[Chem. Pharm. Bull. 22(9)2136—2141(1974)]

UDC 547.466.1.057

Solid Phase Synthesis of Isariin, a Long Chain β-Hydroxy Acid-containing Cyclodepsipeptide, and Its Diastereoisomer[1,2]

Kozo Okada, Yotaro Kurosawa, and Minoru Hiramoto

Faculty of Pharmaceutical Sciences, Kanazawa University[3]

(Received March 8, 1974)

The method of solid phase synthesis was applied to the preparation of isariin (Ia), a β-hydroxydodecanoic acid-containing cyclodepsipeptide metabolite of *Isaria cretacea*, and its isomer (Ib). The open-chain depsipeptide, H–Val–D–Leu–Ala–Val–DL–Hyd–Gly–OH, was synthesized by coupling successively BOC–Val–DL–Hyd–OH, alanine, and Z–Val–D–Leu–OH to resin-bound glycine using dicyclohexylcarbodiimide (DCC) or DCC+hydroxysuccinimide (HOSu) as coupling agent. After cleavage from the resin the depsipeptide was cyclized by means of DCC+HOSu. The cyclization product was successfully resolved, after chromatographic purification on Dowex 50×4 and Sephadex LH-20 column, by preparative thin-layer chromatography on silica gel to afford Ia and Ib in moderate yields.

In recent year, depsipeptides have been isolated with increasing frequency from various microbial sources. Some of them have received much attention owing to their biological or physico-chemical activities such as antimicrobial or ion transport inducing potency.[4] Although several natural depsipeptides have been synthesized by conventional methods,[4] generally, chemical synthesis of depsipeptide is considerably troublesome. For synthetic work of depsipeptides in relation to their biological functions, the use of solid phase synthesis[5] has been suggested to be advantageous (*e.g.* unprecedented speed).[6] By using this method Semkin, *et al.*[7] were the first who prepared an angiotensin analog and a tetradepsipeptide containing α-hydroxy acids. The antibiotic valinomycin, a cyclic dodecadepsipeptide containing two α-hydroxy acids, was also prepared by two groups[6,8] using a fragment condensation of aminoacylhydroxy acid subunits on the polymer support.

Unfortunately, there is very few information on the solid phase synthesis of depsipeptides except mentioned ones, which contain α-hydroxy acids as hetero component.

---

1) Part of this paper was presented at the 10th Symposium of Peptide Chemistry at Hokkaido University, Sapporo, Japan, 10th, September, 1972.

2) The amino acid residues except glycine are of the L-configuration unless otherwise stated. D-Leucine was purchased from Tanabe Amino Acid Research Foundation, Osaka, Japan. The abbreviations used to denote amino acids, peptides and their derivatives are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature; *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967). Hyd= β-hydroxydodecanoyl, $CH_3(CH_2)_8\underset{O-}{C}H–CH_2–CO–$.

3) Location: *Takara-machi, Kanazawa.*

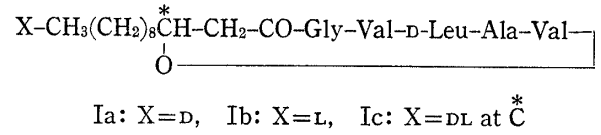
4) M.M. Shemyakin, E.I. Vinogradova, M. Yu. Feigina, N.A. Aldanova, N.F. Loginova, I.D. Ryabova, and I.A. Pavlenko, *Experientia*, **21**, 548 (1965); D.W. Russell, *Quart. Rev.* (London), **20**, 559 (1966); J.S. Davies, "Amino-acids, Peptides, and Proteins," Vol. 3, ed. by G.T. Young, The Chemical Society, London, 1971, pp. 292—302.

5) R.B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); *idem, ibid.*, **86**, 304 (1964); *idem, Science*, **150**, 178 (1965); *idem, Recent Progr. Hormone Res.*, **23**, 451 (1967).

6) B.F. Gisin, R.B. Merrifield, and D.C. Testeson, *J. Am. Chem. Soc.*, **91**, 2691 (1969).

