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Solution Conformation of Cyclic Dipeptides Having Aliphatic Side Chains

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Cyclic dipeptides having aliphatic side chains, *cyclo*(L-Val)$_2$, *cyclo*(L-Val–D-Val), *cyclo*(L-Leu)$_2$, and *cyclo*(L-Leu–D-Leu) were synthesized and their conformation in solution was investigated by circular dichroism (CD) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. ^{1}H NMR spectra in $(CD_3)_2SO$ of *cyclo*(L-Val–D-Val) and *cyclo*(L-Leu–D-Leu) indicated that the diketopiperazine ring of these *trans* cyclic dipeptides takes a planar or a chair conformation and the molecules take a center-symmetric conformation. ^{1}H NMR spectra in $(CD_3)_2SO$ and CD spectra in CH_3OH and H_2O of *cyclo*(L-Val)$_2$ and *cyclo*(L-Leu)$_2$ indicated that the conformation of these *cis* cyclic dipeptides is planar-axial and flagpole-boat-type respectively. It is suggested that *cis* cyclic dipeptides having aliphatic side chains take such conformations in polar solvents because of the stabilization by attractive forces between aliphatic side chains.

It is well-known that the secondary structure of enzymes plays an important role in exerting their catalytic activities. Biologically active oligopeptides such as peptide antibiotics involve cyclic peptides containing unusual D-amino acids or *N*-substituted amino acids. These examples also show the importance of the secondary structure determined by these unusual amino acids on the biological activity. In order to investigate the structure/activity relationship of these polypeptides and oligopeptides, the use of synthetic cyclic peptides is very helpful.[1,2] In cyclic peptides side chain functional groups may be fixed in a specific arrangement owing to the rigid conformation of the ring moiety. The reduced freedom of conformation enables a detailed analysis of the conformation in solution to be made. The accumulation of information on the structure/activity relation will make possible the design of a molecule having a specific activity.

With this in mind we have synthesized *cyclo*(D-Leu–L-His) and *cyclo*(D-Val–L-His); these are cyclic dipeptides carrying a hydrophobic and a nucleophilic side chain, and we have investigated their hydrolytic activities toward carboxylic acid *p*-nitrophenyl esters. As consequence, these *trans* cyclic dipeptides proved to be effective catalysts for the hydrolyses of $CH_3(CH_2)_{10}COOC_6H_4NO_2(p\text{-})$ and $CH_3(CH_2)_8COOC_6H_4NO_2(p\text{-})$. On the other hand, the diastereomeric *cis* cyclic dipeptides were nearly inactive in the same reactions.[3,4] The conformation of these cyclic dipeptides in aqueous solution was investigated by nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopy.[4,5] It was found that in these cyclic dipeptides the aromatic side chain of the histidine residue stacks over the diketopiperazine ring due to aromatic–amide interaction, and that in *trans* cyclic dipeptides the two side chains are situated on the opposite sides of the nearly planar diketopiperazine ring. Furthermore in *cis* cyclic dipeptides the two side chains compete for the space on the same side of the boat-type diketopiperazine ring. In the case of *cyclo*(L-Leu–L-His) the X-ray structural analysis of a single crystal revealed a conformation very similar to that observed in solution.[6]

In order to get some more general information about the conformational properties of cyclic dipeptides in solution, it is necessary to investigate the solution conformation of cyclic dipeptides having only aliphatic side chains, so that the aromatic–amide interaction is absent. It has been assumed[7] without firm experimental support that side chains of these cyclic dipeptides tend to take a pseudoequatorial position in a bowsprit-boat conformation of the diketopiperazine ring because of steric interactions between the side chains or between side chain and diketopiperazine ring. However, in some papers which have been published on the experimental studies of solution conformation before we started the present investigation, experimental evidence for a flagpole-boat conformation was presented. Hooker, Jr., *et al.*[8] carried out the CD investigation of *cyclo*(L-Ala)$_2$ and observed a flagpole-boat conformation in aqueous solution. Davies and Khaled[9] have measured ^{1}H NMR spectra of a number of *cyclo*(Gly–Xyz) and *cyclo*(Sar–Xyz) in $(CD_3)_2SO$ or D_2O and determined the superposition angle β of the ring from ^3J(H–C$^\alpha$–N–H) and ^5J(H–C$^\alpha$–C′–N–C$^\alpha$–H). They have found a boat-type conformation with many kinds of Xyz except of L-Pro and D-Abu.

