Studies on Cyclic Dipeptides. I. Thermodynamics of the Cis–Trans Isomerization of the Side Chains in Cyclic Dipeptides[1]

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Abstract: In a cyclic dipeptide, there are cis and trans isomers with respect to the configuration of two side chains on the diketopiperazine ring. The isomerization between these two forms was examined in ethanolic sodium ethoxide at 30–75° as well as in aqueous solutions at 250°. The thermodynamic constants (K, ΔG, $\Delta H°$, and $\Delta S°$) in the equilibrium of this isomerization were determined for various cyclic dipeptides and discussed with relation to the conformations of these compounds. The composition of the cis and trans isomers in the equilibrium state widely varied depending upon the types of cyclic dipeptides. The amount of the cis isomer was almost equal to that of the trans isomer for *cyclo*(Ala-Ala) (**1**), *cyclo*(Leu-Leu) (**2**), and *cyclo*(Phe-Phe) (**3**). For *cyclo*(Pro-Ala) (**5**), *cyclo*(Pro-Leu) (**6**), *cyclo*(Pro-Phe) (**7**), and *cyclo*(Pro-Val) (**8**), the trans isomers were more stable than the cis isomers. Both the free energy difference and the enthalpy difference between the cis and trans isomers became larger with the bulkiness of the side chain of the amino acid residue. For *cyclo*(Pro-Pro) (**9**) and *cyclo*(Hyp-Hyp) (**10** and **11**), on the other hand, only the cis isomers were found in equilibrium.

For a cyclic dipeptide, there are four possible isomers, as shown in Figure 1. The four isomers are classified into cis and trans isomers. These isomers are known to isomerize easily into each other under alkaline conditions.[2–6] Ott, Frey, and Hofmann reported that 90–95% of *cyclo*(L-Pro-L-Phe) was isomerized into *cyclo*(D-Pro-L-Phe) in dilute sodium hydroxide at room temperature for a short time.[5] Mauger found that the equilibrium mixture in methanolic sodium methoxide contained mainly the trans isomer.[6] The conformations of cyclic dipeptides, on the other hand, have extensibly been studied by means of X-ray diffraction,[7–12] nmr,[13–16] ORD, CD,[17–19] ir,[20,21] and quantum mechanical calculation.[19,22,23] Now a study on the thermodynamics of the isomerization between the cis and trans forms of cyclic dipeptides seems to be required for conformational analysis of cyclic dipeptides. This is the subject of this paper.

Results and Discussion

The isomerization experiments were carried out in 0.1 N ethanolic sodium ethoxide at three temperatures, 30, 50, and 75°. Under these conditions, no reaction other than isomerization was observed. The percentages of the trans isomer in the equilibrium mixtures at 30° are shown in Table I. These values were reproducible within ±0.3%. From the equilibrium constants (K = [trans isomer]/[cis isomer]) determined at the three temperatures, thermodynamic constants ($\Delta H°$ and $\Delta S°$) of the cis–trans isomerization of cyclic dipeptides were obtained by plotting log K against $1/T$ and by adopting the least-squares method. The results are summarized in Table II.

The isomerization experiments were also carried out in aqueous solutions at 250°. Under this condition, some side reactions such as hydrolysis occurred besides the isomerization, and substances positive for ninhydrin test were produced. The percentages of the trans isomer in the equilibrium mixtures are shown also in Table I. These values were reproducible within ±1%.

***cyclo*(Pro-Pro) Type of Cyclic Dipeptides (9, 10 and 11).** Four sets of racemic cyclic dipeptides and two meso-type cyclic dipeptides can be made by the combinations of four isomers of 4-hydroxyproline, DL-Hyp, and DL-*a*Hyp, as shown in Figure 2. The configurations around the asymmetric carbons (C_4 and $C_{4'}$) in the pyrrolidine rings do not change at all under both of these isomerization conditions. Therefore, the isomerization reactions in eq 1–3 should take place (see Figure 2).

