Fungal Metabolites. IX.[1,2] Synthesis of a Membrane-Modifying Peptide, Hypelcin A-III, from *Hypocrea peltata*

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A membrane-modifying peptide antibiotic having uncoupling activity on rat liver mitochondria, hypelcin A-III, has been synthesized by assembling five peptide fragments *via* the *N,N'*-dicyclohexylcarbodiimide method. The synthesized hypelcin A-III was identical with the natural product.

Keywords *Hypocrea peltata*; peptaibol; hypelcin; α-aminoisobutyric acid; isovaline

Hypelcins A I—IX[1] from *Hypocrea peltata* are peptide antibiotics containing unusual amino acids, α-aminoisobutyric acid (Aib) and/or isovaline (Iva), and having the N- and C-terminal residues protected by an acetyl group and an amino alcohol, respectively. They belong to the class of membrane-modifying peptides named peptaibols, like alamethicin.[3] The uncoupling activity of hypelcins A on rat liver mitochondria[4] presumably results from ion influx through the modified biomembrane, suggesting that the peptides may be useful in studies of biomembrane functions. In view of the low abundance of these compounds in the natural source, we have developed a total synthesis of hypelcin A-III, one of the major components of hypelcins A, with the aim of making available a sufficient amount for further investigation of the biological activities.

Results and Discussion

Hypelcin A-III[1] has the following primary structure: Ac–Aib–Pro–Aib–Ala–Aib–Aib–Gln–Aib–Leu–Aib–Gly–Aib–Aib–Pro–Val–Aib–D-Iva–Gln–Gln–Lol. The optically active amino acids are all of L-form except for Iva. Due to the steric hindrance of the unusual amino acids, Aib and Iva, solution-phase synthesis is the method of choice[5] for the synthesis of hypelcin A-III. The synthetic strategy was based on our previous work on the synthesis of trichosporin B—V,[6] and is summarized in Fig. 1. Fragments [**2**]—[**5**] were designed such that Aib or Iva was placed at the C-terminal in order to avoid racemization during activation and deprotection.

For protection of all N-terminals, the Z group was used, except for the N-terminal fragment [**5**] and the C-terminal fragment [**1**]. In the case of fragment [**1**], Z–Gln–Gln–Lol was insoluble in methanol and gave undesired products during deprotection, so the Boc group was used for protection. Fragments [**2**], [**3**], [**4**] and [**5**] were synthesized by using the DCC–HOBt method as shown in Fig. 2. In the synthesis of fragment [**2**], racemic Iva prepared according to the reported method[7] was used. No fragment containing racemic Iva could be optically resolved, even on HPLC.

Fragments [**2**]—[**5**] were condensed successively according to the route shown in Fig. 1. All fragment condensation steps were carried out by the DCC–HOBt procedure in DMF at room temperature. The condensation of the amine component from fragment [**1**] with the peptide acid from fragment [**2**] gave the C-terminal heptapeptide (positions