We considered that the conformation of cyclic dipeptides consisting only of aliphatic α-amino acids in solution should be investigated in more detail. For this purpose, *cyclo*(L-Leu)$_2$, *cyclo*(L-Leu–D-Leu), *cyclo*(L-Val)$_2$, and *cyclo*(L-Val–D-Val) were synthesized and the relationship between the properties of aliphatic side chains and the solution conformation have been investigated.

Experimental

Cyclic Dipeptides. The *N*-terminal of an α-amino acid was blocked by benzyloxycarbonyl group and the *C*-terminal of the other α-amino acid was blocked by an ethyl ester group. Both were coupled with dicyclohexylcarbodiimide as a condensing agent to yield a linear dipeptide, both terminals of which were blocked. The benzyloxycarbonyl group was removed by catalytic hydrogenation, and the product was refluxed in CH_3OH to yield the cyclic dipeptides. Needle-like crystals were obtained by the recrystallization from CH_3OH/H_2O mixed solvent. The products subjected to thin layer chromatography with effluents $CHCl_3$(95)/CH_3OH(2)/AcOH-(3) and *n*-BuOH(15)/AcOH(10)/H_2O(12)/pyridine(13) were ninhydrin-negative and colored with iodine (one spot). *cyclo*(L-Val)$_2$, mp 268 °C. Found: C, 60.33; H, 9.29; N, 13.86%.

Calcd for $C_{10}H_{16}N_2O_2$: C, 60.58; H, 9.15; N, 14.13%. *cyclo*(L-Val-D-Val), mp 266—268 °C. Found: C, 60.14; H, 9.31; N, 14.12%. *cyclo*(L-Leu)$_2$, mp 276 °C. Found: C, 63.42; H, 9.98; N, 12.13%. Calcd for $C_{12}H_{22}N_2O_2$: C, 63.66; H, 9.82; N, 12.38%. *cyclo*(L-Leu-D-Leu), mp 275—278 °C. Found: C, 63.57; H, 9.69; N, 12.14%.

NMR Spectra. 100 MHz and 270 MHz ^{1}H NMR spectra were measured at room temperature with a Varian HA-100 and a Bruker WH-270 spectrometer, respectively. The solvent was $(CD_3)_2SO$ and the internal standard was $(CH_3)_4Si$. The sample concentration was 20 mg/0.4 cm^3 in 100 MHz measurement and 5 mg/0.4 cm^3 in 270 MHz NMR measurement, The NMR measurement in aqueous solution was impossible because of the extremely low solubility of cyclic dipeptides in water.

The NMR signals were assigned by the spin decoupling method in the 100 MHz or 270 MHz spectrum. The chemical shift and the coupling constant were determined from the 270 MHz NMR spectrum. The signal resolution was about $\pm$0.18 Hz.

CD Spectra. The CD spectra of the cyclic dipeptides in aqueous or methanolic solution were measured at room temperature using a JASCO J-20 spectropolarimeter. The concentration of the cyclic dipeptides was about 4×10^{-4} mol dm^{-3}, and quartz cells having optical path lengths 1 cm and 1 mm were used.

A Gaussian Cotton effect curve was assumed, and the spectra obtained were resolved into each Cotton effect component by a curve-fitting method. The molar elipticity [θ] was calculated by assuming the maximum wavelength, the half width, and the rotational strength, which were changed by $\pm$0.5, $\pm$0.5, and $\pm$0.1%, respectively. The FACOM M-190 system of Computer Center of Kyoto University was used for the computation. The computation was repeated until the standard deviation, which is the root-mean square of the difference between the calculated [θ] and the observed [θ] at each wavelength, became smaller than 10^3 deg cm^2 dmol^{-1}.

