Studies on Cyclic Dipeptides. II.[1] Isomerization among *cyclo*-(-4-Hydroxyprolyl-4-hydroxyprolyl-)s and a Novel Conversion of D-*allo*-Hydroxyproline to L-Hydroxyproline[2]

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(Received March 26, 1974)

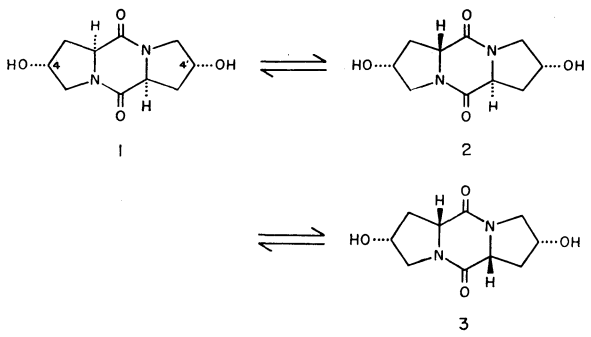
The isomerization among *c*-L-Hyp-L-Hyp (**1**), *c*-L-Hyp-D-*a*Hyp (**2**) and *c*-D-*a*Hyp-D-*a*Hyp (**3**) has been examined in ethanolic sodium ethoxide at 25—78 °C as well as in aqueous solutions at 75—250 °C. At equilibrium state, **1** was predominant and **2** was not found at all in the reaction mixture. By the use of this isomerization reaction and the difference of solubility between **1** and **3**, **3** could be converted almost quantitatively to **1**. The mixture of **1** and **3** was obtained in good yield directly from D-*a*Hyp or L-Hyp by heating in water or ethylene glycol. **1** was hydrolyzed quantitatively to L-Hyp by acid. Thus, L-Hyp was obtained with the yield of 59% from D-*a*Hyp by combination of preparation, isomerization and acid hydrolysis of cyclic dipeptide.

In our previous work,[1] we have determined the thermodynamic constants (K, $\Delta G°$, $\Delta H°$, and $\Delta S°$) of the *cis-trans* isomerization of cyclic dipeptides and found that, for *c*-Pro-Pro type of cyclic dipeptides, *e.g.*, *c*-Pro-Pro, *c*-Hyp-Hyp, only the *cis*-isomer occurs at equilibrium. This finding suggested a new method of obtaining L-Hyp from D-*a*Hyp, which involves an application of the isomerization reaction among *cyclo*(-4-hydroxyprolyl-4-hydroxyprolyl-)s. Our further study conducted on this suggestion is a subject of this paper.

So far, the interconversion between L-Hyp and D-*a*Hyp has been made by three methods: (A) heating an aqueous solution of the amino acid in the presence of excess barium hydroxide at 200 °C for several hours,[3] (B) treating the amino acid with excess acetic anhydride in refluxing glacial acetic acid,[4] and (c) heating an aqueous solution of the amino acid at 200 °C for 2 hr.[5] The method of obtaining L-Hyp from D-*a*Hyp is important to our new process to synthesize L-Hyp from D-Glu.[5] However, the reaction mixture at equilibrium state contained about 50% of L-Hyp in method (A), about 25% of L-Hyp in method (B), and about 55% of L-Hyp in method (C). Thus, it was not efficient to obtain L-Hyp from D-*a*Hyp by these methods. By our present method, on the other hand, the reaction mixture at equilibrium state contains about 97% of L-Hyp, therefore, L-Hyp is very easily obtained from D-*a*Hyp as will be described below.

Results and Discussion

Isomerization among c-L-*Hyp*-L-*Hyp* ***(1)***, c-L-*Hyp*-D-a*Hyp* ***(2)***, *and* c-D-a*Hyp*-D-a*Hyp* ***(3)***. The *cis-trans* isomerization among **1**, **2**, and **3** (Scheme 1) has been examined in 0.1 M ethanolic sodium ethoxide at three temperatures, 25, 50, and 78 °C. A part of the results is shown in Fig. 1. It was found that **2** is very rapidly isomerized to **1** and **3**. For example, when the isomerization was started from **2** at 25 °C, after only two min **2** was not found at all in the reaction mixture any more. Thus, under these conditions, the isomerization among *cis*-isomers (**1** and **3**) and *trans*-isomer (**2**) was actually equivalent to the isomerization between only two *cis*-isomers. The equilibrium constants (K) between **3** and **1** were determined at 25, 50, and 78 °C,



Scheme 1.