7) E.P. Semkin, A.P. Smirnova, and L.A. Shchukina, *Zh. Obshch. Khim.*, **37**, 1169 (1967); L.A. Shchukina, E.P. Semkin, and A.P. Smirnova, *Khim. Prir.* Soedin., **3**, 358 (1967); *Chem. Abstr.*, **68**, 87567 (1968).

8) G. Loss and H. Klengel, *Tetrahedron*, **27**, 1423 (1971).



Ia: X=D, Ib: X=L, Ic: X=DL at C*

We now describe the application of solid phase method to the synthesis of the β-hydroxy acid-containing depsipeptides, isariin (Ia) (a cyclodepsipeptide metabolite of *Isaria cretacea*[9]) and its diastereoisomer (Ib). The former had been synthesized by an alternative conventional method.[10]

The synthesis was achieved by a combination of the fragment condensation and the stepwise procedure, as outlined in Chart 1.

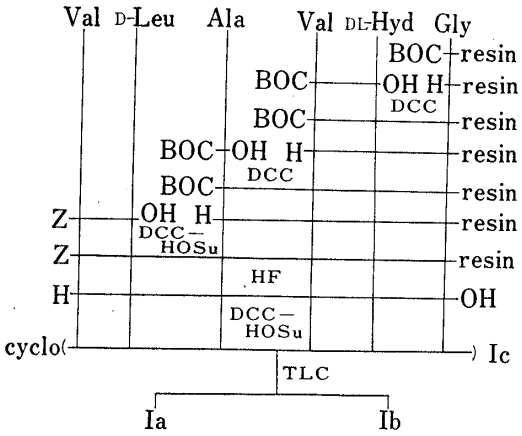


Chart 1. Scheme of the Solid Phase Synthesis of Isariin (Ia) and Its Isomer (Ib)

In our synthetic study, DL-β-hydroxydodecanoic acid was employed without optical resolution, since a preliminary experiment[11] indicated that a diastereoisomeric mixture of the cyclohexadepsipeptide (Ic), product from the racemic hydroxy acid, could be easily resolved by thin-layer chromatography (TLC) over silica gel into the diastereoisomers, Ia and Ib. Taking into account the previous suggestions[12] that both the formation of ester bond on the polymer support and the coupling of valine to a resin-bound peptide gave far less satisfactory result, the two fragments required for the synthesis, Z–Val–D-Leu–OH and BOC–Val–DL-Hyd–OH, were prepared by the conventional methods.

DL-β-Hydroxydodecanoic acid was converted into its benzyl ester by treating in benzyl alcohol with HCl. Esterification of hydroxyl group of the resulting ester with BOC–Val–OH was achieved by use of N,N′-carbonyldiimidazole (CDI)[13] in a good yield. The mixed anhydride method with benzenesulfonyl chloride[14] did not give a detectable amount of the product. BOC–Val–DL-Hyd–OBzl was then debenzylated by catalytic hydrogenolysis. TLC of the product showed that the hydrogenolysis produced, besides the desired BOC–Val–DL-Hyd–OH, a small amount of BOC–Val–OH as a by-product which could be eventually removed from the major product by washing with aqueous bicarbonate. The other fragment Z–Val–D-Leu–OH was prepared by saponification of its methyl ester with standard manner.

The solid phase synthesis of the protected linear hexadepsipeptide having the sequence of isariin was carried out as shown in Chart 1. In order to minimamize racemization in the ultimate cyclization step, glycine was chosen as the C-terminal residue. Thus BOC–Gly–OH was firstly bound to the chloromethylated copolymer of styrene with 2% divinylbenzene in the usual way. The amino acyl resin was treated with 4N HCl in dioxane and then neutralized with triethylamine. The resulting free amino acyl resin was allowed to react with the foregoing didepsipeptide, BOC–Val–DL-Hyd–OH, by use of dicyclohexylcarbodiimide (DCC).