Results

NMR Spectra. cyclo*(L-Val)$_2$* *and* cyclo*(L-Val-D-Val)*: 270 MHz NMR spectra of $(CD_3)_2SO$ solution of cyclic dipeptides consisting of two valyl residues were measured. Chemical shifts and coupling constants were determined on the basis of the NMR spectra and are shown in Table 1. In the NMR spectra, only one set of signals was observed for the amide protons, $C^\alpha H$, $C^\beta H$ and the $C^\gamma H_3$ for either cyclic dipeptide. This indicates that *cyclo*(L-Val)$_2$ assumes a C_2-symmetric conformation and *cyclo*(L-Leu-D-Leu) assumes a center-symmetric conformation.

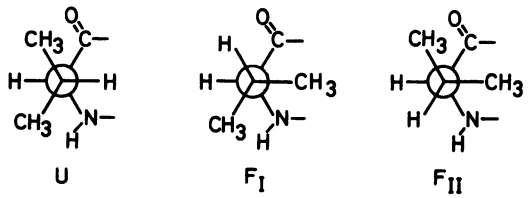

Fig. 1. Rotational isomers around C^α–C^β bond of valyl residue in cyclic dipeptide.

With regard to the side chain conformation of the valyl residues, three kinds of conformational states *U*, F_I, and F_{II} ($U+F_I+F_{II}=1$) corresponding to energy minima of internal rotation around C^α–C^β bond are available; these are illustrated in Fig. 1. The proportions of *U*, F_I, and F_{II} were calculated assuming the coupling constants between a pair of protons which are *gauche* and *trans* with regard to C^α–C^β bond to be 2.60 and 13.56 Hz, respectively.[10] On the above basis, F_I+F_{II} values amount to 94% and 97% for *cyclo*(L-Val)$_2$ and *cyclo*(L-Val-D-Val), respectively. It is somewhat surprising that 94% of the available conformations of *cyclo*(L-Val)$_2$ are folded, because this type of conformation should suffer from steric crowding between side chains.

With regard to the conformation of diketopiperazine ring, information on the rotational angle ϕ of the ring can be obtained from $J_{H\text{-}C^\alpha\text{-}N\text{-}H}$. Since the diketopiperazine ring of the cyclic dipeptides are symmetric and if a planar *cis* peptide bond is assumed, the conformation of diketopiperazine ring is determined by ϕ. Karplus-type equations have been proposed[11-13] for the relationship between the H-C^α-N-H coupling constant and the dihederal angle θ [$\theta=|\phi-60|$]. However, the usual $J_{H\text{-}C^\alpha\text{-}N\text{-}H}$ values (2 Hz) of cyclic dipeptides give only scattered values of θ by the Karplus-type equations, and hence the determination of unequivocal θ value is in general difficult. In the 270 MHz NMR spectra of *cyclo*(L-Val)$_2$ and *cyclo*(L-Val-D-Val) the NH signal is a broad singlet. This makes the determination of $J_{H\text{-}C^\alpha\text{-}N\text{-}H}$ extremely difficult and inaccurate. $J_{H\text{-}C^\alpha\text{-}N\text{-}H}$ is about 1.5 Hz for either cyclic dipeptide as is seen in Table 1. Substitution of this value into the

Table 1. Chemical shifts[a] and coupling constants[b] in 270 MHz proton nuclear magnetic resonance spectra[c] of *cyclo*(L-Val)$_2$ and *cyclo*(L-Val-D-Val)

	δ_{NH}	$\delta_{C^\alpha H}$	$\delta_{C^\beta H}$	$J_{H\text{-}C^\alpha\text{-}N\text{-}H}$	$J_{H\text{-}C^\alpha\text{-}C^\beta\text{-}H}$
cyclo(L-Val)$_2$	7.91	3.68	2.18	$\approx$1.5	3.31
cyclo(L-Val-D-Val)	7.98	3.65	2.19	$\lesssim$1.5	2.94

a) δ in ppm below $(CH_3)_4Si$. b) J in Hz. c) In $(CD_3)_2SO$, $(CH_3)_4Si$ as internal standard.