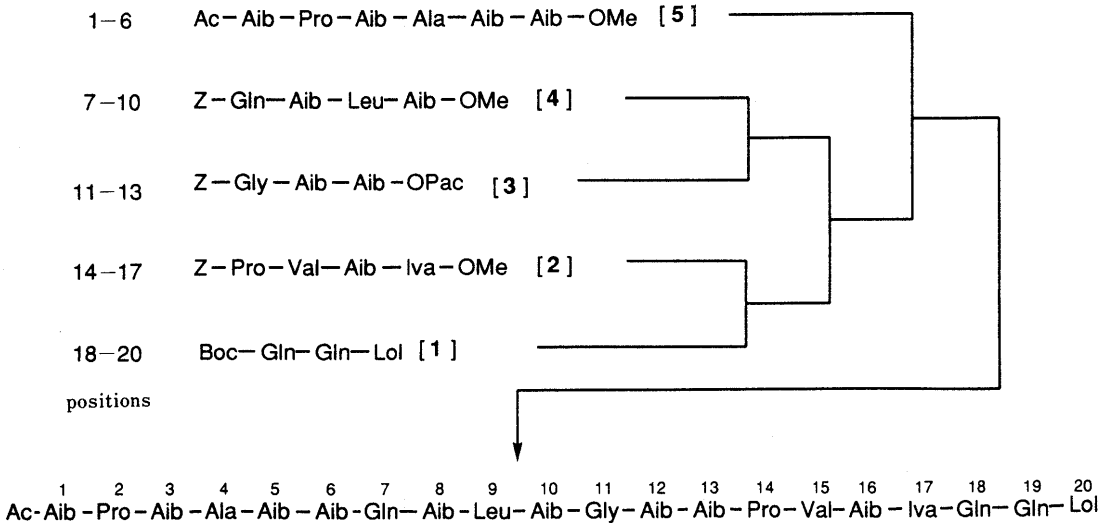

Fig. 1. Synthetic Route to [DL-Iva[17]]Hypelcin A-III

This paper is dedicated to Professor Yoshifumi Maki on the occasion of his retirement from Gifu Pharmaceutical Unversity in March 1994.

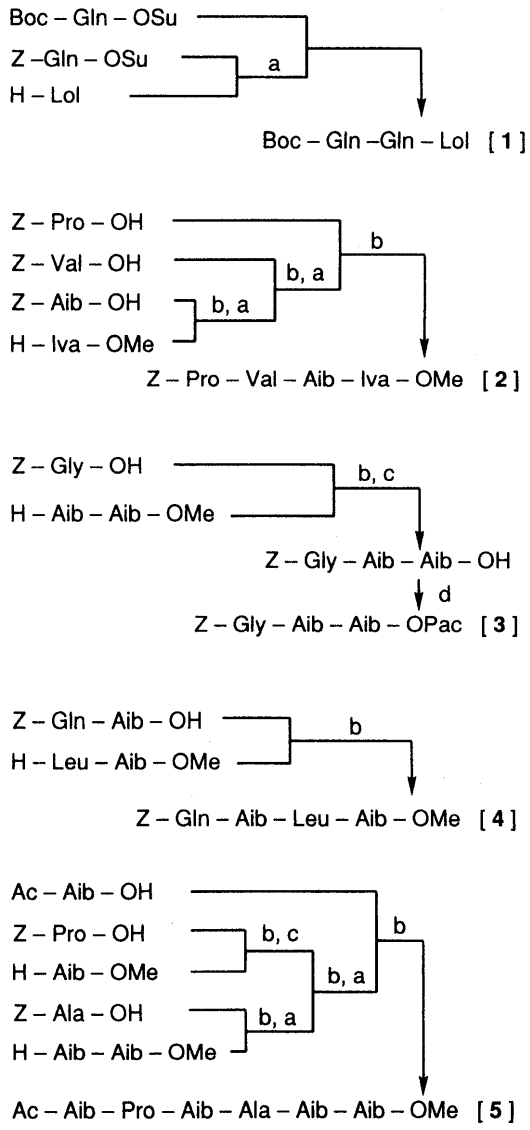

Fig. 2. Synthetic Schemes for Fragments [1]—[5]
Reagents: a, H_2/Pd–C; b, DCC–HOBt; c, 1 N NaOH; d, PacBr.

14—20). The amino-protecting group, Z, was removed by hydrogenation over palladium-on-charcoal to give the deprotected heptapeptide. The heptapeptide (positions 7—13) located in the middle of the hypelcin A-III molecule was obtained by condensation of the peptide derivatives from fragments [3] (positions 11—13) and [4] (positions 7—10). In a previous study, we found that the methyl ester, Z–(7—13)–OMe, did not give a good results when the sample was hydrolyzed with alkali.[6] The phenacyl group of the heptapeptide (positions 7—13) was removed by Zn powder in AcOH to give the heptapeptide acid in satisfactory yield. The tetradecapeptide (positions 7—20) was obtained by the condensation of the heptapeptide acid (positions 7—13) with the C-terminal amine component (positions 14—20). The yield of this coupling reaction was low (34.5%) because the acid was insufficiently activated. The N-terminal hexapeptide acid, fragment [5] (positions 1—6), and the above amine component (positions 7—20) were coupled to give [DL-Iva[17]]hypelcin A-III.

