl·2H$_2$O, $C_{40}H_{52}N_{10}O_8Cl_2$: C, 55.11; H, 6.01; N, 16.07; Cl, 8.13. Found: C, 55.23; H, 5.90; N, 16.13; Cl, 7.70.

Results

The tables and figures present the pmr data collected in this study. The conclusions and discussion below are based on analyses of the spectra of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ in dimethyl sulfoxide, hexafluoro-2-propanol, chloroform, and pyridine, of *cyclo*(L-Orn(HCl)-L-Pro-D-Phe)$_2$ in dimethyl sulfoxide, hexafluoro-2-propanol, and water, and of *cyclo*(L-His-(HCl)-L-Pro-D-Phe)$_2$ in dimethyl sulfoxide and water. Examples of the spectra are given in Figures 1–4. The alanine peptide was insufficiently soluble in butyl alcohols to allow an investigation of the effects of solvent isomerism like that carried out for its retro isomer.[2]

The peptides exist in two observable forms that exchange slowly on the pmr time scale. Both forms have C_2 symmetry, differing in cis–trans isomerism about the L-XXX-L-Pro peptide bond. The situation is like that described by Torchia, Wong, Deber, and Blout for

(5) T. Shioiri, K. Ninomiya, and S. Yamada, *J. Amer. Chem. Soc.*, **94**, 6203 (1972).

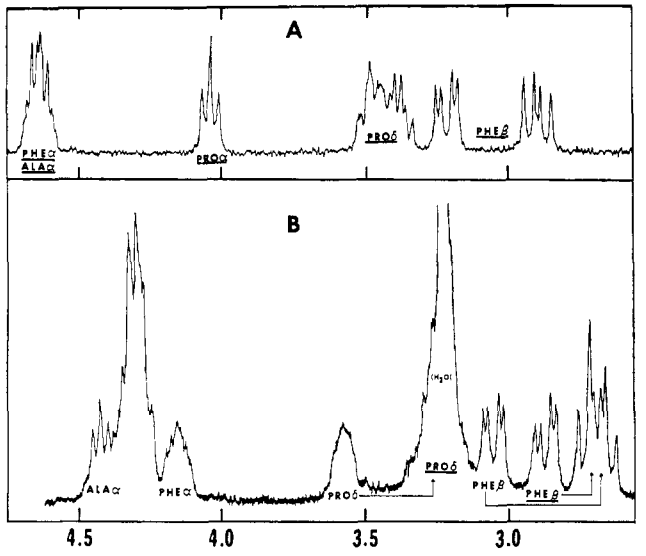

Figure 1. Central regions of the 250-MHz proton spectra of *cyclo*-(L-Ala-L-Pro-D-Phe)$_2$: (A) in chloroform, 7 mg/ml, 30° (only one component is present); (B) in dimethyl sulfoxide-d_6, 50 mg/ml, 30° (two components are present). Assignments referring to the major component are underlined. The reference is internal tetramethylsilane.

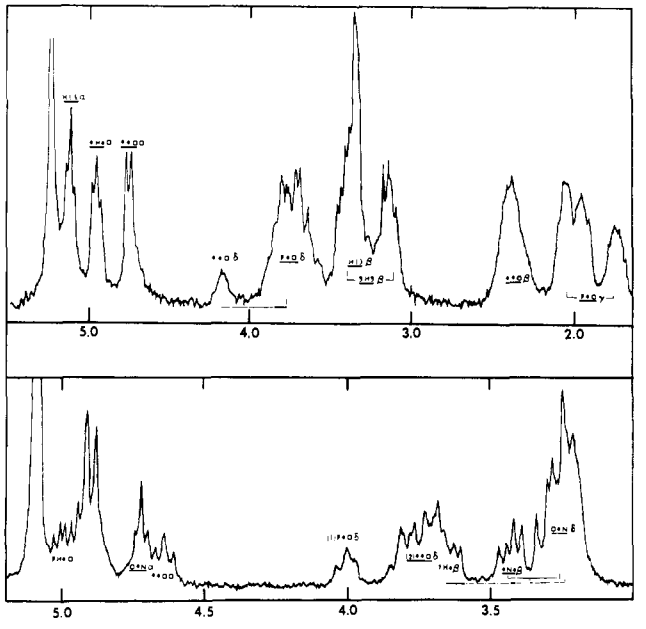

Figure 2. Upper: higher field portion of 270-MHz spectrum of *cyclo*(L-His-L-Pro-D-Phe)$_2$ hydrochloride in deuterium oxide, 70 mg/ml, 20°. Only the resonance of one of its proline δ protons at about 4.22 ppm indicates the presence of the minor component. Lower: central region of the 250-MHz spectrum of *cyclo*(L-Orn-L-Pro-D-Phe)$_2$ hydrochloride in deuterium oxide containing 5% acetic acid-d_4, 50 mg/ml, 30°. The reference in both cases is capillary hexamethyldisiloxane. Assignments to the major component are underlined.

cyclo(L-Ser-L-Pro-Gly)$_2$,[6] to which the present peptides are closely related, if glycine can be considered a D-series residue.

One form, called hereafter conformation A, is the only one observed in hexafluoro-2-propanol and chloroform and is favored 4:1 in pyridine solutions of the

(6) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 616 (1972).