Table 2. Chemical shifts[a] and coupling constants[b] in 270 MHz proton nuclear magnetic resonance spectra[c] of *cyclo*(L-Leu)$_2$ and *cyclo*(L-Leu-D-Leu)

	δ_{NH}	$\delta_{C^\alpha H}$	$\delta_{C^\beta H^1}$	$\delta_{C^\beta H^2}$	$J_{H\text{-}C^\alpha\text{-}N\text{-}H}$	$J_{H\text{-}C^\alpha\text{-}C^\beta\text{-}H^1}$	$J_{H\text{-}C^\alpha\text{-}C^\beta\text{-}H^2}$
cyclo(L-Leu)$_2$	8.15	3.71	1.45	1.59	$\approx$2.4	8.46	5.15
cyclo(L-Leu-D-Leu)	8.07	3.75	1.51	1.60	$\lesssim$1.5	7.35	5.15

a) δ in ppm below $(CH_3)_4Si$. b) J in Hz. c) In $(CD_3)_2SO$, $(CH_3)_4Si$ as internal standard.

Ramachandran equation[11] or the Bystrov equation[12] led to $\theta>60°$ ($\phi<0°$), while that into the Cung equation[13] gave $\theta\leq60°$ ($\phi\geq0°$). Therefore, the ring conformations are not very explicit but seem to be nearly planar.

The $C^{\alpha}H$ signals of *cyclo*(L-Val)$_2$ and *cyclo*(L-Val–D-Val) in 270 MHz NMR spectra are a broad doublet. The latter signal does not become much sharper when it is decoupled with NH. This observation as well as the broad singlet NH signals of *cyclo*(L-Val)$_2$ and *cyclo*(L-Val–D-Val) in 270 MHz NMR spectra could be interpreted if the symmetric ring conformations of *cyclo*(L-Val)$_2$ and *cyclo*(L-Val–D-Val) fluctuate around the C^{α}–N bond on the NMR time scale.

cyclo*(L-Leu)$_2$ and* cyclo*(L-Leu–D-Leu)*: 270 MHz NMR spectra of $(CD_3)_2SO$ solution of cyclic dipeptides consisting of two leucyl residues were measured. The chemical shifts and coupling constants were determined on the basis of the NMR spectra, and are shown in Table 2. Similarly to the case of cyclic dipeptides of valine, only one set of signals was observed for each proton. Therefore, it can be concluded that *cyclo*(L-Leu)$_2$ assumes a C_2-symmetric conformation and *cyclo*(L-Leu–D-Leu) assumes a center-symmetric conformation.

With regard to the side chain conformation of the leucyl residues, similarly to the case of the cyclic dipeptides of valine, three kinds of conformational states F, U_{I} and U_{II} ($F+U_{\mathrm{I}}+U_{\mathrm{II}}=1$) corresponding to energy minima of internal rotation around C^{α}–C^{β} bond are available, which are illustrated in Fig. 2. Similarly to the case of cyclic dipeptides of valine, the proportions of each conformer were calculated from $J_{\mathrm{H\text{-}C^{\alpha}\text{-}C^{\beta}\text{-}H}}$ values listed in Table 2 using the theoretical coupling constants of *trans* and *gauche* proton pairs. Consequently, F% were 23.5 and 33.6 for *cyclo*(L-Leu)$_2$ and *cyclo*(L-Leu–D-Leu), respectively. Since the conformation of *cyclo*(L-Leu)$_2$ and *cyclo*(L-Leu–D-Leu) is C_2-symmetric and center-symmetric, respectively, the proportions of F–F conformation are also 23.5 and 33.6%, respectively. When the C^{α} substituent changed from the isopropyl group of valine to the isobutyl group of leucine, the fraction of folded conformation decreased. However, still one quarter of the available conformations of *cyclo*(L-Leu)$_2$ are folded ones. These should be sterically crowded because of the two bulky side chains accommodated in the same side of diketopiperazine ring. Therefore, this observation suggests that in *cyclo*(L-Leu)$_2$ some kind of stabilization must occur between side chains or between a side chain and the diketopiperazine ring.