The molecular weight and the amino acid sequence of the [DL-Iva[17]] hypelcin A-III were confirmed by ion spray mass spectrometry (ISP-MS) and mass spectrometry/mass spectrometry (MS/MS) (Fig. 3). The acid hydrolysate of the synthetic product revealed the presence of D- and L-IVa (1 : 1) on HPLC using a chiral column. The mixture of D- and L-Iva[17] hypelcin A-III showed one peak on analytical HPLC (Fig. 4), while on recycle HPLC using an ODS column, the peak was divided into two parts. The ratio of D- and L-Iva in the first part was *ca.* 5 : 1. The fraction was repeatedly chromatographed and divided in the same manner to give pure [D-Iva[17]]hypelcin A-III. The ^1H and ^{13}C resonances of the synthetic [D-Iva[17]]hypelcin A-III were in good agreement with those of the natural hypelcin A-III (Fig. 5a and b). On the other hand, the resonances of the purified synthetic [L-Iva[17]]hypelcin A-III were different from those of [D-Iva[17]]hypelcin A-III (Fig. 5c). An examination of the biological activities of both isomers is in progress.

Experimental

General Methods All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. ^1H- and ^{13}C-NMR spectra were recorded on JEOL JNM-FX200, Bruker AC-300, and Bruker AM-600 spectrometers. Samples were dissolved in $CDCl_3$, CD_3OD, or CD_3OH containing tetramethylsilane as an internal standard. For brevity, assignments of individual resonances are omitted. EI-MS was performed on a JEOL 01-SG. FAB-MS was performed on a JEOL MS-DX-300. ISP-MS was performed on an API III (Perkin Elmer Sciex). Samples were dissolved in acetonitrile–H_2O (1 : 1) containing 1% TFA. MS/MS experiments were carried out by collision-induced dissociation. Argon atoms were used as the collision gas for MS/MS. TLC was performed on silica gel (Kieselgel $60F_{254}$, Merck). The *Rf* values refer to the following solvent systems (v/v): Rf_1 = $CHCl_3$–MeOH (95 : 5), Rf_2 = $CHCl_3$–MeOH (9 : 1), Rf_3 = $CHCl_3$–MeOH (8 : 2), Rf_4 = $CHCl_3$–MeOH–H_2O (5 : 4 : 1). For column chromatography on silica gel, Silica gel 60 (70—230 mesh, Merck) and for gel filtration, Sephadex LH-20 (Pharmacia) were used. Analytical and preparative HPLC procedures were performed on a Shimadzu LC-6A system, using the solvent system acetonitrile–H_2O (49 : 51). Recycle HPLC was performed on a Shimadzu LC-8A system. Amino acid analyses were done with a Hitachi model 835 amino acid analyzer.

Coupling Reactions Unless otherwise stated, coupling reactions were performed by the DCC–HOBt method at room temperature for 24—72 h and the mixtures were worked up according to procedure A or B after removal of DCU and the solvent.

Procedure A: EtOAc-soluble protected peptides were each dissolved in EtOAc and the solution was washed successively with 1 N HCl, 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4 and concentrated. The residue was usually recrystallized or precipitated from appropriate solvents.

Procedure B: EtOAc-insoluble protected peptides were purified by gel-filtration of Sephadex LH-20 in MeOH.

Hydrolysis of Z-Peptide Methyl Esters (Procedure C) Z-Peptide methyl esters were hydrolyzed with 1 N NaOH (2—3 eq) in MeOH below 35 °C. After complete saponification, the solution was neutralized with 1 N HCl and evaporated to remove MeOH. The residual solution was acidified to pH 3 and extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na_2SO_4 and concentrated. The residue was usually employed in the following step without further purification.

Catalytic Hydrogenation (Procedure D) The benzyloxycarbonyl group, Z, was removed by the use of H_2 gas over 10% palladium-on-charcoal with stirring. After removal of the catalyst by filtration, the filtrate was concentrated and used in the next step without further purification.