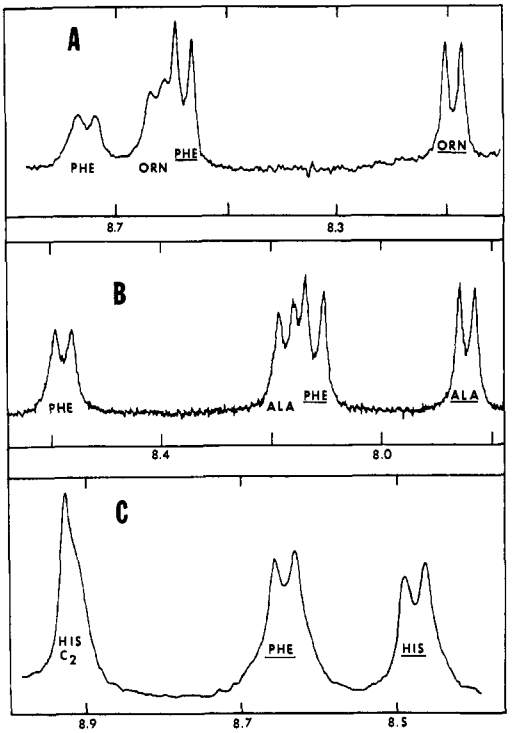

Figure 3. Peptide proton region of the spectra of the cyclic peptides: (A) *cyclo*(L-Orn-L-Pro-D-Phe)$_2$ hydrochloride in water containing 5% acetic acid, 40 mg/ml, 30°, reference capillary hexamethyldisiloxane; (B) *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ in dimethyl sulfoxide, 50 mg/ml, 30°, reference internal tetramethylsilane; (C) *cyclo*-(L-His-L-Pro-D-Phe)$_2$ hydrochloride in water, 70 mg/ml, 20°, reference capillary hexamethyldisiloxane. Assignments to the major component are underlined.

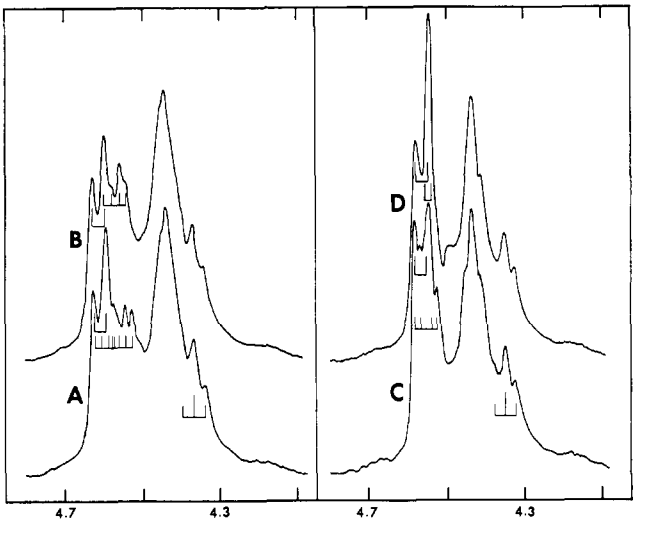

Figure 4. Partial resolution of the α proton absorptions of *cyclo*-(L-Orn-L-Pro-D-Phe)$_2$ in dimethyl sulfoxide, 30°. These spectra were obtained at 250 MHz using the multiple rapid scan correlation technique of Dadok, Sprecher, and Bothner-by:[3] (A) peptide in dimethyl sulfoxide; (B) peptide with irradiation into the more strongly coupled (J = 10 Hz) β proton resonances of phenylalanine residues, at 2.76 ppm; (C) peptide with exchangeable protons replaced by deuterons; (D) exchanged peptide with more strongly coupled β protons of phenylalanine decoupled. Indicated by the lines are the doublet and triplet proline α resonances of the two conformers and the idealized patterns of the phenylalanine α protons of the major conformer.

alanine peptide. In this form the L-xxx-L-Pro peptide bond is trans, according to the correlation suggested

Table I. Proton Chemical Shifts and Coupling Constants of *cyclo*(D-Phe-L-xxx-L-Pro)$_2$[a]

Solvent	D-Phe H_N δ	D-Phe H_N $J_{\alpha-NH}$	D-Phe H_α δ	D-Phe H_α $J_{\alpha-\beta}$	D-Phe H_β[b] δ	L-xxx H_N δ	L-xxx H_N $J_{\alpha-NH}$	L-xxx H_α δ	L-xxx Other	L-Pro H_α δ	L-Pro H_α $J_{\alpha-\beta}$	L-Pro H_δ δ
Conformation A[c]												
xxx = Ala												
DMSO (0.35)	8.63	7.5	4.23	10.0	2.81	8.18	7.0	4.51	β, 1.09	~4.4		3.68
				4.6	3.15							~3.35
Pyr (0.8)	9.60	8.1	5.22		3.35	8.91	7.2	4.87	β, 1.40	4.66	t, Σ ~ 15	~3.3
					3.66							
HFP (1.0)	6.73	7.1	4.60	10.8	2.90	8.31	7.4	4.77	β, 1.35	4.23	t, Σ ~ 15	~3.65
				4.5	3.43							3.58
$CDCl_3$ (1.0)[d]	6.32	8.7	4.72	9.3	2.99	8.01	7.2	4.75	β, 1.28	4.14	t, Σ ~ 15	3.59
				4.8	3.31							3.49
xxx = Orn(HCl)												
DMSO (0.3)	8.40	8.5	4.58		~2.75	8.05	~7	~4.43	β, γ, ~1.66	4.34	t, Σ ~ 15	3.70
				~4	3.22				δ, ~2.75			~3.35
HFP (1.0)	6.80	8.4	4.83	9.3	2.93	8.34	6.4	4.64	β, γ, ~1.93	4.17	t, Σ ~ 15	3.70
				5.9	3.38				δ, ~3.12;			~3.52
									NH_3^+, 8.13			
H_2O (0.45)	8.75	8.0	4.96	~10	3.25	8.63	~7	4.85	δ, ~3.15	4.60	t, Σ ~ 15	3.97
				~6	3.59							~3.70
Conformation B[c]												
xxx = Ala												
DMSO (0.65)	8.17	8.3	4.43	9.9	2.77	7.89	7.0	4.37	β, 1.12	4.39		~3.35
				4.9	2.96							
Pyr (0.2)	8.69	*e*				9.74	9.0	5.15	β, 1.50	4.78		~3.3
xxx = Orn(HCl)												
DMSO (0.7)		~8.1	4.53		~2.75	~8.1		~4.42	β, γ, ~1.66	4.55	d, 8.5	~3.35
				4	3.02				δ, ~2.75			
H_2O (0.55)	8.57	7.5	4.88	8.5	3.26	8.09	7.5	4.69	δ, ~3.15	4.86	d, 8	~3.7
				7.5	3.39							
xxx = His(HCl)												
H_2O (0.8)	8.64	7.0	4.93	9.5	3.10	8.47	7.2	5.09	β, 3.30	4.74	d, 8	~3.7
				6.3	3.39							(β, ~2.4)
												(γ, 1.74, ~2.0)
DMSO (0.75)	(8.20)	~7.5	(4.47)[f]			(8.24)	~7.5	(4.91)[f]		4.62	d, 8	(β, ~1.88)