9) L.C. Vining and W.A. Taber, *Can. J. Chem.*, **40**, 1579 (1962); W.A. Wolstenholme and L.C. Vining, *Tetrahedron Letters*, **1966**, 2785.
10) K. Okada, Y. Kurosawa, and M. Hiramoto, *Tetrahedron Letters*, **1972**, 2693.
11) Y. Kurosawa, K. Okada, and M. Hiramoto, Abstracts of 10th Symposium on Peptide Chemistry, Sapporo, Sept. 1972, p. 126.
12) V. Gut and J. Rudinger, "Peptide, 1968," North-Holland Pub. Co., Amsterdam, 1968, p. 185; H. Yajima, H. Kawatani, and H. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **18**, 1279 (1970); Y. Kishida and S. Sakakibara, *Tampaku Kakusan Koso*, **16**, 459 (1971).
13) H.A. Staab and K. Wendel, *Chem. Ber.*, **96**, 3374 (1963).
14) M.M. Shemyakin, N.A. Aldanova, E.I. Vinogradova, and M. Yu. Feigina, *Tetrahedron Letters*, **1963**, 1921; M.M. Shemyakin, Yu. A. Ovchinnikov, V.T. Ivanov, and A.A. Kiryushkin, *Tetrahedron*, **19**, 581 (1963); P. Quitt, R.O. Studer, and K. Vogler, *Helv. Chim. Acta*, **47**, 166 (1964); G. Loss and H. Raue, *Chem. Ber.*, **101**, 1532 (1968).

Table I. *Rf* Values of Depsipeptides on PPC or TLC

Depsipeptides	PPC[a]	TLC		
		Rf_1	Rf_2	Rf_3
H–Val–DL–Hyd–OH	0.28			
H–Val–DL–Hyd–Gly–OH	0.75			
H–Ala–Val–DL–Hyd–Gly–OH				0.56
H–Val–D–Leu–Ala–Val–DL–Hyd–Gly–OH		0.10	0.07	0.84
Ia		0.35	0.44	0.90
Ib		0.20	0.33	0.83

a) solvent system: *n*-butanol–AcOH–H_2O (4: 1: 5, v/v)

Addition of the succeeding BOC–Ala–OH was performed by repeating such cycle (reaction time 2 hr). For the subsequent introduction of the dipeptide Z–Val–D-Leu–OH, the free depsipeptide resin (H–Ala–Val–DL-Hyd–Gly–resin) was treated for 3 hr with 3 equivalents of the dipeptide in the presence of each 3 equivalents of DCC plus N-hydroxysuccinimide (HOSu)[15] which could minimamize possible racemization during carboxyl activation. The resulting peptide resin was again allowed to react with an equimolar mixture of fresh Z–Val–D-Leu–OH, DCC and HOSu in exactly the similar manner as above, since the bulky side chain of leucine is supposed to retard the reaction,[12] moderate introduction of the dipeptide was therefore achieved as a result. After each coupling step a sample of the peptide-resin was cleaved by hydrogen fluoride and the liberated depsipeptide was subjected to TLC or paper chromatography (PPC) and amino acid analysis. All of the three intermediates proved to be practically homogeneous (Table I) and gave theoretical amino acid ratios corresponding to the respective expected intermediates. Finally, the protected linear hexadepsipeptide with isariin sequence was cleaved from the resin by anhydrous hydrogen fluoride.[16] This procedure was found to remove the Z group at the same time but not damage the ester bond in the depsipeptide chain. The product, when purified by passing through a Sephadex LH-20 column, gave the pure linear hexadepsipeptide, H–Val-D-Leu–Ala–Val-DL-Hyd–Gly–OH, in 18% overall yield, based on the starting material BOC–glycyl–resin. It was homogeneous by TLC in different solvent systems, its *Rf* values (Table I) being fully coincident with those of an authentic sample.[11] An acid hydrolysate of the product also gave amino acid ratios in full accord with the theoretical values. Next the linear hexadepsipeptide was subjected to cyclization by the DCC plus HOSu method[17] under high dilution condition. The product (Ic) was purified by successive column chromatographies on Dowex 50×4 and Sephadex LH-20 followed by resolution with preparative TLC over silica gel. The optically pure cyclohexadepsipeptides, Ia and Ib, were obtained as colorless crystals in yields of 28% and 23%, respectively. Each of them was homogeneous by TLC in different solvent systems (Table I), gave expected quantitative amino acid and elemental analysis, and was shown to be identical with the respective authentic samples[10,11] in their mp, mixed mp, optical rotatory dispersion (ORD), infrared and mass spectra, and in chromatographic behaviours.