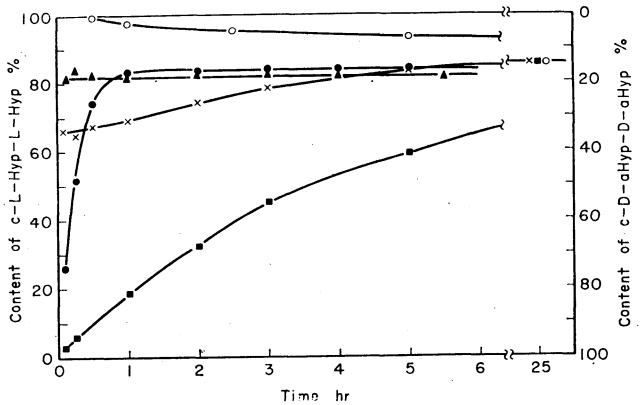


Fig. 1. Contents of *c*-L-Hyp-L-Hyp and *c*-D-*a*Hyp-D-*a*Hyp in the isomerization mixture in 0.1 M ethanolic sodium ethoxide: (○), **1** (starting material), 25 °C (reaction temperature); (×), **2**, 25 °C; (■), **3**, 25 °C; (●), **3**, 50 °C; (▲), **3**, 78 °C.

and then the thermodynamic constants ($\Delta H°$ and $\Delta S°$) of this isomerization were obtained by plotting log K against $1/T$ and by adopting the least-squares method. The results are summarized in Table 1.

Table 1. Thermodynamic constants for the equilibrium, *c*-D-*a*HYP-D-*a*HYP (**3**) ⇌ *c*-L-HYP-L-HYP (**1**)

K_{298}	$= 5.94$ (85.6% of **1**)
K_{323}	$= 5.10$ (83.6% of **1**)
K_{351}	$= 4.43$ (81.6% of **1**)
ΔG_{323}	$= -1.04 \pm 0.01$ kcal/mol
$\Delta H°$	$= -1.15 \pm 0.2$ kcal/mol
$\Delta S°$	$= -0.29 \pm 0.3$ e.u.

The conformation of the diketopiperazine (DKP) ring of **3** is probably similar to that of **1**, which is a boat form with C^{α}–C^{β} bond in the pyrrolidine ring setting at the quasi-equatorial position of the DKP ring.[1,6-8] The enthalpy difference between **1** and **3** is considered to be due to intramolecular dipole-dipole interactions. The vector sum of the dipole moments of two amide groups in the DKP ring is considered to be oriented along the perpendicular direction of the DKP ring. In **1**, both dipole moments of the C–O bonds on the two pyrrolidine rings are directed opposite to that of the amide groups. While, in **3**, the two C–O dipoles are oriented along the same direction with the net dipole of the amide groups.

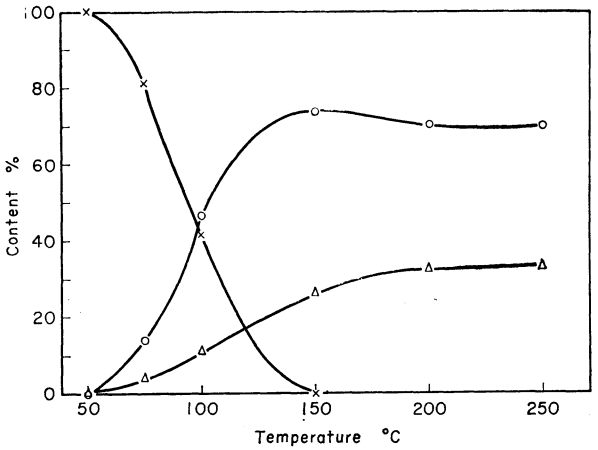


Fig. 2. Contents of *c*-L-Hyp-L-Hyp (○), *c*-L-Hyp-D-*a*Hyp (×), and *c*-D-*a*Hyp-D-*a*Hyp (△), in the isomerization mixture: starting material, 10% aqueous solution of **2**; heating time, 2 hr.

The isomerization experiment was also carried out in an aqueous solution in the temperature range of 75—250 °C. The results of the experiment in which the reaction was started from **2** are shown in Fig. 2. When an aqueous solution of **2** was heated for 2 hr at 150 °C, **2** was completely isomerized into a mixture of 74% of **1** and 26% of **3**. On the other hand, when an aqueous solution of **1** or **3** was heated, **2** was not found at all in the reaction mixture at any temperature. The equilibrium mixture at 250 °C contained 71% of **1** and 29% of **3**.