[a] Coupling constants are in Hz and chemical shifts are ppm below internal tetramethylsilane, except that the reference for the aqueous solution is capillary hexamethyldisiloxane. For comparison, chemical shift values for the aqueous solution can be converted to an internal trimethylsilylpropionate reference by subtraction of 0.31 ppm. The symbol ~ indicates a range of ±0.05 ppm in chemical shift and an uncertainty of ±0.5 Hz in a coupling constant. Only resonances completely identified as to residue and conformer are tabulated. Peptide concentration is 30–50 mg/ml, and temperature 20°, except for aqueous solution for which 30° data are given. [b] $J_{\beta\beta}$ of the phenylalanine residues was about 13.5 Hz in dimethyl sulfoxide, 14 Hz in water, and 14.5 Hz in the other organic solvents. [c] The resonances of the two coexisting conformations are separated here for clarity. The value in parentheses after the solvent indicates the mole fraction of the conformer, estimated by integration of regions of the spectra where there are clean separations between corresponding absorptions of the two conformers. [d] Saturated solution, about 7 mg/ml. [e] Obscured by residual proton resonance of solvent. [f] Certain assignment of α protons to histidine or phenylalanine residues is not possible here because of overlaps of the β proton resonances.

by Patel.[7] The proline α resonance is a triplet, with the sum of the H^α–H^β couplings about 15 Hz. The second form, called B, exhibits a doublet proline α proton resonance with an apparent 8-Hz splitting, from which the L-xxx-L-Pro peptide bond is inferred to be cis. Where both forms occur it was possible to estimate the proportions from the spectrum integral, since one or another resonance of the minor form, *e.g.*, an α proton, a Phe β proton, a Pro δ proton, or H^2 of an imidazole ring, stood well separated from the stronger absorptions. The mole fractions so determined are given in Table I, along with the chemical shift and coupling data.

We were unable to find a solvent in which only conformation B occurs. In dimethyl sulfoxide solutions of the alanine peptide, conformations A and B are present in a 1:2 ratio. In mixtures with chloroform, the fraction of B decreases about linearly with the decreasing volume fraction of dimethyl sulfoxide. In dimethyl sulfoxide alone, the ornithine peptide divides itself between the two conformations 1:2.3 and the histidine peptide 1:3. In aqueous solutions both conformations are also present; they are almost equal in the ornithine case, but B is favored 4:1 when L-xxx is histidine.

The effects of sodium, potassium, and rubidium thiocyanates on the A ⇌ B equilibrium were examined for dimethyl sulfoxide solutions of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$, with the results shown in Table II. The potassium salt caused the only significant shift of equilibrium, slightly increasing the stability of conformation B. All of the salts moved the alanine N–H resonance of conformation B downfield and its phenylalanine N–H slightly upfield, if at all; they had no effect whatever on the N–H resonances of conformation A.

In conformation A, the peptide proton of the variable residue is shielded from the solvent. This is shown,

(7) D. J. Patel, *Biochemistry*, **12**, 667 (1973).

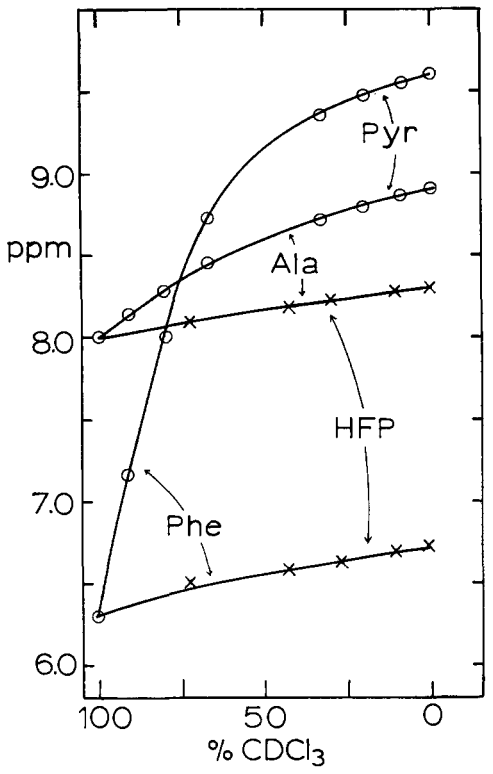

Figure 5. Chemical shift of the N–H protons of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ in chloroform–pyridine and chloroform–hexafluoro-2-propanol mixtures, 20°. The resonances followed are those of conformation A, which is the only one present in chloroform or hexafluoro-2-propanol and is predominant in pyridine. Reference is internal tetramethylsilane.