Depsipeptides containing a β-hydroxy acid are relatively uncommon, to the author's knowledge, there has been very few synthetic work of this kind of depsipeptides, with the two exceptions of serratamolide[18] and isariin, the both of which were prepared *via* the conven-

15) F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch.*, **21b**, 426 (1966); F. Weygand and U. Ragnarsson, *ibid.*, **21b**, 1141 (1966); G.S. Omenn and C.B. Anfinsen, *J. Am. Chem. Soc.*, **90**, 6571 (1968).

16) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965); S. Sakakibara, Y. Kishida, R. Nishizawa, and Y. Shimonishi, *ibid.*, **41**, 438 (1968); J. Leonard and A.B. Robinson, *J. Am. Chem. Soc.*, **89**, 181 (1967).

17) T. Wieland, C. Birr, and F. Flor, *Ann. Chem.*, **727**, 130 (1969).

18) M.M. Shemyakin, Yu. A. Ovchinnikov, V.K. Antonov, A.A. Kiryushkin, V.T. Ivanov, V.I. Shchelokov, and A.M. Shkrob, *Tetrahedron Letters*, **1964**, 47.

tional methods. Therefore, this paper constitutes the first example of the solid phase synthesis of such a depsipeptide. Although overall yields of the synthesized depsipeptides were rather low compared with those reported for peptides by solid phase synthesis, we believe that the application of the principles of this method is quite useful as a rapid and simple way for the preparation of depsipeptide containing a long chain β-hydroxy acid as hetero component.

Experimental[19]

General experimental methods employed are essentially the same as described in a previous paper.[20]

TLC was performed on silica gel (Kieselgel G. Merck). *Rf* values refer to the following solvent systems; Rf_1 $CHCl_3$-MeOH–AcOH (95: 5: 3, v/v), Rf_2 $CHCl_3$–MeOH–pyridine (95: 5: 3, v/v), Rf_3 *n*-butanol–AcOH–H_2O (4: 1: 5, v/v), Rf_4 ether–petr. ether–AcOH (30: 70: 2, v/v).

DL-H-Hyd-OBzl——A solution of DL-β-hydroxydodecanoic acid (2.2 g, 10 mmole) in benzyl alcohol (20 ml) was saturated with dry HCl and allowed to stand overnight at room temperature. After evaporation and addition of CH_2Cl_2 (20 ml), the solution was washed with aqueous sodium bicarbonate, water, dried over $MgSO_4$, and then evaporated. The oily residue was crystallized from petr. ether; yield 2.1 g (68%), mp 20—30°, Rf_4 0.27. IR ν_{max}^{Nujol} cm^{-1}: 1720 (ester carbonyl). This appeared to be homogeneous by TLC and, therefore, was submitted to subsequent synthesis without further purification.

BOC-Val-DL-Hyd-OH——BOC–Val–OH (3.4 g, 15.5 mmole) and CDI (2.7 g, 16 mmole) in CH_2Cl_2 (10 ml) were stirred at 3° for 1 hr according to Staab and Wendel,[14] then DL-H–Hyd–OBzl (2.6 g, 11.9 mmole) was added. The mixture was allowed to react for 2 days and at room temperature for additional 4 days, then poured into ether (70 ml). The solution thereby formed was successively washed with 1N HCl, saturated NaCl, 5% $NaHCO_3$, saturated NaCl, and dried over $MgSO_4$. The solvent was removed under a reduced pressure to yield colorless oil (BOC–Val–DL-Hyd–OBzl) which was homogeneous by TLC (Rf_1 0.87); yield 5.6 g (94%).