As shown in Tables I and II, only the cis isomer is found in equilibrium for this type of cyclic dipeptide

cyclo(L-Hyp-L-Hyp) (**10a**) ⇌ *cyclo*(L-Hyp-D-*a*Hyp) (**10b**) ⇌ *cyclo*(D-*a*Hyp-D-*a*Hyp) (**10c**) (1)

cyclo(D-Hyp-D-Hyp) ⇌ *cyclo*(D-Hyp-L-*a*Hyp) ⇌ *cyclo*(L-*a*Hyp-L-*a*Hyp) (2)

(1) The abbreviations for amino acids and peptides recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, 241, 2491 (1966), have been used throughout. In addition: Hyp = 4-hydroxyproline, *a*Hyp = *allo*-4-hydroxyproline.

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Table I. Equilibrium Composition in the Cis–Trans Isomerization of Cyclic Dipeptides

Cyclic dipeptide		R	Content of trans isomer, % Condition A[a]	Condition B[b]
cyclo(Ala-Ala) type				
	1 *cyclo*(Ala-Ala)	CH_3	47.1	52
	2 *cyclo*(Leu-Leu)	$CH_2CH(CH_3)_2$	41.7	52
	3 *cyclo*(Phe-Phe)	CH_2Ph	50.1	54
	4 *cyclo*(Val-Val)	$CH(CH_3)_2$	75.9	67
cyclo(Pro-Ala) type				
	5 *cyclo*(Pro-Ala)	CH_3	57.2	52
	6 *cyclo*(Pro-Leu)	$CH_2CH(CH_3)_2$	85.0	73
	7 *cyclo*(Pro-Phe)	CH_2Ph	90.9	79
	8 *cyclo*(Pro-Val)	$CH(CH_3)_2$	92.4	81
		R_1 R_2		
cyclo (Pro-Pro) type				
	9 *cyclo*(Pro-Pro)	H H	<0.5	<0.5
	10 *cyclo*(Hyp-Hyp)(A)[c]	OH(*R*)[d] OH(*R*)	<0.5	<0.5
	11 *cyclo*(Hyp-Hyp)(B)[c]	OH(*R*) OH(*S*)	<0.5	<0.5

[a] Condition A: at 30° in 0.1 *N* ethanolic sodium ethoxide. [b] Condition B: at 250° in H_2O. [c] See Figure 2. [d] The symbols (*R*) and (*S*) represent the chirality of C_4 or $C_{4'}$ in the pyrrolidine ring.

Table II. Thermodynamic Constants for the Equilibrium, Cis Isomer ⇌ Trans Isomer of Cyclic Dipeptides[a]

Compound	K_{303}[b]	ΔG_{303}, kcal/mol	$\Delta H°$, kcal/mol	$\Delta S°$,[c] eu
cyclo(Ala-Ala)	0.892	$+0.07 \pm 0.005$	$+0.22 \pm 0.1$	$+0.49 \pm 0.2$
cyclo(Leu-Leu)	0.716	$+0.20 \pm 0.005$	$+0.46 \pm 0.1$	$+0.87 \pm 0.2$
cyclo(Phe-Phe)	1.003	-0.002 ± 0.03		
cyclo(Val-Val)	3.16	-0.69 ± 0.01	-0.44 ± 0.1	$+0.82 \pm 0.3$
cyclo(Pro-Ala)	1.34	-0.17 ± 0.005	-0.12 ± 0.1	
cyclo(Pro-Leu)	5.67	-1.04 ± 0.01	-1.00 ± 0.2	
cyclo(Pro-Phe)	10.03	-1.39 ± 0.02	-1.28 ± 0.2	
cyclo(Pro-Val)	12.09	-1.50 ± 0.02	-1.40 ± 0.2	
cyclo(Pro-Pro)	<0.005	> +3.2		
cyclo(Hyp-Hyp)(A)[d]	<0.005	> +3.2		
cyclo(Hyp-Hyp)(B)[d]	<0.005	> +3.2		

[a] The reaction was run at 30, 50, and 75° in 0.1 *N* ethanolic sodium ethoxide. [b] K = [trans isomer]/[cis isomer]. [c] In the *cyclo*(Pro-Ala) type, the entropy difference was generally small and the projected error of $\Delta S°$ was larger than the measured values ($\Delta S°$). Thus, data were deleted from the table. [d] See Figure 2.