Table II. Effect of Alkali Thiocyanates on Dimethyl Sulfoxide Solutions of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$, *ca.* 0.045 *M*, 20°

Salt	Concn, *M*	Conf A, %[a]	ΔδN–H, ppm (conf B)
None		43	
Na	0.21	41	Ala, 0.19 down Phe, none
K	0.23	25	Ala, 0.20 down Phe, 0.08 up
Rb	0.23	38	Ala, 0.08 down Phe, 0.03 up

[a] From Phe β proton region, estimated error ±3–4%.

first, by the data of Figures 5 and 6. Addition of 10–20% of such hydrogen bond accepting agents as pyridine or dimethyl sulfoxide to chloroform solutions of the alanine peptide results in large downfield shifts of the phenylalanine peptide proton resonance but has relatively little effect on the resonance of the alanine peptide proton. Going in the opposite direction, the peptide proton resonance of the phenylalanine residue moves upfield 1.9 ppm on going from pure dimethyl sulfoxide to pure hexafluoro-2-propanol, while that of alanine moves only 0.1 ppm downfield. If the backbone remains unchanged by this large solvent variation, this also indicates solvent exposure of the phenylalanine proton and sequestering of the alanine peptide proton.[8,9] Analogous changes occur for the ornithine peptide.

(8) T. P. Pitner and D. W. Urry, *J. Amer. Chem. Soc.*, **94**, 1399 (1972).

(9) K. D. Kopple and T. J. Schamper, "Chemistry and Biology of Peptides," J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 75.

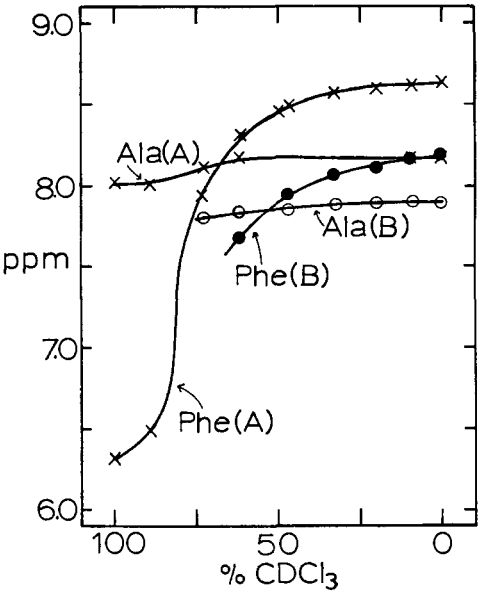

Figure 6. Chemical shift of the N–H protons of both conformations of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ in chloroform–dimethyl sulfoxide mixtures, 20°, reference internal tetramethylsilane. The fraction of conformation B is 65% in 100% dimethyl sulfoxide, 25% in 60% chloroform, and near zero in pure chloroform.

The peptide proton resonances of the minor component of the histidine peptide have not been assigned, but they occur at 8.33 and 8.73 ppm in dimethyl sulfoxide and at 6.8 and 8.47 ppm in hexafluoro-2-propanol. In this case also, one moves upfield 1.5–2 ppm and the other changes only slightly, again indicating one type of solvent-exposed and one type of solvent-shielded peptide proton. A second indication of peptide proton exposure is given by the line broadening effect of an added free radical.[2,10] Low concentrations of 3-oxyl-2,2,5,5-tetramethyloxazolidine have only a minor line-broadening effect on the alanine peptide proton of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ in chloroform solutions, about 1 Hz at half-height per per cent radical added, but have a large effect on the phenylalanine peptide proton, about 8 Hz/%. In pyridine, the corresponding figures are about 1 and 2.5 Hz/%. In hexafluoro-2-propanol, however, the radical apparently associates with the acidic solvent rather than with the peptide, so that both peptide protons show line broadening of only about 2 Hz/%. In solvents where both conformations are present, overlaps of the broadened lines rule out quantitative estimates of the effect of the radical.

The H–C$^\alpha$–N–H coupling constants observed for conformation A correspond to dihedral angles of 155 ± 10° for the phenylalanine and 145 ± 5° for the alanine or ornithine residues.[11]

A model that has planar amide groups and the usual bond distances and angles, and is consistent with the pmr data, is readily constructed for conformation A. The model has two β turns of the LD type, with transannular hydrogen bonds between the L-xxx residues,

(10) K. D. Kopple and T. J. Schamper, *J. Amer. Chem. Soc.*, **94**, 3644 (1972).

(11) G. N. Ramachandran, R. Chandrasekaran, and K. D. Kopple, *Biopolymers*, **10**, 2113 (1971). In this range the recently refined version of the correlation of V. F. Bystrov, V. T. Ivanov, S. L. Portnova, T. A. Balashova, and Yu A. Ovchinnikov, *Tetrahedron*, **29**, 873 (1973), would give the same results.

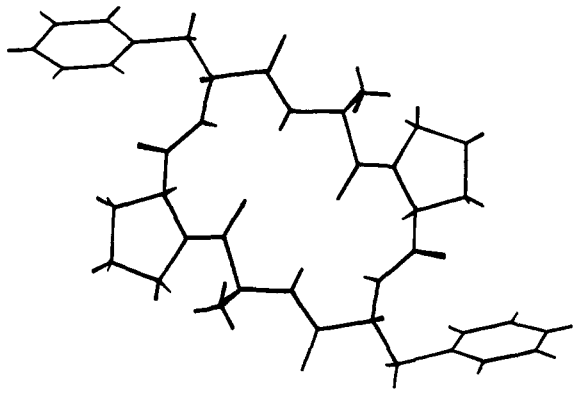

Figure 7. Suggested conformation of *cyclo*(L-xxx-L-Pro-D-Phe)$_2$ with trans xxx-Pro peptide bonds, referred to as conformation A in the text. This is a retouched photograph of a Kendrew model.

is available suggests that the variable residue N–H is more exposed than that of the phenylalanine. Figure 8 shows the effects of added nitroxyl on the peptide proton resonances of aqueous solutions of the histidine and ornithine peptides. The phenylalanine resonance of the major component, conformation B, is less broadened than the histidine or ornithine doublet. In the alanine peptide there occurs a large downfield shift, 1.8 ppm, of the alanine peptide proton resonance on changing solvent from dimethyl sulfoxide to pyridine. This shift, which is at least twice as large as the corresponding ehange for the other three kinds of peptide protons (see Table I), could be interpreted to suggest a particularly favorable hydrogen bonded association with pyridine.