The benzyl ester (466 mg, 0.93 mmole) was dissolved in MeOH (8 ml) and hydrogenated for 1 hr in the presence of 10% Pd on charcoal (100 mg). After filtration and evaporation of the solvent the residue was dissolved in ether (10 ml) and washed with 5% $NaHCO_3$, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to yield the N-protected didepsipeptide (319 mg, 83%) as a colorless oil which was homogeneous by TLC (Rf_1 0.60; Rf_2 0.77).

Z-Val-D-Leu-OMe——To a cooled solution (−10°) of D-H–Leu–OMe [prepared from the hydrochloride 1.8 g (10 mmole) and Et_3N 1.4 ml (10 mmole)] in a mixture of dimethylformamide (DMF) (5 ml) and AcOEt (50 ml) was added Z–Val–ONp (3.7 g, 10 mmole). The mixture was stirred at room temperature for 3 days and washed with 1N HCl, 5% $NaHCO_3$, H_2O, and dried over $MgSO_4$, then the solvent evaporated. The crude product obtained as needles was recrystallized from AcOEt–petr. ether; yield 3.2 g (83%), mp 130—131°, $[\alpha]_D^{22}$ +21.8° (c=1.0, EtOH), Rf_2 0.74. *Anal.* Calcd. for $C_{20}H_{30}O_5N_2$: C, 63.47; H, 7.99; N, 7.40. Found: C, 63.45; H, 7.81; N, 7.65.

Z-Val-D-Leu-OH——A solution of Z–Val–D-Leu–OMe (2.0 g, 5.28 mmole) in MeOH (16 ml) was treated with 1N NaOH (6.3 ml) at room temperature for 2 hr. The mixture was acidified with 1N HCl and evaporated *in vacuo* to remove MeOH. The resulting residue was dissolved in AcOEt (50 ml) which was washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent gave an oily residue which was crystallized from AcOEt–petr. ether; yield 3.2 g (67%), mp 135—136°, $[\alpha]_D^{15}$ +4.3° (c=1, MeOH), Rf_1 0.53; Rf_2 0.12. *Anal.* Calcd. for $C_{19}H_{28}O_5N_2$–1/2H_2O: C, 61.11; H, 7.83; N, 7.50. Found: C, 61.69; H, 7.59; N, 7.56.

Esterification of Glycine onto the Resin——BOC–Gly–OH (1.3 g, 7.2 mmole), Et_3N (1.0 ml, 7.2 mmole), and chloromethylated copolystyrene–2% divinylbenzene[21] (3.0 g, chlorine content 0.8 mmole/g[22]) in a mixture of EtOH (8 ml) and $CHCl_3$ (5 ml) were refluxed for 50 hr. The resin was then filtered and washed with MeOH, H_2O, AcOH, and MeOH. Hydrolysis[refluxing for 20 hr in dioxane–12N HCl (1: 1, v/v)] of a part of the resin gave 0.24 mmole/g of glycine (yield 30%).

BOC-Val-DL-Hyd-Gly-Resin——BOC–Gly–resin (2 g, 0.48 mmole) swelled in dioxane was treated with 4N HCl–dioxane (10 ml) at room temperature for 30 min and washed with dioxane and DMF (10 ml × 2 times each). The resin was subsequently treated with Et_3N (2.4 mmole) in DMF (10 ml) for 10 min. After being washed with $CHCl_3$ and CH_2Cl_2 (10 ml × 2 times each), the resin was mixed with a solution of BOC–Val–DL-

19) All melting points were uncorrected.
20) M. Hiramoto, K. Okada, and S. Nagai, *Chem. Pharm. Bull.* (Tokyo), **19**, 1315 (1971).
21) Prepared from the copolystyrene–2% divinylbenzene (100—200 mesh, purchased from Foundation for Protein Research, Osaka) according to Merrifield [R.B. Merrifield, *Biochemistry*, 3, 1385 (1964)].
22) Analyzed by the modified Volhard method (J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman and CO., San Francisco, 1969, p. 55).