Almost Quantitative Conversion of 3 to 1. The solubility of **1** in ethanol or water is lower than that of **3**, so **1** and **3** are easily separated by fractional crystallization. Thus almost quantitative conversion of **3** to **1** may be accomplished by letting the isomerization reaction and the fractional crystallization proceed simultaneously in one vessel.

The compound, **3** (181 mg) was heated in 0.1 M ethanolic sodium ethoxide (2 ml) for 15 min at 78 °C, so that it was completely dissolved. The solution was cooled slowly to room temperature for 4 hr under stirring. The crystals thus produced were filtered, washed with absolute ethanol. The mother liquor was immediately neutralized with hydrochloric acid. The contents of **1** and **3** in the crystals, those in the washing solution and those in the mother liquor were analyzed by glc (Expt. No. 1 in Table 2). The crystals were shown to be pure **1** (78% yield). The ratio of **1** to **3** in the mother liquor was found to be equal to the ratio of the equilibrium at room temperature, so the equilibrium between **1** and **3** is considered to be established in the solution before the end of the reaction. As shown in Fig. 1, only about 83% of **3** would be able to be converted to **1** if only the equilibrium reaction between **1** and **3** at room temperature was used. However, 97% of **3** could, in fact, be converted to **1** in our experiment as shown in Table 2 (Expt. No. 1). These results show that the isomerization and the fractional crystallization simultaneously took place in practice.

A mixture of 58% of **3** and 42% of **1** in crystalline states could also be converted to 96% of **1** and 4% of **3** on the whole as shown in Table 2 (Expt. No. 2). During this experiment, which is different from the case of Expt. No. 1, there were always some amount of crystals remaining in the solution.

By this method, any mixture composed of **1**, **2**, and **3** would be converted almost quantitatively to **1** in one vessel.

One-step Preparation of Cyclic Dipeptide from the Amino Acid. A cyclic dipeptide is known to be prepared by heating the corresponding amino acid in ethylene glycol, however, no detailed examination has yet been made on the composition of the product, that is, the

Table 2. Conversion of *c*-D-*a*Hyp-D-*a*Hyp (**3**) to *c*-L-Hyp-L-Hyp (**1**)

Expt. No.	Substrate	Composition of cyclic dipeptide[a]				
			Product			
		Starting material	Crystal	Washing solution	Mother liquor	Total
1[b]	**1** (mg)	0.0	141.9	8.1	25.4	175.4
	3 (mg)	181.0	0.0	0.2	5.2	5.4
	[**1**]/[**3**]	0/100	100/0	98/2	83/17	97/3
2[c]	**1** (g)	2.84	5.10	1.28		6.38
	3 (g)	3.96	0.04	0.21		0.25
	[**1**]/[**3**]	42/58	99/1	86/4		96/4

a) The amounts of cyclic dipeptides were estimated by glc. b) The solution of cyclic dipeptide in 0.1 M ethanolic sodium ethoxide (2 ml) was heated at 78°C for 15 min and cooled to room temperature for about 4 hr. c) The solution of cyclic dipeptides in 0.1 M ethanolic sodium ethoxide (43.6 ml) was treated in a similar manner to Expt. No. 1.

Table 3. One-step preparation of cyclic dipeptides from the corresponding amino acid by heating

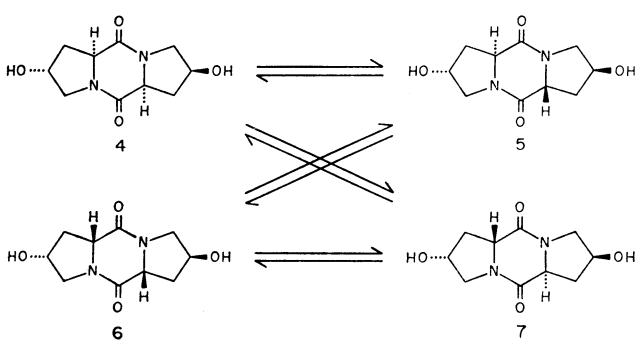
Compd	Reaction condition[a]	Cyclic dipeptides, %[b]		
		Yield	Composition	
L-Hyp	A	88	**1**	70
			3	30
D-*a*Hyp	A	90	**1**	72
			3	28
L-*a*Hyp	A	87	**8**	71
			10	29
DL-Hyp + DL-*a*Hyp[c]	A	84	(**1** + **8**)	35
			(**3** + **10**)	15
			(**4** + **6**)	50
D-*a*Hyp	B	87	**1**	77
			3	23
L-Pro	B	80	(**11** + **12**)	100

a) A: a 20—25% solution of amino acid in water was heated at 260—290°C for 4 hr. B: a 5—10% solution of amino acid in ethylene glycol was refluxed for 7—10 hr. b) The content was estimated by glc. c) A mixture of equal amounts of DL-Hyp and DL-*a*Hyp.