The coupling constants of the H–C$^\alpha$–N–H systems

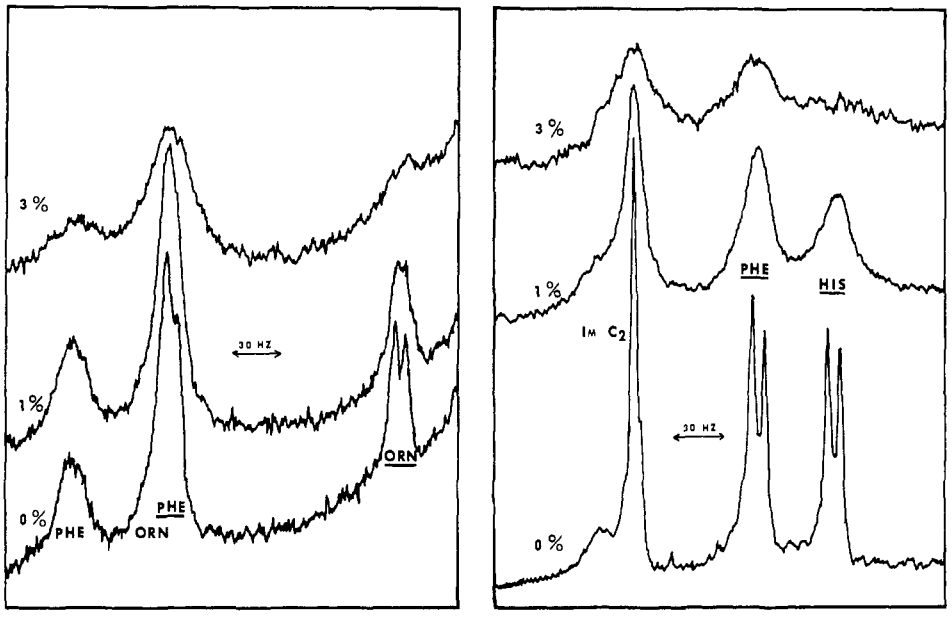

Figure 8. Effect of added 3-oxyl-2,2,5,5-tetramethyloxazolidine on aqueous solutions of *cyclo*(L-Orn-L-Pro-D-Phe)$_2$ in water, containing 5% acetic acid, and *cyclo*(L-His-L-Pro-D-Phe)$_2$ hydrochloride in water, both at 20°. Radical concentration by volume is indicated for each trace; peptide concentrations are about 50 mg/ml. Resonance assignments to the major component, conformation B, are underlined.

which are in the extended (β) conformation, $\phi = -150°$, $\psi = 150°$. The reversals of peptide chain direction occur in the L-Pro-D-Phe sequences, which have the backbone dihedral angles $\phi_{Pro} = -50°$, $\psi_{Pro} = 100°$, $\phi_{Phe} = 150°$, $\psi_{Phe} = -40°$. In this model the N···O distances of the transannular hydrogen bonds are near 2.9 Å, and the H–N···O angles are about 20°.

The H–C$^\alpha$–C$^\beta$–H vicinal coupling constants of the phenylalanine residues of conformation A indicate that the most probable side chain rotation angle χ_1 is either near 60° or near 180°. Reference to a space-filling model indicates that it is more likely that $\chi_1 = 60°$, which places the aromatic ring over the Pro-Phe peptide bond. There is no unusual splitting of the proline β proton resonances to serve as confirmation of this choice, however, nor does the model necessarily predict one. A model of conformation A is shown in Figure 7.

For conformation B there are more limited data on the effects of added hydrogen bond acceptors, but what

Table III. Conformations for *cyclo*(L-xxx-L-Pro-D-Phe)$_2$ Referred to in the Text

Conformation	Backbone dihedral angles in degrees						
	L-xxx			L-Pro		D-Phe	
	ϕ	ψ	ω	ϕ	ψ	ϕ	ψ
A	−150	150	180	−50	100	150	−40
B-1	−150	150	0	−70	120	90	−110
B-2	−150	70	0	−60	150	150	170
B-3	−90	140	0	−60	120	90	90

of conformation B correspond to dihedral angles close to 150°.[11]

These backbone conformations with cis xxx-Pro peptide bonds and 150° H–C$^\alpha$–N–H dihedral angles that we have considered are numbered B-1, -2, -3 in Table III. In none of these is solvation of the phenylalanine peptide proton hindered by the backbone; we were not able to construct a model with the phenylalanine N–H directed inward. From the standpoint of the conformational energies of the individual dipeptide

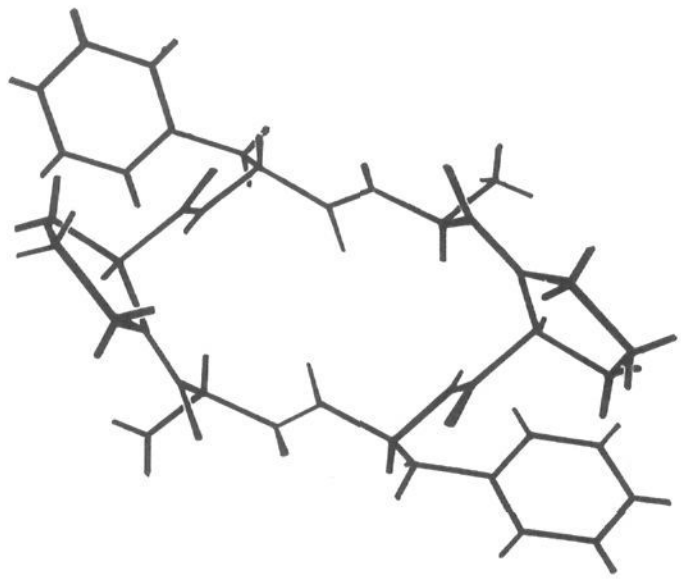

Figure 9. Suggested conformation of *cyclo*(L-xxx-L-Pro-D-Phe)$_2$ with cis xxx-Pro peptide bonds, referred to as conformation B-1 in the text. This is a retouched photograph of a Kendrew model.