In the 270 MHz NMR spectra of *cyclo*(L-Leu)$_2$, the NH signal is a relatively sharp doublet, giving $J_{\mathrm{H\text{-}C^{\alpha}\text{-}N\text{-}H}}$ to be 2.4 Hz as seen in Table 2. This value leads to $\theta\leq60°$ ($\phi\geq0°$) through any of the three Karplus-type equations. It was therefore concluded that *cyclo*(L-Leu)$_2$ assumes a flagpole-boat conformation in which side chains occupy pseudoaxial positions. The $C^{\alpha}H$ signal of *cyclo*(L-Leu)$_2$ in 100 MHz NMR spectrum is a broad triplet, and it becomes a sharp quartet when it is decoupled with NH. These observations suggest that the C_2-symmetric conformation of *cyclo*(L-Leu)$_2$ is rigid on the NMR time scale.

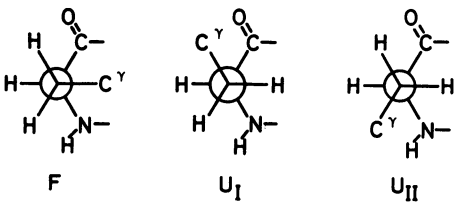

Fig. 2. Rotational isomers around C^{α}–C^{β} bond of leucyl residue in cyclic dipeptide.

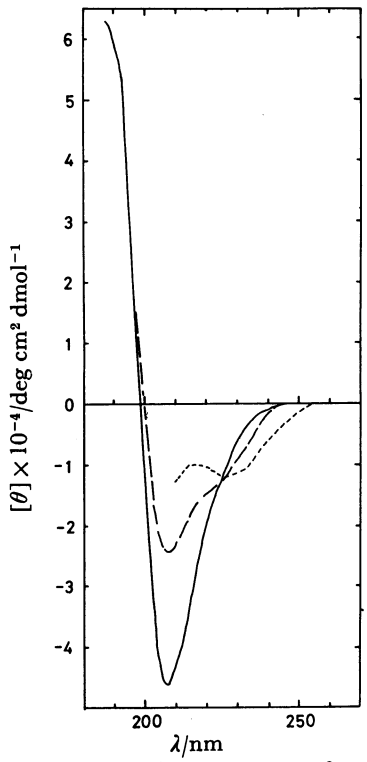

Fig. 3. Circular dichroism spectra of *cyclo*(L-Leu)$_2$. (——), in H_2O: (– – –), in CH_3OH: (------), in dioxane.

On the other hand, the NH signal of *cyclo*(L-Leu–D-Leu) in 270 NHz NMR spectra is a broad singlet, which makes the determination of $J_{\mathrm{H\text{-}C^{\alpha}\text{-}N\text{-}H}}$ extremely difficult and inaccurate. As seen in Table 2, $J_{\mathrm{H\text{-}C^{\alpha}\text{-}N\text{-}H}}$ of *cyclo*(L-Leu–D-Leu) is about 1.5 Hz, which leads to $\theta>60°$ ($\phi<0°$) by the Ramachandran equation[11] and the Bystrov equation[12] and $\theta\leq60°$ ($\phi\geq0°$) by the Cung equation.[13] Therefore, the ring conformation is not explicit but seems to be nearly planar. In 100 MHz NMR spectra of *cyclo*(L-Leu–D-Leu), the $C^{\alpha}H$ signal and the NH signal are a broad triplet and a broad singlet, respectively. The former signal becomes a sharp triplet by the decoupling with NH, and the latter signal becomes a sharp singlet by the decoupling with $C^{\alpha}H$. These observations suggest that the center-symmetric conformation of *cyclo*(L-Leu–D-Leu) is rigid on the NMR time scale.

CD Spectra. With NMR spectroscopy, except in the case of *cyclo*(L-Leu)$_2$, the coupling constant $J_{\mathrm{H\text{-}C^{\alpha}\text{-}N\text{-}H}}$ was not precisely determined and the dihedral angle θ was scattered according to the choice of Karplus-type equations. Consequently, the details of the symmetric

Table 3. Circular dichroism of *cyclo*(L-Leu)$_2$ in aqueous and methanol solution

Solvent	Amide π-π^* transition high energy lobe			Amide π-π^* transition low enenrgy lobe			Amide n-π^* transition		
	λ_{max} nm	Half width nm	Rotational strength[a]	λ_{max} nm	Half width nm	Rotational strength[a]	λ_{max} nm	Half width nm	Rotational strength[a]
H_2O	190	9	0.39	205	10	−0.28	218	12	−0.10
CH_3OH	195	10	0.35	202	10	−0.30	222	12	−0.09

a) In Debye-Bohr-magneton unit.