cyclo(L-Hyp-L-*a*Hyp) (**11a**) ⇌ *cyclo*(L-Hyp-D-Hyp) (**11b**)

cyclo(D-*a*Hyp-D-Hyp) (**11c**) ⇌ *cyclo*(D-*a*Hyp-L-*a*Hyp) (**11d**) (3)

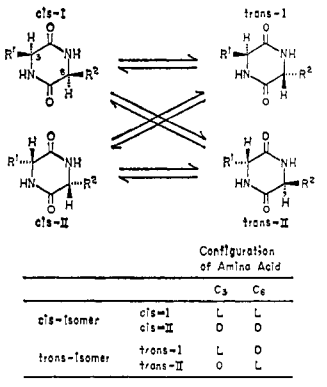

Figure 1. Four isomers of a cyclic dipeptide and their classifications.

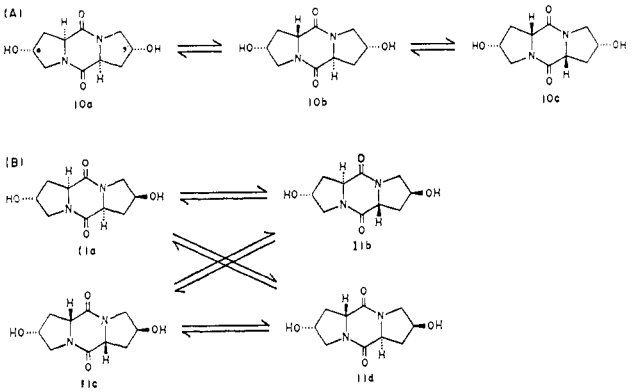

Figure 2. Cis–trans isomerization of a cyclic dipeptide composed of two 4-hydroxyproline residues.

irrespective of the presence of hydroxy groups on C_4 and $C_{4'}$ in the pyrrolidine rings and the configurations around the asymmetric carbons (C_4 and $C_{4'}$). The trans isomer could not be detected in equilibrium; however, its amount was found to be <0.5% by a comparison with that of an authentic mixture; <0.5% of the trans isomer could not be detected exactly by the adopted glc analytical procedures. Since the difference in the compositions at 30, 50, and 75° could not be

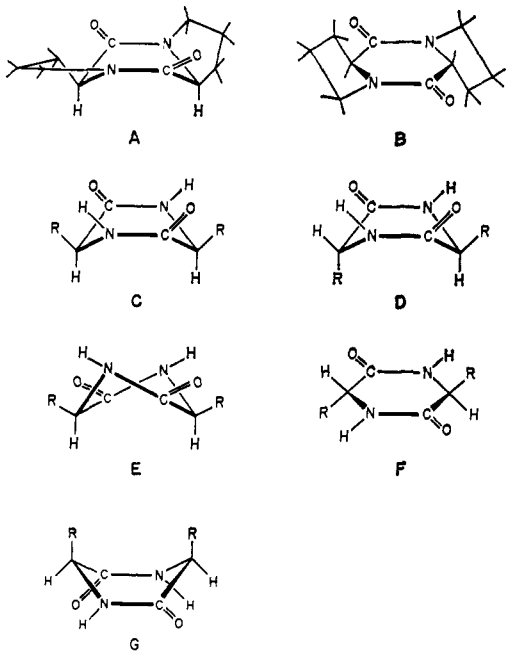

Figure 3. Boat (A, C, D, and G), planar (B and F), and slightly twist-boat (E) conformations for the diketopiperazine rings of cyclic dipeptides.

observed from one another beyond the experimental error of glc, $\Delta H°$ and $\Delta S°$ could not be determined for these dipeptides.

No crystallographic study has ever been made of this type of cyclic dipeptide. On the basis of nmr studies,[14,16] however, the diketopiperazine (DKP) ring is considered to have a boat form (Figure 3A) in the cis isomer and a planar form (Figure 3B) in the trans isomer. The ring was actually found to have a boat form in *cyclo*(L-3,4-dehydroprolyl-L-3,4-dehydroprolyl), whose structure was analyzed by X-ray diffraction.[7] A Dreiding model also indicates that the boat form can be stable for the cis isomer.