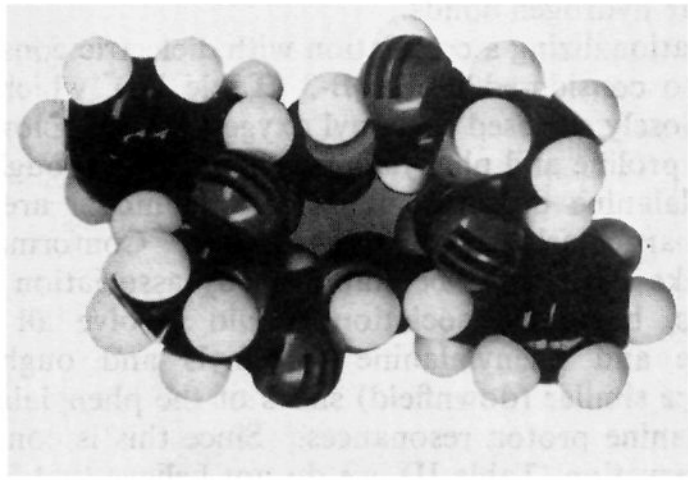

Figure 10. CPK model of conformation of Figure 9, shown from the same side.

units, conformation B-1 is perhaps most satisfactory. Within the precision of estimates made by model building, this is the same conformation as that suggested by Torchia, Wong, Deber, and Blout for the conformation of *cyclo*(L-Ser-L-Pro-Gly)$_2$ with two cis Ser-Pro bonds.[6] In model B-1 the phenylalanine peptide protons are somewhat sequestered by the side chains of the variable residues and of the phenylalanines, particularly when the latter have χ_1 near 60°.

The ring backbone of conformation B-1 is folded, not almost planar as in the structure with two β turns, and the alanine peptide protons are directed to the concave side (Figures 9 and 10). The concave side forms a pocket into which a pyridine ring may fit, with the alanine N–H's at its edge so that one of them can form a hydrogen bond to the pyridine nitrogen. Such specific solvation would explain the strong downfield shift of the alanine peptide protons of conformation B in pyridine solution. A similar effect, but without the hydrogen bond, is seen on comparing spectra of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ in benzene–dimethyl sulfoxide mixtures and in dimethyl sulfoxide alone; in 36% by volume benzene, the two peptide protons of conformation A are moved downfield 0.15 ppm. Since the alanine proton of conformation A is definitely solvent shielded, this must be a nonspecific effect. The phenylalanine proton of conformation B is shifted downfield only 0.04 ppm, which suggests that on the average it sees more of the face of benzene molecules, while the alanine proton is moved 0.26-ppm downfield, indicating that it tends to face an edge.

Weak coordination of potassium ion with the carbonyl oxygens of the Phe-Ala peptide bonds could stabilize conformation B-1 and cause the observed downfield shifts of the alanine peptide protons. The phenylalanine carbonyl oxygens are on the convex side of the folded peptide ring, as are the phenylalanine peptide protons, which are shifted upfield by potassium thiocyanate.

Conformations B-2 and B-3, which we consider less likely, are discussed in the subsequent section.

Discussion

We take up below first factors influencing the relative stability of conformations A and B and second the chemical shift of internal peptide protons.

Relative Stability of Conformations A and B. Coexistence of comparable amounts of conformations A and B indicates that there is only a small energy difference between them in solution. Experimentally, the balance depends on the solvent and on the nature of the variable residue. Taking the solvent variation first, the most obvious observation is that conformation A, which contains good transannular hydrogen bonds, is most favored in the solvents that are poor hydrogen bond acceptors, chloroform and hexafluoro-2-propanol. Solutions in solvents that can form good hydrogen bonds to the peptide protons, pyridine, dimethyl sulfoxide, and water, contain significant amounts of conformation B. The peptide protons are accessible to solvent in all our models of conformation B, although in some instances they may be hindered by side chains from associating with the bulky 1-oxyl-2,2,5,5-tetramethyloxazolidine. Hydrogen bonding to solvent is not the only factor in solvent effects on the A–B equilibrium, however. Importance of something in addition is hinted by the fact that the A/B ratio for *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ is 4 in pyridine and 0.5 in dimethyl sulfoxide. By spectroscopic[12] and calorimetric[13] criteria pyridine ought to accept hydrogen bonds at least as strongly as does dimethyl sulfoxide. Also, pyridine is no more hindered in approaching the peptide protons. Therefore, if solvent hydrogen bonding alone were determining, the A/B ratio in the two solvents ought to be more nearly the same.

Dielectric constant is a bulk solvent property that correlates roughly with the A/B ratio. Water (78) and dimethyl sulfoxide (47) have high dielectric constants, and conformation B is favored in them. Chloroform (4.8), pyridine (12.4), and hexafluoro-2-propanol (16.6)[14] have lower dielectric constants, and conformation A is favored in them. Examination of the orientation of the amide dipoles in a model of conformation B-1 does suggest that B-1 might be stabilized by small dipolar solvent molecules with accessible regions of partial positive charge. The phenylalanine carbonyl oxygens of conformation B-1 are somewhat opposed, without internal dipolar compensation,

(12) D. P. Eyman and R. S. Drago, *J. Amer. Chem. Soc.*, **88**, 1617 (1966).

(13) K. F. Purcell, J. A. Stikeleather, and S. D. Brunk, *J. Amer. Chem. Soc.*, **91**, 4019 (1969).

(14) J. Murto, A. Kivinen, and E. Lindell, *Suom. Kemistilehti B*, **43**, 28 (1970).

whereas in conformation A, the opposed carbonyls of the variable residues are involved in strong transannular hydrogen bonds.