amounts of *cis*- and *trans*-isomers.[9,10] In the course of the present studies, it was found that cyclic dipeptides can be formed even in an aqueous solution (instead of ethylene glycol) directly from the amino acid at high temperature in good yield. We therefore examined, in the next step, the composition of *cis*- and *trans*-isomers in such products. The results are shown in Table 3 for the case of 4-hydroxyproline and proline heated in an aqueous solution or ethylene glycol.

The composition of cyclic dipeptides produced by heating an aqueous solution of L-Hyp or D-*a*Hyp was found to be equal to that in the equilibrium of the isomerization between **1** and **3** at the same temperature. Pure **1** was easily obtained by the recrystallization of these cyclic dipeptides mixture from ethanol or water.

When a mixture of equal amounts of DL-Hyp and DL-*a*Hyp was heated in an aqueous solution, only the *cis*-isomers were formed. This *cis*-isomer mixture contained 50% of racemic modification of **1** and **3**, and 50% of racemic modification of *c*-L-Hyp-L-*a*Hyp (**4**) as shown in Table 3. Therefore, three isomerization reactions, *i.e.*, the reaction shown in Scheme 1, the reaction among *c*-D-Hyp-D-Hyp (**8**), *c*-D-Hyp-L-*a*Hyp (**9**) and *c*-L-*a*Hyp-L-*a*Hyp (**10**) (the enantiomeric reaction of Scheme 1), and the reaction among *c*-L-Hyp-L-*a*Hyp (**4**), *c*-L-Hyp-D-Hyp (**5**), *c*-D-*a*Hyp-D-Hyp (**6**), and *c*-D-*a*Hyp-L-*a*Hyp (**7**) (Scheme 2), occurred and



Scheme 2.

independently reached the equilibrium without mutual intersection.

By refluxing in ethylene glycol, L-Pro was coverted to optically inactive *c*-Pro-Pro,[10] which was found in the present studies to be racemic form, *i.e.*, equimolar mixture of *c*-L-Pro-L-Pro (**11**) and *c*-D-Pro-D-Pro (**12**), but not to be *meso* form, *c*-L-Pro-D-Pro (**13**).

Acid Hydrolysis of Cyclic Dipeptides. In order to obtain L-Hyp from D-*a*Hyp through cyclic dipeptide, **1** should be hydrolyzed into L-Hyp in its last step. Therefore, it is desirable that no racemization takes place in this procedure. It was reported, however, that the amino acids produced by acid hydrolysis of cyclic dipeptides were in general partially racemized.[11,12] We therefore made a slightly more detailed examination on this point.

Table 4. Acid hydrolysis of cyclic dipeptides

Compd	Reaction condition[a]	Amino acid, %		
			Yield[b]	Degree of racemization[c]
1	A	L-Hyp	97.4	2.1
		D-*a*Hyp	2.1	
2	A	L-Hyp	52.6	52.1
		D-*a*Hyp	48.2	
3	A	L-Hyp	3.3	3.3[d]
		D-*a*Hyp	96.4	
11	A	Pro	101.9	3.1[e]
14[f]	B	Ala	99.4	1.9[e]
15[g]	C	Val	69.9	1.2[e]

a) A: a 5% solution of cyclic dipeptide in 6 M hydrochloric acid was heated for 14 hr at 110°C in an open vessel B: a 0.5% solution of cyclic dipeptide in 6 M hydrochloric acid was similarly heated. C: a 0.3% solution of cyclic dipeptide in 6 M hydrochloric acid was similarly heated in a closed vessel. b) The analyses were carried out by amino acid analyzer. c) [D]/[L+D]. d) [L]/[L+D]. e) The analyses were performed by the method of Halpern and Westley (Refs. 15 and 16). f) *c*-L-Ala-L-Ala (**14**). g) *c*-L-Val-L-Val (**15**).