Z–Gln–Lol Z–Gln–OSu (11.35 g, 30.0 mmol) was added to a solution of Lol (3.52 g, 1 eq) in DMF (150 ml), and the solution was stirred at room temperature for 30 h. After evaporation of the solvent, the residue was dissolved in a small amount of methanol and precipitated with excess water, followed by washing with water to give the pure Z–Gln–Lol; yield 6.76 g (59.4%), mp 151—154 °C, $[\alpha]_D^{24} = -25.6°$ ($c = 0.67$, MeOH), Rf_3 0.44. EI-MS *m/z*: 379 (M^+), 263 ($M^+ - Lol$), 235 (263 – CO), 107 (235 – $C_5H_8N_2O_2$), 91 (C_7H_7). *Anal.* Calcd for $C_{19}H_{29}N_3O_5 \cdot 1/2H_2O$: C, 58.75; H, 7.78; N, 10.82. Found: C, 58.96; H, 7.76; N, 10.94.

H–Gln–Lol Z–Gln–Lol (6.66 g, 17.5 mmol) in MeOH (50 ml) was hydrogenated for 2 h according to precedure D to give H–Gln–Lol; yield 4.30 g (99.9%), mp 135—138 °C, Rf_3 0.34.

Boc–Gln–Gln–Lol [1] Boc–Gln–OSu (2.33 g, 9.49 mmol) added to a

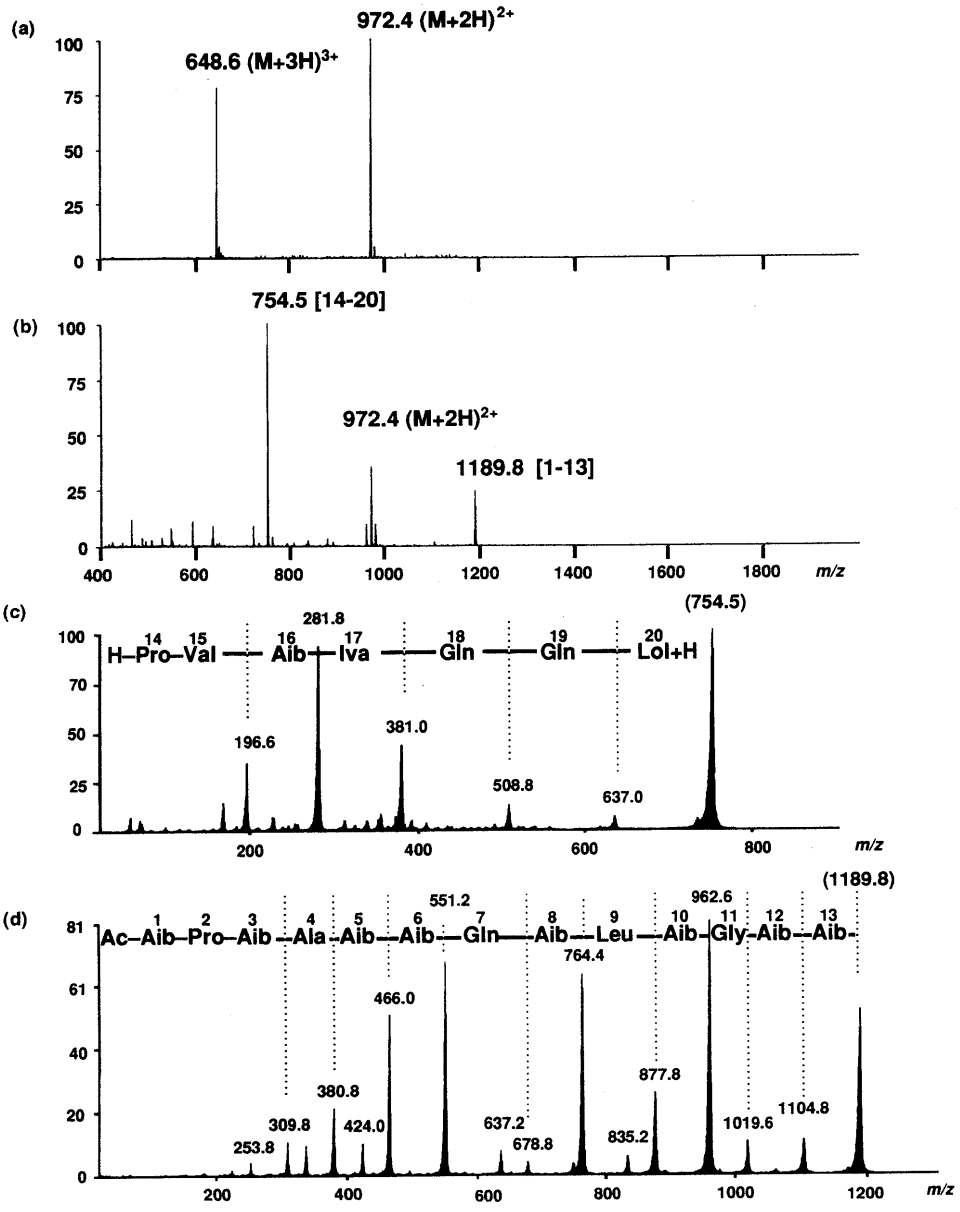

Fig. 3. ISP-MS and MS/MS of the [DL-Iva[17]]Hypelcin A-III

(a) At 60 V orifice voltage, 2- and 3-fold charged molecular ions were found at *m/z* 972.5 and *m/z* 648.6, respectively. The molecular mass, 1942.8, estimated from these ions was in agreement with that of the natural product ($C_{90}H_{155}N_{23}O_{24}$: calculated average mass; 1943.37). (b) At 120 V orifice voltage, two ions at *m/z* 754.5 and *m/z* 1189.8 were observed. These ions arise from the C-terminal (positions 14—20) and N-terminal (positions 1—13) fragments, respectively. (c) and (d) MS/MS spectra of *m/z* 754.5 and *m/z* 1189.8, respectively.