Hyd–OH (266 mg, 0.64 mmole) in CH_2Cl_2 (10 ml×2 times), and stirred for 10 min. A solution of DCC (0.13 g, 0.64 mmole) in CH_2Cl_2 (10 ml) was added and the mixture was shaken at room temperature for 3 hr. The resin was washed with CH_2Cl_2 (10 ml×2 times). A part of the dried resin was submitted to acid hydrolysis (110°, 18 hr). Amino acid ratios of Gly and Val were 1.00 and 0.93, respectively. Depsipeptide content calculated from the recovery of Val, taking the recovery of Gly as the standard: 0.22 mmole/g.

BOC-Ala-Val-DL-Hyd-Gly-Resin——BOC–Val–DL-Hyd–Gly–resin (1.9 g, 0.418 mmole) was coupled with BOC–Ala–OH (121 mg, 0.64 mmole) using the essentially same way as described in the preparation of BOC–Val–DL–Hyd–Gly–resin. The average recovery of Gly, Val and Ala in an acid hydrolysate was 0.2 mmole/g (coupling yield: 91%).

H-Val-D-Leu-Ala-Val-DL-Hyd-Gly-OH——A. Z–Val–D-Leu–Ala–Val–DL-Hyd–Gly–Resin: The foregoing resin (1.8 g, 0.36 mmole) was deprotected by treatments with 4N HCl-dioxane (10 ml) and 0.24N Et_3N in $CHCl_3$ (10 ml). The resulting resin, after washing with CH_2Cl_2 (10 ml×3), was soaked for 10 min with a solution of Z–Val–D-Leu–OH (0.33 g, 1.14 mmole) and HOSu (0.13 g, 1.14 mmole) in CH_2Cl_2 (10 ml). A solution of DCC (0.24 g, 1.14 mmole) in CH_2Cl_2 (1.4 ml) was added and the mixture was shaken at room temperature for 3 hr. After the resin was filtered and washed with CH_2Cl_2 (10 ml×2), the resin was again treated in exactly the same procedure with fresh Z–Val–D–Leu–OH (3 equi-moles) plus DCC (3 equi-moles) in the presence of HOSu (3 equi-moles). Then the resin was collected by filtration, washed with CH_2Cl_2 and MeOH (each 10 ml×2) and dried over P_2O_5.

B. H–Val–D-Leu–Ala–DL-Hyd–Gly–OH: Z–Val–D-Leu–Ala–Val–DL-Hyd–Gly–resin was treated with anhydrous HF (about 20 ml) in the presence of anisole (0.5 ml) at 0° for 1 hr. HF was evaporated *in vacuo* and the residue was kept on standing over KOH pellets *in vacuo* overnight, followed by addition of 1% AcOH (20 ml). The resin was removed by filtration and the filtrate was evaporated under reduced pressure to give an amorphous yellowish mass. The crude product thus obtained was dissolved in MeOH (5 ml) and the solution was applied to a Sephadex LH-20 column (2.5×80 cm). The column was developed with MeOH. The fractions containing the desired product was pooled and the solvent was evaporated *in vacuo* to furnish the pure material as a white powder; yield 54 mg (25% from BOC–Ala–Val–DL-Hyd–Gly–resin). Amino acid ratios in an acid hydrolysate: Gly 1.06, Ala 1.03, Leu 1.00, Val 2.00. This product was indistinguishable with a specimen obtained by the HF-treatment of BOC–Val–D-Leu–Ala–Val–DL-Hyd–Gly–OBzl[10] on TLC in different solvent systems (Table I).

Analysis of the Intermediate Depsipeptides——The intermediates were cleaved from small samples of the resin by anhydrous HF, as described above, and were checked for purity by PPC or TLC (Table I) and for constituent amino acids after acid hydrolysis (110°, 18 hr).