For the trans isomer, on the other hand, the boat conformation cannot be constructed as long as the amide bonds are kept planar. A Dreiding model strongly suggests that only the planar form (Figure 3B) can be stable. With the planar DKP ring, the pyrrolidine rings should have a half-chair conformation with four carbon atoms in a plane and the nitrogen atom out of the plane on the same side as the carbonyl group. This conformation of the pyrrolidine ring is greatly strained as long as the amide bonds are kept planar. Such a strain may explain why the trans isomer is less stable than the cis isomer.

***cyclo*(Pro-Ala) Type of Cyclic Dipeptides (5–8).** For this type of cyclic dipeptide, the trans isomer is mostly predominant in equilibrium. Both the free energy difference and the enthalpy difference between the cis and trans isomers become larger as the bulkiness of the side chain (R) of the amino acid residue increases in the neighborhood of the α carbon, and then changes in $\Delta G°$ owing to R are almost controlled by changes of $\Delta H°$ (see Tables I and II).

On the basis of the X-ray crystallographic data of *cyclo*(L-Pro-L-Leu)[7] and nmr data,[15] the DKP rings of both the cis and trans isomers are considered to have boat conformations (Figure 4H and I). In Figure 4H, the R group is on a quasiequatorial bond of the DKP

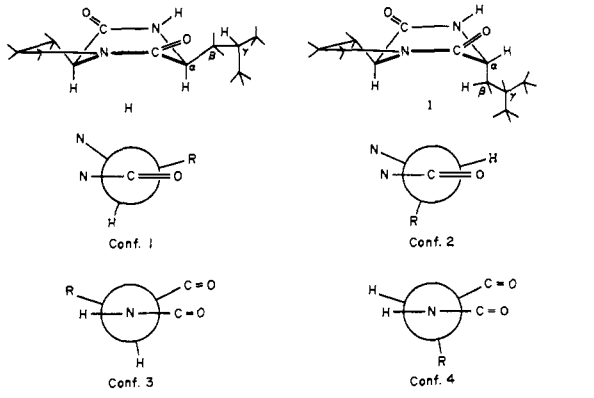

Figure 4. Boat conformations (H and I) of the *cyclo*(Pro-Ala) type of cyclic dipeptides, *e.g.*, *cyclo*(Pro-Leu) (R = $CH_2CH(CH_3)_2$). Conformations 1 and 2 show the conformation around the C′–C^α bond. Conformations 3 and 4 show the conformation around the N–C^α bond.

ring and eclipses the carbonyl group around the C′–C^α bond (Figure 4, conformation 1)[24] or the N′–H bond of amide group around the N′–C^α bond (Figure 4, conformation 3). On the other hand, the R group in Figure 4I is on a quasiaxial bond of the DKP ring and is skew to the carbonyl group (Figure 4, conformation 2) or to the N′–H bond of the amide group (Figure 4, conformation 4).

In the carbonyl compounds such as CH_3CH_2COR, the conformation in which the methyl group eclipses the carbonyl group seems more stable than the conformation in which the methyl group is skew to the carbonyl group, *e.g.*, the stabilization energy of the eclipsed conformer from the skew conformer is 900 cal/mol for CH_3CH_2CHO,[25] 1000 cal/mol for $CH_3CH_2COCH_3$,[26] and 75 ± 50 cal/mol for $CH_3CH_2CO_2CH_3$.[27]

On the other hand, the enthalpy difference between the cis and trans isomers in the *cyclo*(Pro-Ala) type of cyclic dipeptides suggests that conformation 2 is more stable than conformation 1. Therefore, on the basis of above knowledge, one might suspect that what we found is somewhat abnormal. It is not necessarily so, however, in view of a recent finding of Koyama.[28] For $CH_3CH_2CONHCH_3$, he found by means of gas electron diffraction and ir that a conformation in which the methyl group eclipses the OC–NH bond (*i.e.*, the methyl group is staggered to the carbonyl group) is the most stable, but the conformation in which the methyl group eclipses the carbonyl group is not observed at all in gas, liquid, and solid states. This difference of $CH_3CH_2CONHCH_3$ from other carbonyl compounds in the conformation of the $CH_3CH_2\frac{\alpha}{\beta}CO$- axis is considered to be due to partial double bond character of the OC–N bond and partial single bond character of the C=O bond in the amide group. On the basis of this finding, it is quite understandable

(24) The abbreviations and symbols for the description of the conformation of polypeptide chains proposed by the IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, 9, 3471 (1970), have been used throughout.