The *c*-Pro-Pro type of cyclic dipeptides were hydrolyzed under acidic condition and then the degree of racemization during hydrolysis was examined. The results are shown in Table 4. When a solution of the cyclic dipeptide in 6 M hydrochloric acid was heated for 14 hr at 110 °C, the degree of racemization was about 2—3%. This value is not so much larger than that found for other peptides.[13,14] Therefore, cyclic dipeptides are not necessarily considered to be easily racemized under acidic condition. Pure L-Hyp was easily obtained by recrystallization of crude L-Hyp produced by hydrolysis of **1**.

Conversion of D-a*Hyp to* L-*Hyp.* On the basis of our examination so far described, it has now been made clear that, by combination of isomerization and hydrolysis of cyclic dipeptide, L-Hyp could be readily obtained from D-*a*Hyp or any mixture of D-*a*Hyp and L-Hyp. Actually L-Hyp was obtained in 59% yield from D-*a*Hyp by the procedures. Here, the preparation of cyclic dipeptides was made in an aqueous solution by heating and the isomerization was made

in ethanolic sodium ethoxide. L-Hyp was also very easily obtained in 50% yield from D-*a*Hyp by combination of preparation of cyclic dipeptides by heating in ethylene glycol and acid hydrolysis of cyclic dipeptides. These procedures have been also applied to the conversion of L-*a*Hyp to D-Hyp with practically the same yield in the conversion of D-*a*Hyp to L-Hyp, as expected.

Experimental

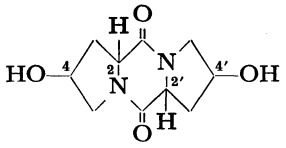
All melting points are uncorrected. IR spectra were recorded in KBr disk on a Jasco Model IR-S Infrared Spectrophotometer. NMR spectra were measured in DMSO-d_6 solution with a Varian Spectrometer T-60 using TMS as internal standard. Mass spectra were obtained using a Hitachi RMU-6E Mass Spectrometer. Optical rotations were measured with a Jasco Model DIP-SL automatic polarimeter. The thin layer chromatography (tlc) was carried out on Merck silica gel 60 using the following solvent systems: (A) chloroform: methanol: acetic acid: water=68: 28: 1: 3; (B) *n*-butanol: acetic acid: water=4: 1: 1. The analyses of amino acid were carried out using a Hitachi KLA-3B Automatic Amino Acid Analyzer (column size, 50×0.9 cm; resin, Hitachi Type 2612; column flow rate, 60 ml/hr; buffer, citrate (0.2 N) pH 3.25; temperature, 55 °C). Glc analyses were performed using a Hewlet Packard 402 High Efficiency Gas Chromatogram equipped with FID and a 4 ft×1/4 in. i.d. glass column packed with 5% QF-1 on Chromosorb G (AW-DMC), 80—100 mesh.

Preparation of Cyclic Dipeptides. The physical constants of *c-Pro-Pro* type of cyclic dipeptides are summarized in Table 5.

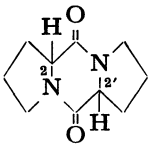
c-L-*Hyp*-L-*Hyp* (**1**) and *c*-L-*Pro*-L-*Pro* (**11**) were prepared by the method of Kapfammer and Matthes.[17] *c*-L-*Pro*-D-*Pro* (**13**) was prepared by the method of Siemion.[6]

c-L-*Hyp*-D-*aHyp* (**2**). A mixture of D-*allo*-hydroxyproline methyl ester hydrochloride (6.90 g, 39.0 mmol) and equimolar triethylamine in 30 ml of chloroform was added at −5 °C with vigorously stirring to a mixture of *N*-carbobenzoxy-L-hydroxyproline (10.1 g, 38.0 mmol), methyl chloroformate (4.10 g, 38.0 mmol) and equimolar triethylamine in 100 ml of chloroform. The mixture was stirred at 0 °C for 4 hr and washed with water, 2 M hydrochloric acid, water, saturated sodium bicarbonate and water. The chloroform solution was dried over sodium sulfate. Evaporation gave *N*-carbobenzoxy-L-hydroxyprolyl-D-*allo*-hydroxyproline methyl ester (4.90 g) in 33% yield as oil. This dipeptide ester was hydrogenated in methanol solution at atmospheric pressure in the presence of 10% Pd-C (50 mg) for 10 hr and the filtrate was evaporated. Water (10 ml) was added to the residue and the aqueous solution was passed through an ion exchange column carrying 5 ml of IR-120 resin (acid form) to remove substrates positive for ninhydrin test. The passed solution was decolorized with active charcoal and evaporated to dryness under