Table 4. Circular dichroism of *cyclo*(L-Val)$_2$ in aqueous and methanol solution

Solvent	Amide π-π^* transition			Amide n-π^* transition		
	λ_{max} nm	Half width nm	Rotational strength[a]	λ_{max} nm	Half width nm	Rotational strength[a]
H_2O	199	11	−0.41	222	11	0.12
CH_3OH	200	11	−0.47	224	13	0.13

a) In Debye-Bohr-magneton unit.

conformations of the diketopiperazine ring are often difficult to clarify. However, CD spectroscopy seems to be helpful to determine the ring conformation of cyclic dipeptides, as we succeeded in the conformational analysis of *cyclo*(L- or D-Leu-L-His).[5] The usefulness of CD spectroscopy is related with a result of computation that the Cotton effect due to the amide π-π^* transition changes the sign at $\theta=60°$.[8,14]

Figure 3 shows CD spectra of *cyclo*(L-Leu)$_2$ in aqueous solution and organic solvents. In aqueous solution, a positive maximum at 190 nm region and a negative minimum at 208 nm region are observed. In CH_3OH solution the negative minimum at 208 nm decreases in intensity and a shoulder appears at 225 nm region. In dioxane, which is less polar, a clear minimum exsists at 227 nm. The shoulder appearing in the 225 nm region has been observed in CD spectra of *cyclo*(L-Ala)$_2$ in CH_3OH and $(EtO)_3PO$ solutions by Hooker, Jr., *et al.*[8] They observed a week positive Cotton effect at 240 nm region in addition to the shoulder at 225 nm region, but they did not discuss the origin of the shoulder. With the present *cyclo*(L-Leu)$_2$ we did not observe any positive Cotton effect at 240 nm region. The same situation as in the case of *cyclo*(L-Leu)$_2$ was met in the CD spectrum of *cyclo*(Gly-L-Leu).[5] Therefore, we ascribe the negative minimum which shifts to the red in nonpolar solvents to the Cotton effect due to the amide π-π^* transition.[8] Furthermore, we ascribed the positive Cotton effect at 190 nm and the negative Cotton effect at 208 nm to a pair of Cotton effects due to the splitting of the amide π-π^* transition. According to this assignment, the CD spectra shown in Fig. 3 were resolved into the component Cotton effects, and are shown in Table 3. A pair of Cotton effects due to an exciton splitting should be opposite in sign and same in the absolute values of the rotational strength. In *cyclo*(L-Leu)$_2$ the couplet of lower wavelength is somewhat stronger than its pair, which should have been caused by the contribution from a Cotton effect at much lower wavelength. Since among two Cotton effects due to the exciton splitting of the amide π-π^* transition

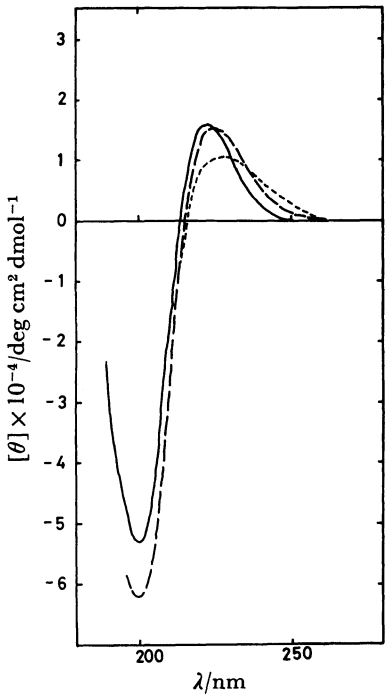

Fig. 4. Circular dichroism spectra of *cyclo*(L-Val)$_2$. (——), in H_2O: (-----), in CH_3OH: (------), in dioxane.

the one at the longer wavelength is negative and the other at the shorter wavelength is positive, the rotational angle around N–C$^\alpha$ bond ϕ should be positive. This conclusion is supported by the computation of the CD spectrum of *cyclo*(L-Ala)$_2$ by Hooker, Jr., *et al.*[8] and that of a dipeptide having two substituted *cis* peptide bonds by Bayley, *et al.*[14] It is therefore concluded from CD spectra that the diketopiperazine ring of *cyclo*(L-Leu)$_2$ assumes a flagpole-boat conformation. This conclusion agrees with that obtained from the NMR spectra in $(CD_3)_2SO$ solution. The general conclusion is that *cyclo*(L-Leu)$_2$ assumes a flagpole-boat conformation in aqueous, CH_3OH and $(CD_3)_2SO$ solutions.