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(27) J. Bowles and W. O. George, *Chem. Commun.*, 103 (1970).

(28) Y. Koyama, The Kwansei Gakuin University, personal communication, 1973.

that conformation 2 is more stable than conformation 1. Thus, what we found here for the cyclic dipeptides is an interesting coincidence with what was recently found for a linear amide.

Koyama has also found that, in $CH_3CONHCH_2CH_3$, the methyl group is skew to the N–H bond (*i.e.*, CH_3CH–H bond eclipses the N–H bond) in its gas, liquid, and solid states.[28] This conformation corresponds to conformation 4. Therefore, it is understandable that conformation 4 is more stable than conformation 3 and the energy difference between these two conformations increases with the bulkiness of the R group.

***cyclo*(Ala-Ala) Type of Cyclic Dipeptides (1–4).** The amounts of cis and trans isomers in equilibrium are nearly equal in this type of dipeptide. The enthalpy ($\Delta H°$) of the cis isomer → trans isomer reaction is slightly positive in *cyclo*(Ala-Ala) (**1**) and *cyclo*(Leu-Leu) (**2**). In *cyclo*(Val-Val) (**4**), on the other hand, it is negative as in the case of *cyclo*(Pro-Val) (**8**).

This type of cyclic dipeptide is known to be very flexible and is suggested to have various boat and planar conformations which are not discrete and can easily interconvert.[10,11,17,21] However, the stable conformation of the cis isomer is generally considered to be the boat form (Figure 3C[17] or G[19,21]) or a slightly twisted boat form (Figure 3E),[8,10,13] and that of trans isomer is considered to be a boat form (Figure 3D)[17] or a planar form (Figure 3F).[8,9,21]

By X-ray crystallographic studies,[8,10] *cyclo*(L-Ala-L-Ala) is known to have a slightly twisted-boat conformation (Figure 3E). Its torsional angles, $\psi_{C^\alpha-C'}$ and $\phi_{N'-C^\alpha}$, are found to be smaller by about 10° than those of *cyclo*(L-Pro-L-Leu),[29] so the DKP ring of *cyclo*(L-Ala-L-Ala) is nearer to a planar structure than that of *cyclo*(L-Pro-L-Leu). Thus, the dihedral angles, $\theta_1(C^\beta-C^\alpha-C'-O)$ and $\theta_2(C^\beta-C^\alpha-N'-H)$, in two alanine residues are all greater than the corresponding angles of *cyclo*(L-Pro-L-Leu).[30] Therefore, *cyclo*(L-Ala-L-Ala) has not nearly eclipsed conformations (conformations 1 and 3) anymore.

On the other hand, by the results of ORD[19] and consistent force field calculations,[21] *cyclo*(L-Ala-L-Ala) is suggested to have a shallow boat form (Figure 3G), in which two methyl groups are on the quasiaxial bonds of the DKP ring and are skew to the carbonyl group and to the N′–H bond.

Since the *cyclo*(Ala-Ala) type of cyclic dipeptide is much more flexible than the *cyclo*(Pro-Ala) type of cyclic dipeptide, the following factors should be further considered in the *cyclo*(Ala-Ala) type of dipeptide about $\Delta H°$ in addition to conformational energies for the $-CO\overset{\alpha}{\frown}CHR-$ and $-NH\overset{\alpha}{\frown}CHR-$ axes: (a) energy of distortion from planarity of the amide bond,[8,22] (b) intramolecular interactions of nonbonded atom pairs of the two side chains[18] and similar interactions between a side chain and the DKP ring.[12,13,23]

However, if the cis isomer of this type of cyclic dipeptides has generally a stable conformation similar to that of *cyclo*(L-Ala-L-Ala) (Figure 3E or G), the energy difference between the cis and trans isomers due to the conformations around the $C'-C^\alpha$ and $N'-C^\alpha$ bonds decreases in this type of cyclic dipeptide. It is understandable, therefore, that the cis isomer is not so unstable in these cyclic dipeptides as in the *cyclo*(Pro-Ala) type of cyclic dipeptide.