In rationalizing a correlation with dielectric constant, we also considered model B-3 (Table III), which has four closely opposed carbonyl oxygens, those belonging to the proline and phenylalanine residues, although the phenylalanine dipeptide units of this model are not in a particularly stable arrangement. Conformation B-3, like B-1, might be stabilized by association with cations, but the association should involve all four proline and phenylalanine carbonyls and ought to produce similar (downfield) shifts of the phenylalanine and alanine proton resonances. Since this is contrary to observation (Table II), we do not believe that B-3 is a likely conformation.

An internal factor influencing the stability of the conformations of *cyclo*(L-xxx-L-Pro-D-Phe)$_2$ may be the torsional freedom of the backbone. In conformation A, the dihedral angles 150 and $-40°$ put the D-phenylalanine units at the edge of a region of stability on conformational energy plots,[15] so that a backbone torsional oscillation that increases $\phi_{\text{D-Phe}}$ and/or decreases $\psi_{\text{D-Phe}}$ may be of high energy. In conformation B-1, however, the dihedral angles of the phenylalanine may oscillate widely (20–30°) about the suggested values, 90°, $-110°$, at very little change in the potential energy of local interactions.

The residue preceding the proline is constrained in both trans (A) and cis (B) conformers. Calculated dipeptide energy maps for alanyl joined to proline by a trans peptide bond place a residue with our suggested dihedral angles, which are -150 and 150° in both A and B-1, near a steep potential wall along ψ = 150–170° that results from interference between the β group of the alanine and the δ methylene of the proline,[16-18] although the interference is reduced when flexibility of the pyrrolidine ring is considered.[18] Maps for *cis*-alanylproline have not yet appeared,[19] but models indicate that at $\psi > 150°$ there is similar interference of the methyl with the proline C^α and C′ groups. At $\psi_{\text{Ala}} \approx 150°$ there is also less permissible variation in ϕ, so that the variable residue is at least as constrained in conformation B as in A, so far as the backbone is concerned.

Side chain torsional freedom is a likely influence on the A–B equilibrium. In dimethyl sulfoxide solution, the fraction of B increases in the order L-xxx = Ala < Orn < His, which is also the order of increasing side chain bulk. There is also more of conformation B in an aqueous solution of the histidine peptide than in one of the ornithine peptide. Although CPK models of conformations A and B-1 of *cyclo*(L-His-L-Pro-D-Phe)$_2$ give but faint indication, the experimental observations make it reasonable to suppose that, when $\chi_1 = 180°$, the xxx side chain interferes more strongly in conformation A with the proline δ-C–H than it interferes in conformation B with the proline α-C–H. This interference does not prohibit $\chi_1 = 180°$ (all three minima of χ_1 can be taken up), but it narrows the permissible variation of χ_2 and therefore may provide an additional entropic factor in the A–B balance.

Chemical Shift of Internal Peptide Protons. In a number of cyclic peptides the resonance of the internal N–H proton of a β turn, that proton directed to the inside of the bend and shielded from solvent, occurs at the high-field end of the range of N–H resonances, even though the proton is presumably involved in a transannular hydrogen bond. The high-field position of this resonance has been ascribed to diamagnetic shielding by the amide group that joins the two residues (usually numbered 2 and 3) making the bend.[20,21] However, the results of this work, taken with previously reported data, indicate that the principal determinant of the position of this resonance within the N–H region is more likely to be the strength or weakness of the transannular hydrogen bond.

In conformation A of *cyclo*(L-Ala-L-Pro-D-Phe)$_2$, the peptide protons of the alanine residues come into resonance at 8.2 ppm in dimethyl sulfoxide solution. The position of this resonance is not much changed when the peptide is transferred to poor hydrogen bond acceptors, being 8.0 ppm in chloroform and 8.3 ppm in hexafluoro-2-propanol. In the *retro* isomer, *cyclo*(L-Ala-D-Phe-L-Pro)$_2$, the alanine protons appear at 7.0 ppm in dimethyl sulfoxide and again are not much affected by transfer to chloroform (7.0 ppm) or hexafluoro-2-propanol (7.1 ppm). The constancy of the resonance positions in both cases indicates that in both cases the protons are in an internal environment, unexposed to hydrogen bonding with dimethyl sulfoxide. Other measures of peptide proton solvent exposure confirm this. Our model for conformation A of the *cyclo*(L-xxx-L-Pro-D-Phe)$_2$ peptides involves the internal peptide proton in a good hydrogen bond with an N–H···O distance of about 2.9 Å. On the other hand, our estimate for the retro isomer backbone places the internal peptide proton in a weaker bond, with an N–H···O distance of about 3.8 Å. The correlation between the 1–1.2 ppm difference in chemical shift and the internal hydrogen bond stands out.

In support may be cited the pmr data on aluminum analogs of the ferrichromes, obtained by Llinas, Klein, and Neilands,[22] which can be correlated with the crystal structure of ferrichrome A.[23] The cyclic hexapeptide backbone of ferrichrome A, like the peptides above, may be described as composed of two β turns. One of the corresponding internal protons is involved in a transannular hydrogen bond with N–H···O = 3.0 Å. For the other the N–H···O distance is considerably less favorable.[23] In the aluminum analog, which can safely be argued to be very similar in structure,[22] the better hydrogen bonded proton appears at 8.0 ppm in dimethyl sulfoxide solutions, but the other shows up at higher field, 7.3 ppm.