Figure 4 shows CD spectra of *cyclo*(L-Val)$_2$ in aqueous

solution and organic solvents. In aqueous solution two distinct Cotton effects were observed, one being negative at 200 nm and the other positive at 222 nm. On change of solvent from H_2O to CH_3OH and further to dioxane, the positive Cotton effect at 222 nm red shifted and a slight hypochromic effect was observed. The positive Cotton effect at 222 nm region is ascribed to the amide n-π^* transition allowing for its wavelength and intensity. On the other hand, the negative Cotton effect at 200 nm region is ascribed to the amide π-π^* transition allowing for its wavelength and intensity. In the present case no evidence for the splitting of the amide π-π^* transition was obtained. This has not been usual with many cyclic dipeptides. In fact, the curve-fitting method using various parameters was unable to realize a pair of Cotton effects having different sign in the 180—210 nm region. Therefore, it was considered that *cyclo*(L-Val)$_2$ shows the only Cotton effect without the splitting of the amide π-π^* transition. The results of spectral resolution by the curve-fitting method and the assignments of spectrum are shown in Table 4.

The methods of computation reported by Hooker, Jr., *et al.*[8] and Bayley, *et al.*,[14] which were applied to *cyclo*(L-Leu)$_2$, cannot be applied in the present case because of the absence of the splitting of the amide π-π^* transition. Taking into account the fact that the splitting of the π-π^* transition occurs with a nonplanar diketopiperazine ring, a planar ring ($\phi=0°$) was considered for *cyclo*(L-Val)$_2$. Under such conditions the rotational strength of the amide π-π^* transition due to the exciton splitting is zero, and the observed Cotton effect of the amide π-π^* transition should have appeared due to an unclarified mechanism. No theoretical support can be provided for this explanation and a further investigation is necessary.

With *cyclo*(L-Val–D-Val) and *cyclo*(L-Leu–D-Leu) no optical activity was observed in 190—300 nm region. As was shown by NMR spectra in $(CD_3)_2SO$ solution, these cyclic dipeptides are considered to assume a symmetric conformation in aqueous solution on the time scale of the CD spectrum. In other words, their diketopiperazine ring should assume either a planar or a chair-like conformation rather than a boat-type conformation.

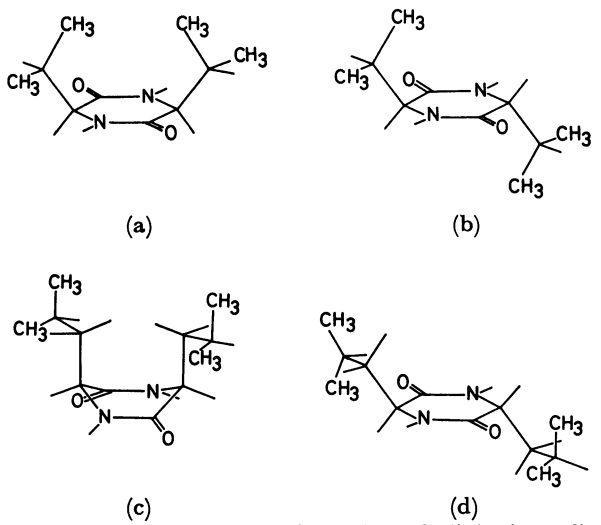

Fig. 5. Most probable conformation of aliphatic cyclic dipeptides in solution. (a), *cyclo*(L-Val)$_2$: (b), *cyclo*(L-Val–D-Val): (c), *cyclo*(L-Leu)$_2$: (d), *cyclo*(L-Leu–D-Leu).

Based on these experimental results, the most probable conformations of the four cyclic dipeptides were considered and are shown in Fig. 5.