(29) *cyclo*(L-Ala-L-Ala): Slettern reported that the two torsional angles, $\psi_{C^\alpha-C'}$ and $\phi_{N-C'}$, were +20.6 and −32.4°, respectively, in one alanine residue and +26.9 and −25.7°, respectively, in another alanine residue.[8] Benedetti, Corradini, and Pedone reported that $\psi_{C^\alpha-C'}$ and $\phi_{N-C'}$ were +20 and −25°, respectively, in one alanine residue and +28 and −33°, respectively, in another alanine residue.[10] *cyclo*(L-Pro-L-Leu): $\psi_{C^\alpha-C'}$ and $\phi_{N-C'}$ were +33.8 and −41.5°, respectively, in the leucine residue and +33.7 and −41.5°, respectively, in the proline residue.[6]

(30) *cyclo*(L-Ala-L-Ala): The dihedral angles, $\theta_1(C^\beta-C^\alpha-C'-O)$ and $\theta_2(C^\beta-C^\alpha-N-H)$, were calculated to be −36.6 and +38.4°, respectively, in one alanine residue and −31.6 and +35.4°, respectively, in another alanine residue on the basis of Slettern's X-ray data.[8] From the X-ray data of Benedetti, Covadini, and Pedone,[10] $\theta_1(C^\beta-C^\alpha-C'-O)$ and $\theta_2(C^\beta-C^\alpha-N-H)$ were calculated to be −39.1 and +40.0°, respectively, in one alanine residue and −33.1 and −34.3°, respectively, in another alanine residue. *cyclo*(L-Pro-L-Leu): From the X-ray data of Karle,[6] $\theta_1(C^\beta-C^\alpha-C'-O)$ and $\theta_2(C^\beta-C^\alpha-N-H)$ were calculated to be −24.6 and +19.9°, respectively, in the leucine residue and −28.1 and +17.4°, respectively, in the proline residue.

Experimental Section

Cyclic Dipeptides. The cyclic dipeptides were prepared according to published procedures,[14,15,31] They were all analytically pure and homogeneous according to glc and thin-layer chromatographic criteria. Preparation of new cyclic dipeptides, *e.g.*, *cyclo*(L-Hyp-D-*a*Hyp), *cyclo*(D-*a*Hyp-D-*a*Hyp), *cyclo*(L-Hyp-L-*a*Hyp), *cyclo*(L-Hyp-D-Hyp), and *cyclo*(D-*a*Hyp-L-*a*Hyp), will be described in a subsequent paper in this series.

Analysis of the Cis and Trans Isomers by Glc. Compositions of the cis and trans isomers were determined by glc using a Hewlet Packard 402 high efficiency gas chromatogram equipped with FID and glass columns. The gas chromatographic method has been applied to analyze the cis and trans isomers as unmodified compounds,[6,32] as the trimethylsilyl derivatives,[6] as the trifluoroacetyl derivatives,[33] and as the *N*-methyl derivatives.[6] We modified these methods and analyzed these isomers as follows: (A) cyclic dipeptide (8–10 mg) in dry methanol (2 ml) was injected directly; (B) cyclic dipeptide (10–13 mg) was heated with *N*,*O*-bis(trimethylsilyl)acetamide (1 ml) and dry pyridine (1 ml) for 5–10 min at 80°. The mixture was injected directly; (C) cyclic dipeptide (7–12 mg) in trifluoroacetic anhydride (2 ml) and chloroform (1 ml) was allowed to react for 2 days at room temperature in a closed vessel and then it was injected directly.

The isomerization of cyclic dipeptide was not at all observed during these procedures. The compositions of the cis and trans isomers were reproducible within 0.1% in these analytical procedures. The analytical procedures, column, column temperature, and retention times are shown in Table V.[34]

The conditions sufficiently necessary to analyze the cis and trans isomers of *cyclo*(Phe-Phe) (**3**) could not be found in these procedures, so the glc method was not adopted for **3**.