Isariin (Ia) and Its Isomer (Ib)——To a solution of the foregoing hexadepsipeptide (422 mg, 0.59 mmole) in DMF (10 ml) were added a solution of HOSu (276 mg, 2.4 mmole) and Et_3N (0.09 ml) in CH_2Cl_2 (300 ml) and the mixture was cooled to 0°. Then a cold solution of DCC (340 mg, 1.3 mmole) in CH_2Cl_2 (5 ml) was added, and the mixture was stirred at 0° for 9 hr and at room temperature for additional 48 hr. The precipitated dicyclohexylurea was removed by filtration and the filtrate was evaporated *in vacuo* and reevaporated twice with addition of AcOEt. The residue was dissolved in AcOEt (60 ml) and washed with 1N HCl, 5% $NaHCO_3$, H_2O, and dried over $MgSO_4$, then evaporated *in vacuo*. The residue was taken up in MeOH (5 ml) and the solution was passed through a column of Dowex 50×4 (H^+ form, 3×4.5 cm) using MeOH–dioxane–H_2O (25:25:10, v/v) (150 ml) as developing solvent. After removal of the solvent from the eluate, the residue was further purified by gel filtration on Sephadex LH-20 (1.5×50 cm) using MeOH as an eluant. The eluates containing the desired material were pooled and the solvent was removed *in vacuo* to yield the cyclization product, cyclo(Val–D-Leu–Ala–Val–DL-Hyd–Gly) (Ic); yield 72 mg (19.2%). The product was dissolved in a small amount of EtOH and subjected to preparative TLC over silica gel with $CHCl_3$–MeOH–pyridine (95:5:3, v/v) as a solvent system (three plates, 20×20 cm, were developed). Zones corresponding to *Rf* 0.44 and *Rf* 0.33 were separated from the chromatograms and extracted with MeOH. The respective extracts were evaporated *in vacuo* to yield crystalline residues, which were recrystallized from EtOH–H_2O to afford Ia (24 mg) and Ib (20 mg) as colorless crystals. Ia: mp 249—251°, $[\alpha]_D^{20}$ −2.7° (c=1.0, MeOH). [Isariin: mp 249—251°, $[\alpha]_D^{20}$ −3.0° (c=1.0, MeOH)]. There was no mixture melting point depression with isariin. ORD[23] (c=0.075, EtOH) $[\alpha]^{20}$ (mμ): 0° (224), −2020° (230), −3030° (240), (trough), −2020° (250), −1280° (260), −105° (400). IR ν_{max}^{KBr} cm^{-1}: 1735 (ester), 1635 (amide I), 1535 (amide II). Mass Spectrum *m/e*: 637 (M^+). *Anal.* Calcd. for $C_{35}H_{59}O_9N_5$: C, 62.11; H, 9.32; N, 10.98. Found: C, 61.81; H, 9.27; N, 10.72. This product gave one spot in three different systems in TLC (for *Rf* values see Table I), which was ninhydrin negative and pale yellow in the iodine–tolidine reaction.[24] The ORD, infrared and mass spectra, and TLC behaviours of this sample were identical with those of the natural specimen. Ib: mp 205—207°, unchanged by admixture with an authentic sample[11] (mp 205—207°) prepared by the conventional method starting from L-β-hydroxydodecanoic acid. A 0.075% solution in EtOH exhibited no optical rotation at a range of 220 mμ to 700 mμ. IR ν_{max}^{KBr} cm^{-1}: 1735 (ester), 1634 (amide I), 1535 (amide II).

23) ORD measurements were carried out with a Jasco spectropolarimeter, model ORD IUV-5.
24) R.H. Mazur, B.W. Ellid, and P.S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).

Mass Spectrum *m/e*: 637 (M^+). *Anal.* Calcd. for $C_{33}H_{59}O_9N_5$: C, 62.11; H, 9.32; N, 10.98. Found: C, 62.00; H, 9.50; N, 10.73. The physical constants, spectroscopic and TLC (for *Rf* values see Table I) behaviours were identical with those of the authentic sample.

Acknowledgement The authors are indebted to Mr. Y. Itatani and Miss T. Tsuji of our faculty for elemental analyses and mass spectral measurements. Thanks are also due to Professor Y. Tsuda of Showa College of Pharmacy for his kind advices.