Chemical shift differences as large as 1 ppm should

(15) For example, those shown in the review by G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, 23, 283 (1968).
(16) P. R. Schimmel and P. J. Flory, *J. Mol. Biol.*, 34, 105 (1968).
(17) A. Damiani and P. DeSantis, *Nature (London)*, 226, 542 (1970).
(18) B. Maigret, B. Pullman, and J. Caillet, *Biochem. Biophys. Res. Commun.*, 40, 808 (1970).
(19) A. E. Tonelli, Abstracts of 166th National Meeting of the American Chemical Society, Chicago, August, 1973, paper BIOL 121.
(20) A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Nat. Acad. Sci. U. S.*, 61, 734 (1968).
(21) K. D. Kopple, M. Ohnishi, and A. Go, *Biochemistry*, 8, 4087 (1968).
(22) M. Llinas, M. P. Klein, and J. B. Neilands, *J. Mol. Biol.*, 68, 265 (1972).
(23) A. Zalkin, J. D. Forrester, and D. H. Templeton, *J. Amer. Chem. Soc.*, 88, 1810 (1966).

not be expected to result from differences in position of an internal proton relative to the magnetically anisotropic peptide bond adjacent, in any case. Tigelaar and Flygare have reported the magnetic susceptibility anisotropy of formamide, which may be taken as a model for that of a peptide bond.[24] From their value, it can be calculated that the internal proton, which is 2–2.5 Å above the plane of the peptide bond and a similar distance radially from a perpendicular through the carbonyl carbon, will experience shielding only of the order of 0.1 ppm. If the above argument is correct, it should be possible, once a peptide proton has been established as shielded from the solvent, to deduce from its chemical shift something about its internally hydrogen bonded state, *e.g.*, that the valine peptide protons of gramicidin S (7.2 ppm in dimethyl sulfoxide[20]) are involved in a weak hydrogen bond, while those of the leucines (8.35 ppm[20]) are in a good one.

An Additional Possibility for Conformation B. In model building to fit the basic parameters of conformation B, we obtained another that can be constructed with C_2 symmetry, cis xxx-Pro peptide bonds, 150° H–N–C–H angles for the nonproline residues, and standard bond angles and lengths, B-2 in Table III. This is an esthetically satisfying one in which antiparallel pairs of Pro-Phe and Phe-xxx peptide bonds are stacked in roughly parallel planes, with positive and negative centers of the amide functions paired, H closest to O and C closest to N. These transannular electrostatic interactions could make conformation B-2 stable, even though there is little vibrational freedom for the backbone because of very close packing. Interference between the proline ring and the side chain of the preceding residue is absent.

Conformation B-2 could be stabilized by N–H···O–H···O=C bridges involving a molecule of water and does have all four peptide protons exposed to solvent–hydrogen bonding, in distinction to conformation A. However, the relative orientation of the amide groups does not provide a clear reason why B-2 should be more stable in dimethyl sulfoxide than in pyridine. A more serious objection is that the phenylalanine peptide proton is not at all hindered from association with the stable free radical. We are not prepared to rule conformation B-2 out entirely, however, and have optical experiments underway, using hydrogenated derivatives, that may provide information about amide group stacking.

(24) H. L. Tigelaar and W. H. Flygare, *J. Amer. Chem. Soc.*, **94**, 343 (1972).

A Stereoselective Approach to Eremophilane Sesquiterpenes. A Synthesis of (±)-Nootkatone and (±)-α-Vetivone

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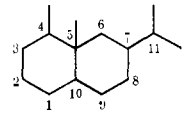
Abstract: A method for the stereoselective construction of the eremophilane sesquiterpene skeleton has been developed. The method's potential is exemplified by a synthesis of (±)-nootkatone and (±)-α-vetivone.

The eremophilane group of sesquiterpenoids possesses the carbon skeleton **1**, which does not conform to the isoprene rule.[1] Two representative members of this group of natural products are nootkatone (**2**) and α-vetivone (**3**). (+)-Nootkatone was originally identified as a heartwood constituent of Alaska yellow cedar (*Chamaecyparis nootkatensis*)[2] and was subsequently shown to be a major bitter principle in grapefruit peel oil (*inter alia*).[3,4] (+)-α-Vetivone is a constituent of vetiver oil[5,6] used extensively in perfumery.



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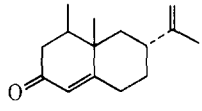

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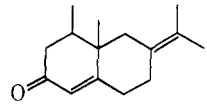

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The commercial interest in these compounds has already resulted in three total syntheses of (±)-nootkatone[7–9] and two of (±)-α-vetivone[9,10] as well as two partial syntheses of (+)-nootkatone.[11,12]

Most synthetic approaches to construction of the eremophilane skeleton have relied on the Robinson annelation reaction.[8–10,13,14] However, the crucial stereospecific establishment of the cis C(4),(5)-dimethyl moiety, and in the case of nootkatone, the equatorial C(7)-isopropenyl functionality, has presented problems in previous syntheses.[7–9] Mixtures of epimers often resulted, and it would not be unreasonable to suggest that a general scheme inherently giving rise to the

(1) For a review, see A. R. Pinder, *Perfum. Essent. Oil Rec.*, **59**, 280 (1968).
(2) H. Erdtman and Y. Hirose, *Acta. Chem. Scand.*, **16**, 1311 (1962).
(3) W. D. MacLeod and N. M. Buignes, *J. Food Sci.*, **29**, 565 (1964).
(4) J. W. Kesterson, R. Hendrickson, R. R. Seiler, C. E. Huffman, J. A. Brent, and J. T. Griffiths, *Amer. Perfum. Cosmet.*, **80**, 29 (1965).
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(7) M. Pesaro, G. Bozzato, and P. Schudel, *Chem. Commun.*, 1152 (1968).
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(9) A. Van Der Gen, L. N. Van Der Linde, J. G. Witeveen, and H. Boelens, *Recl. Trav. Chim. Pays-Bas*, **90**, 1034 (1971).
(10) J. A. Marshall, H. Faubl, and T. M. Warne, *Chem. Commun.*, 753 (1967).
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(12) Firmenich & Cie, Netherlands Patent Appl. 6914545 (1965).
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(14) E. Piers and R. J. Keziere, *Tetrahedron Lett.*, 583 (1968).