Analysis of the Cis and Trans Isomers by Nmr. The cis and trans isomers of *cyclo*(Ala-Ala) (**1**), *cyclo*(Pro-Ala) (**5**), and *cyclo*(Phe-Phe) (**3**) were analyzed by nmr with a Varian XL-100 spectrometer using TMS as internal standard in deuteriotrifluoroacetic acid. The methyl protons of cyclic dipeptides having the alanine residue gave a doublet signal at δ 1.73 ($J_{\alpha\beta}$ = 7.1 Hz) for the cis isomer of **1**, at 1.69 ($J_{\alpha\beta}$ = 7.2 Hz) for trans isomer of **1**, at 1.59 ($J_{\alpha\beta}$ = 7.1 Hz) for the cis isomer of **5**, and at 1.63 ($J_{\alpha\beta}$ = 7.1 Hz) for the trans isomer of **5**. The amounts of the cis and trans isomers were determined from the peak heights of these methyl protons at 250 Hz sweep width by a comparison with that of an authentic mixture. The α protons of the cis and trans isomers of **3** gave multiplet signals at δ 4.56 and 3.95, respectively. The abundance ratio of the cis and trans isomers of **3** was measured by integration of these signals.

Cis–Trans Isomerization Studies in 0.1 *N* Ethanolic Sodium Ethoxide. The isomerization was started from both the cis (*cis*-I or *cis*-II) and trans sides (*trans*-I or *trans*-II). When the abundance ratios of the cis/trans isomers in the two independent reaction mixtures became equal to each other, this was taken as the equilibrium ratio. The time required for reaching the equilibrium was about 20 hr at 30.0°, 3 hr at 50.0°, and 1 hr at 75.0°. The concentration

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(33) G. P. Slater, *J. Chromatogr.*, **64**, 166 (1972).

(34) See paragraph at end of paper regarding supplementary material.

of the cyclic dipeptide was chosen depending upon the solubility, *e.g.*, 0.33 m*M* for *cyclo*(Phe-Phe) (**3**), 3.3 m*M* for *cyclo*(Ala-Ala) (**1**), *cyclo*(Leu-Leu) (**2**), and *cyclo*(Val-Val) (**4**), and 50 m*M* for other cyclic dipeptides.

cyclo(**Val-Val**) (**4**). *cyclo*(L-Val-L-Val) (50 mg, 0.25 mmol) in 0.1 *N* ethanolic sodium ethoxide (75 ml) was allowed to stand at 30.0 ± 0.1°. Samplings were done at least three times between 25 and 40 hr. The aliquot (15 ml) taken out was immediately poured into 2 *N* hydrochloric acid (0.76 ml) cooled with ice to quench isomerization by neutralization, and the slightly acidic solution was evaporated to dryness under reduced pressure. The residue was further dried *in vacuo* on anhydrous potassium hydroxide and analyzed by procedure A. The equilibrium points were determined in a similar manner at 50.0 ± 0.1 and 75.0 ± 0.1°, in which samplings were done at 4–10 hr and at 3–8 hr, respectively. The isomerization was similarly carried out using *cyclo*(L-Val-D-Val). The analytical results are given in Table III.

Table III. Equilibrium Data for the Cis–Trans Isomerization of *cyclo*(Val-Val)

Temp, °K	Content of trans isomer, %: Start from *cyclo*(L-Val-L-Val)	Start from *cyclo*(L-Val-D-Val)	Av
303	75.8, 76.0, 76.1	75.8, 75.7, 76.2	75.9
323	75.1, 75.0, 75.0	74.9, 74.8, 75.1	75.0
348	74.1, 74.1, 74.4	74.3, 74.1, 73.9	74.2

cyclo(**Ala-Ala**) (**1**), *cyclo*(**Leu-Leu**) (**2**), *cyclo*(**Pro-Ala**) (**5**), *cyclo*(**Pro-Leu**) (**6**), *cyclo*(**Pro-Phe**) (**7**), **and** *cyclo*(Pro-Val) (**8**). The isomerization experiments were carried out in a similar manner to that of **4**. In the case of **5**–**8**, the cyclic dipeptides of *cyclo*(L-Pro-L-Ala) type and *cyclo*(L-Pro-D-Ala) type were used as starting materials. The analytical procedures and results by glc were shown in Table IV. By nmr, the equilibrium points of **1** and **5** were at 48% of the trans isomer at 50.0° and at 56% of the trans isomer at 30.0°, respectively.