Table 5. Physical constants of *c*-Pro-Pro type of cyclic dipeptides



Compound	Configuration				°C Mp,	$[\alpha]_D^{28}$ (c 1.0, H_2O)	R_t [a] min	R_f [b]	Found (%) [c]		
	C_2	C_2'	C_4	C_4'					C	H	N
c-L-Hyp-L-Hyp [d]	S	S	R	R	248—249	−152.7	16.7	0.71	53.20	6.36	12.35
c-D-Hyp-D-Hyp	R	R	S	S	248—250	+153.9	16.7	0.71	53.09	6.27	12.49
c-D-*a*Hyp-D-*a*Hyp	R	R	R	R	220—221	+ 65.5	14.2	0.59	53.20	6.37	12.60
c-L-Hyp-D-*a*Hyp	R	S	R	R	210—211	− 18.5	18.2	0.63	53.09	6.24	12.28
c-L-Hyp-L-*a*Hyp	S	S	R	S	192—193	−113.3	14.2	0.63	53.27	6.19	12.53
c-L-Hyp-D-Hyp	S	R	R	S	275—276	0	21.2	0.68	52.88	6.23	12.11
c-D-*a*Hyp-L-*a*Hyp	R	S	R	S	240—241	0	16.6	0.59	53.04	6.17	12.38



Compound	Configuration		Mp, °C	$[\alpha]_D^{24}$ (c 1.0, H_2O)	R_t [e] min	R_f [f]	Found (%) [g]		
	C_2	C_2'					C	H	N
c-L-Pro-L-Pro [h]	S	S	143—144	−149.5	9.0	0.62	61.60	7.20	14.36
c-D-Pro-D-Pro	R	R	141—143	+147.5	9.0	0.62	61.68	7.27	14.25
racemi *c*-Pro-Pro	S (R)	S (R)	149—150	0	9.0	0.62	61.60	7.30	14.50
c-L-Pro-D-Pro [i]	S	R	193—195	0	10.2	0.60	62.00	7.23	14.40

a) Retention time of the trimethylsilyl derivative of cyclic dipeptide in glc (220 °C; He gas, 80 ml/min). b) R_f value of tlc using solvent system A. c) Calcd for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38%. d) Mp 245—246 °C, $[\alpha]_D^{19}$ −153.4 °C (c 2.05, H_2O) (Ref. 17); $[\alpha]_D$ −153 ° (Ref. 18). e) Retention time of unmodified cyclic dipeptide in glc (192 °C; He gas, 80 ml/min). f) R_f value of tlc using solvent system B. g) Calcd for $C_{10}H_{14}O_2N_2$: C, 61.83; H, 7.27; N, 14.42%. h) Mp 146 °C, $[\alpha]_D^{19}$ −147.2 ° (c 2.13, H_2O) (Ref. 17); mp 143 °C, $[\alpha]_D^{20}$ −151.15 ° (c 0.867, H_2O) (Ref. 19). i) Mp 179—181 °C (Ref. 6).

reduced pressure. The residue was recrystallized from ethanol–ether to give 1.22 g (5.40 mmol) of **2** in 43% yield from dipeptide ester: IR 3380 (OH), 1655 and 1625 (C=O) cm^{-1}; NMR δ 1.6—2.2 (4H), 3.40 (4H), 4.2—4.5 (4H) and 5.16 (2H).

c-L-*Hyp*-L-*aHyp* (*4*), *c*-L-*Hyp*-D-*Hyp* (*5*) and *c*-D-*aHyp*-L-*aHyp* (*7*) were prepared in a similar manner to that of **2**. **4**: IR 3350 (OH), 1632 and 1645 (C=O) cm^{-1}; NMR δ 1.9—2.4 (4H), 3.39 (4H), 4.2—4.6 (4H) and 5.10 (2H). **5**: IR 3334 (OH) and 1630 (C=O) cm^{-1}; NMR δ 1.8—2.2 (4H), 3.4 (4H), 4.0—4.5 (4H) and 5.22 (2H). **7**: IR 3390 (OH) and 1635 (C=O) cm^{-1}; NMR δ 1.5—2.0 (4H), 3.40 (4H), 4.0—4.6 (4H) and 5.20 (2H).