Discussion

In the present investigation, conformations of different aliphatic cyclic dipeptides were investigated using ^1H NMR and CD spectroscopy. The same method has been used to determine the solution conformation of *cyclo*(L-Leu–L-His),[5] which was in a good agreement with the crystalline structure investigated by X-ray diffraction analysis.[6] This suggests the validity of the spectroscopy employed in the present investigation to determine the solution conformation of aliphatic cyclic dipeptides.

Vičar, *et al.*[15] investigated the side chain conformation of aliphatic cyclic dipeptide in $(CD_3)_2SO$ by ^{13}C NMR spectroscopy. According to them, *cyclo*(L-Val)$_2$ and *cyclo*(L-Val–D-Val) assume a folded conformation perfectly. On the other hand, the proportions of the folded and the unfolded form of C_2-symmetric *cyclo*(L-Leu)$_2$ were 55 and 45%, respectively. Those of C_2-symmetric *cyclo*(L-Leu–D-Leu) were 50 and 50%, respectively. A folded conformation is in general more important for their results than for the present results. However, both investigations agree in their general aspects.

Exner and Kostelnik[16] investigated the conformation of *cyclo*(L-Leu)$_2$ and *cyclo*(L-Leu–D-Leu) in $(CH_3)_2SO$ or CF_3CO_2H by ^{13}C NMR spectroscopy. They observed that two substituents of *cyclo*(L-Leu)$_2$ were magnetically equivalent and therefore the molecule took a symmetric conformation, which agrees with our conclusion reached by ^1H NMR spectroscopy. While they discussed their experimental results on the assumption that the diketopiperazine ring of *cyclo*(L-Leu)$_2$ takes a bowsprit-boat conformation, they had no experimental evidence to support the assumption. We believe that the conformation of the diketopiperazine ring of *cyclo*(L-Leu)$_2$ is flagpole-boat-type, which is supported by CD spectroscopy. Exner and Kostelnik reported the magnetic nonequivalence of two Leu residues in *cyclo*(L-Leu–D-Leu), which does not agree with our conclusion obtained by ^1H NMR spectroscopy. They synthesized *cyclo*(L-Leu–D-Leu) by the isomerization-cyclodimerization method in which L-leucine was heated at 200 °C in ethylene glycol. Under such drastic conditions, the isomerization of the cyclic dipeptide has been reported.[17,18] Since the isomerization should occur at random, their reaction product might have contained *cyclo*(L-Leu)$_2$ and *cyclo*(D-Leu)$_2$ as well as *cyclo*(L-Leu–D-Leu). This might be a reason for the magnetic nonequivalence observed by Exner and Kostelnik.

According to our experimental results, as shown in Fig. 5, *cyclo*(L-Leu)$_2$ and *cyclo*(L-Val)$_2$ take a flagpole-boat conformation and a planar-axial conformation, respectively. This conclusion differs from previous assumptions that aliphatic cyclic dipeptides in general

take bowsprit-boat conformation.[7] Furthermore, it is somewhat suprising that as far as the side chain conformation is concerned, an attractive force rather than a repulsive force operates between the aliphatic substituents.

The present experimental results can be explained on the basis that the isopropyl groups of *cyclo*(L-Val)$_2$ or the isobutyl groups of *cyclo*(L-Leu)$_2$ solubilized in polar solvents such as water, $(CH_3)_2SO$, and CH_3OH may be stabilized more by solvation of two substituents in a stacked state than by independent solvation of each substituent. In *cyclo*(L-Leu)$_2$ an attractive force between the side chains should be strong enough in polar solvents to confine the solution conformation in that characterized by $\phi > 0°$. This situation has led to a distinct H-C$^{\alpha}$-N-H coupling in NMR spectrum and a distinct splitting of the amide π-π^* transition in the CD spectrum. On the other hand, in *cyclo*(L-Val)$_2$ the conformation of the diketopiperazine ring seems to be planar-axial and to fluctuate around that conformation. This was implied by the facts that the NH signal in 270 MHz spectrum was a broad singlet, and that the splitting of the amide π-π^* transition in the CD spectrum seemed to be absent. However, the attractive force operating between the aliphatic side chains has not been explicitly observed, and a further investigation should be necessary to confirm it.

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