Table IV. Equilibrium Data for the Cis–Trans Isomerization of Cyclic Dipeptides

	Analytical procedure[a]	Content of trans isomer, %: 323°K	348°K
cyclo(Ala-Ala)	C	47.6	48.2
cyclo(Leu-Leu)	C	43.0	44.2
cyclo(Pro-Ala)	A	56.9	56.5
cyclo(Pro-Leu)	A	83.6	82.0
cyclo(Pro-Phe)	B	89.5	88.4
cyclo(Pro-Val)	A	91.3	90.0

[a] See Experimental Section.

cyclo(**Phe-Phe**) (**3**). *cyclo*(L-Phe-L-Phe) or *cyclo*(L-Phe-D-Phe) (36 mg, 0.025 mmol) was dissolved in 0.1 *N* ethanolic sodium ethoxide (75 ml) by warming at about 60° and the clear solution thus obtained was allowed to stand at 30.0 ± 0.1°. After 35 hr, the clear solution was neutralized with 2 *N* hydrochloric acid (3.8 ml) and the solution was evaporated to dryness *in vacuo*. The dried residue was dissolved in deuteriotrifluoroacetic acid (0.5 ml), filtered, and analyzed by nmr. The equilibrium points were observed at 51.1% of the trans isomer in starting from *cyclo*(L-Phe-L-Phe) and at 49.0% of the trans isomer in starting from *cyclo*(L-Phe-D-Phe).

cyclo(**Pro-Pro**) (**9**). The isomerization experiments were carried out in a similar manner to that of **4** using *cyclo*(L-Pro-L-Pro) and *cyclo*(L-Pro-D-Pro) as starting materials. Gas chromatography (procedure A) showed that there was no detectable amount of the trans isomer in the equilibrium mixtures at 30.0, 50.0, and 75.0°. The detectable amount of the trans isomer in the mixture of cis and trans isomers was checked by using authentic mixtures which contained 10, 1, 0.5, 0.1, and 0.01% of the trans isomer: 0.5% of the trans isomer was detectable; however, 0.1 or 0.01% of the trans isomer was not detectable.

cyclo(**Hyp-Hyp**) (A) (**10**). Using *cyclo*(L-Hyp-L-Hyp) (**10a**), *cyclo*(D-*a*Hyp-D-*a*Hyp) (**10c**), and *cyclo*(L-Hyp-D-*a*Hyp) (**10b**) as starting materials, the isomerization experiments were similarly carried out. The trans isomer (**10b**) was not detectable by glc (procedure B) in the equilibrium mixtures at 25–78°. Using authentic mixtures of **10a** and **10b**, the detectable amount of the trans isomer in the mixture was checked; <0.5% of **10b** was not detectable.

cyclo(**Hyp-Hyp**) (B) (**11**). *cyclo*(L-Hyp-L-*a*Hyp) (**11a**), *cyclo*(L-Hyp-D-Hyp) (**11b**), and *cyclo*(D-*a*Hyp-L-*a*Hyp) (**11d**) were used as starting materials. The trans isomers (**11b** and **11d**) could not be detected by glc (procedure B) in the equilibrium mixtures.

Further investigation of the isomerization of **10** and **11** will be described in a subsequent paper in this series.

Cis–Trans Isomerization Studies in Aqueous Solutions. *cyclo*(**Val-Val**) (**4**). *cyclo*(L-Val-L-Val) or *cyclo*(L-Val-D-Val) (20 mg, 0.1 mmol) in water (1 ml) was sealed in a small glass tube and heated at 250 ± 1° for 3 hr. The equilibrium state was found to be accomplished after 2 hr in preliminary experiments. The tube was immediately cooled and opened. The solution was evaporated to dryness under reduced pressure and dried further *in vacuo* over phosphorus pentoxide. The residue was analyzed by glc. The equilibrium point was at 67.0% of the trans isomer in starting from *cyclo*(L-Val-L-Val) and at 67.7% of the trans isomer in starting from *cyclo*(L-Val-D-Val).

The isomerization experiments of other cyclic dipeptides were similarly carried out, in which the concentration of the cyclic dipeptide was chosen as 1–10% depending upon the solubility.

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Supplementary Material Available. Table V 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 $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-3985.