c-D-*aHyp*-D-*aHyp* (*3*). Thionyl chloride (5.45 g, 45.8 mmol) was added to a solution of D-*aHyp* (5.00 mg, 38.2 mmol) in 50 ml of methanol at —5 °C. The mixture was refluxed for 2 hr and evaporated under reduced pressure. Anhydrous ammonia was bubbled into a chloroform solution (50 ml) of the dried residue at 0 °C. After filtration of ammonium chloride, the filtrate was concentrated below 40 °C under reduced pressure. The resulting syrup was allowed to stand at room temperature for two weeks. Water (10 ml) was added and the aqueous solution was treated with IR-120 resin (acid form) and active charcoal in a similar manner to that of **2**. The residue was recrystallized from ethanol to give 3.67 g (16.2 mmol) of **3** in 85% yield: IR 3380 (OH) and 1645 (C=O) cm^{-1}; NMR δ 2.1—2.5 (4H), 3.35 (4H), 4.2—4.5 (4H) and 5.00 (2H); mass spectrum *m/e* 226 (M^+), 198, 140, 112 and 86. Differences among **1**—**10** were not found in mass spectra.

c-D-*Pro*-D-*Pro* (*12*) was prepared from D-Pro in a similar manner to that of **3**.

Racemic c-Pro-Pro. An equimolar mixture of **11** (0.219 g, 1.13 mmol) and **12** (0.219 g, 1.13 mmol) in ethanol was recrystallized to yield racemic *c*-Pro-Pro (0.277 g, 1.43 mmol, 63%).

Isomerization in 0.1 M Ethanolic Sodium Ethoxide. A solution of *c*-D-*a*Hyp-D-*a*Hyp (**3**) (50.0 mg, 0.221 mmol) in absolute ethanol (3.57 ml) was allowed to stand at 50.0±0.1 °C. The isomerization started immediately after 0.85 ml of 0.520 M ethanolic sodium ethoxide was poured into the solution. After 5 min, 0.44 ml of the solution was taken out and rapidly poured into 0.095 ml of 0.5 M hydrochloric acid. The slightly acidic solution was immediately evaporated to dryness under reduced pressure. The residue was further dried *in vacuo* over potasium hydroxide and was heated with *N,O*-bis(trimethylsilyl)acetamide (0.5 ml) and dry pyridine (0.5 ml) for 5 min at 80 °C. The mixture was injected directly into gas liquid chromatogram. The contents of **1**, **2** and **3** were analyzed. The mixture was composed of 26.1% of **1** and 73.9% of **3**. **2** was not detectable. The samplings were done at different times. The following results were obtained: 52.8% of **1** at 15 min, 74.3% of **1** at 30 min, 83.3% of **1** at 1 hr, 83.6% of **1** at 3 hr, 83.7% of **1** at 4 hr, 83.4% of **1** at 5 hr, and 83.6% of **1** at 8 hr. From the isomerization using **1** as a starting material, the following results were obtained at the same temperature: 83.6% of **1** at 3 hr, 83.7% of **1** at 4 hr, and 83.4% of **1** at 8 hr. The isomerizations were similarly carried out at 25.0±0.1 °C and 78.0±0.1 °C by use of **1**, **2** and **3**. The time required for reaching the equilibrium between **1** and **3** was about 20 hr at 25 °C, 2 hr at 50 °C and 0.5 hr at 78 °C.

Isomerization in Aqueous Solution. A solution of *c*-L-Hyp-D-*a*Hyp (**2**) (50 mg, 0.22 mmol) in water (0.5 ml) was sealed in a small glass tube and heated at 100 °C for 2 hr. The tube was immediately cooled and opened. One-eighth of the resulting clear solution was taken out and evaporated under reduced pressure. The residue was treated in a similar manner to that described in the isomerization in ethanolic sodium ethoxide. The product was composed of 46.8% of **1**, 42.0% of **2** and 11.2% of **3**. In a similar manner, the isomerizations were carried out at 50, 75, 100, 150, 200 and 250 °C by use of **1**, **2** and **3**.

Conversion of c-D-aHyp-D-aHyp (3) to c-L-Hyp-L-Hyp (1). The reaction condition and the results are shown in Table 2. The following results were obtained for the crystals in Table 2, Expt. No. 1: mp 243—245 °C; $[\alpha]_D^{25}$ −153.2° (*c* 1.00, H_2O); Found: C, 53.30; H, 6.32; N, 12.40; Na, 0.041%. Calcd for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38%. The following analytical results were also obtained for the crystals in Table 2, Expt. No. 2: mp 245—247 °C; $[\alpha]_D^{28}$ −150.1° (*c* 1.00, H_2O).

Preparation of c-L-Hyp-L-Hyp (1) from D-aHyp. (*A*) A solution of D-*a*Hyp (15.0 g, 115 mmol) in water (80 ml) was heated at 250—270 °C for 4 hr. The mixture was passed through an ion exchange column carrying 15 ml of IR-120 resin (acid form) to remove substrates positive for ninhydrin test. The passed solution was decolorized with active charcoal and evaporated to dryness under reduced pressure. The residue was further dried *in vacuo* over phosphorus pentoxide. The residue weighed 11.6 g and, by the results of glc analyses, was composed of 72% of **1** and 28% of **3**. A solution of 10.0 g of the cyclic dipeptide mixture thus obtained in 0.1 M ethanolic sodium ethoxide (70.0 ml) was heated by stirring at 78 °C for 30 min. The solution was cooled by gently stirring to room temperature for 4 hr. The crystals thus produced were filtered, washed with cooled absolute ethanol and dried *in vacuo* to give 7.54 g (33.4 mmol) of **1** in 68% yield from D-*a*Hyp: mp 247—249 °C; $[\alpha]_D^{24}$ −152.0° (c 1.00, H_2O); Found: C, 53.08; H, 6.26: N, 12.34%. Calcd for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38%.

(*B*) A solution of D-*a*Hyp (30.0 g, 229 mmol) in ethylene glycol (500 ml) was heated under refluxing for 10 hr. The mixture was evaporated under reduced pressure. Water (150 ml) was poured into the residue. The aqueous solution was similary treated with IR-120 resin and active charcoal, and evaporated. By the results of glc analyses, the residue contained 22.3 g (99 mmol, 87%) of cyclic dipeptides composed of 77% of **1** and 23% of **3**. This residue was recrystallized from water to give 14.8 g (65.5 mmol) of **1** in 57% yield: mp 248—249 °C; $[\alpha]_D^{26}$ −155.3° (c 1.00, H_2O); Found: C, 53.00; H, 6.26; N, 12.41%. Calcd for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38%.

Preparation of c-D-Hyp-D-Hyp (8) from L-aHyp. A solution of L-*a*Hyp (7.85 g, 59.9 mmol) in water (70 ml) was heated at 270—290 °C for 2 hr and was similarly treated with IR-120 resin. By the results of glc analyses, the passed solution contained 5.89 g (26.1 mmol, 87%) of cyclic dipeptides composed of 71% of **8** and 29% of *c*-L-*aHyp*-L-*aHyp* (**10**). The passed solution was decolorized with active charcoal, concentrated and cooled. The crystals thus produced were filtered, washed with cold water and dried *in vacuo* to give 3.68 g (16.3 mmol) of **8** in 54% yield: mp 248—250 °C; $[\alpha]_D^{28}$ +153.9° (c 1.17, H_2O); Found: C, 53.09; H, 6.27; N, 12.49%. Calcd for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38%. IR 3290 (OH), 1655 and 1635 (C=O)cm^{-1}. NMR δ 1.9—2.1 (4H). 3.32 (4H), 4.1—4.7 (4H) and 5.05 (2H).

Hydrolysis of c-L-Hyp-L-Hyp (1). A solution of **1** (5.00 g, 22.1 mmol) in 6 M hydrochloric acid (100 ml) was heated for 14 hr at 110 °C. The contents of amino acids in the mixture were analyzed by automatic amino acid analyzer. L-Hyp (5.65 g, 43.1 mmol, 97.4% yield) and D-*a*Hyp (0.12 g, 0.93 mmol, 2.1% yield) were formed. The mixture was evaporated under reduced pressure. The residue was dissolved in water (30 ml) and the aqueous solution was

applied to an ion exchange column carrying 100 ml of IR-120 resin (acid form). The resin was washed with water and the absorbed amino acids were eluted with 0.2 M ammonium hydroxide. The elute was decolorized with active charcoal and concentrated under reduced pressure. Ethanol was poured into the residue to yield precipitates. The precipitates were filtered, washed with ethanol and dried *in vacuo* to give 5.03 g (38.4 mmol) of L-Hyp in 87% yield: mp 276—277 °C; $[\alpha]_D^{29}$ —76.0° (c 1.01, H_2O); Found: C, 45.92; H, 6.75; N, 10.84%. Calcd for $C_5H_9O_2N$: C, 45.80; H, 6.92; N, 10.68%.

The authors wish to express their thanks to Prof. Masamichi Tsuboi and Prof. Naoki Inamoto in University of Tokyo for their valuable suggestions, and to Dr. Takehiko Ichikawa, Dr. Yuji Iwashita and Dr. Masatsune Kainosho for many helpful discussions.

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