solution of H–Gln–Lol (2.31 g, 1 eq) in DMF (150 ml), and the solution was stirred at room temperature for 12 h. The solvent was removed by evaporation, and the residue was treated with MeOH–H_2O. The filtrate was evaporated to dryness and purified by Sephadex LH-20; yield 2.52 g (56%), mp 203—205 °C, $[\alpha]_D^{26} = -37.8°$ ($c = 1.0$, MeOH), Rf_3 0.55. EI-MS *m/z*: 474 (MH^+), 443 ($MH^+ - CH_2OH$), 357 ($M^+ - Lol$), 257 ([357 − Boc] + H), 229 (357 − Gln), 101 (Boc). *Anal.* Calcd for $C_{21}H_{39}N_5O_7 \cdot H_2O$: C, 51.31; H, 8.41; N, 14.25. Found: C, 51.30; H, 8.35; N, 14.23.

H–Gln–Gln–Lol Boc–Gln–Gln–Lol (484 mg, 1.02 mmol) was treated with anisole (0.27 ml, 2.3 eq) for 6 min and then TFA (1.04 ml, 1.7 eq) was added at 0 °C. The mixture was stirred for 2 h and treated with dry ether. The resulting precipitate was collected by filtration, dissolved in MeOH and treated with Amberlite IRA-400 to give the deprotected tetrapeptide H–Gln–Gln–Lol, which was further purified by recrystallization from MeOH; yield 321.3 mg (84.3%), Rf_3 0.28, mp 174—176 °C.

Z–Aib–DL-Iva–OMe Z–Aib–OH (43.18 g, 182 mmol), HOBt (24.5 g, 1 eq) and DCC (37.55 g, 1 eq) were added successively to a solution of HCl·H–DL-Iva–OMe (30.48 g, 1 eq) in DMF (200 ml) containing TEA (24.5 ml, 1 eq) with stirring. After 48 h, the solution was worked up according to procedure A and the residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 9 : 1); yield 55.97 g (93%), mp 63—64 °C, Rf_2 0.79. EI-MS *m/z* 350 (M^+), 291 ($M^+ - COOCH_3$), 259 ($M^+ - C_7H_7$), 192 ($291 - C_5H_9NO$), 91 (C_7H_7). *Anal.* Calcd for $C_{18}H_{26}N_2O_5$: C, 61.7; H, 7.48; N, 7.99. Found: C, 61.4; H, 7.62; N, 7.84.

HCl·H–Aib–DL-Iva–OMe Z–Aib–DL-Iva–OMe (1.00 g, 2.90 mmol) in 90% MeOH (15 ml) containing 1 N HCl (2.9 ml, 1 eq) was hydrogenated according to procedure D to give HCl·H–Aib–DL-Iva–OMe; yield 721 mg (98%), Rf_2 0.30.

Z–Val–Aib–DL-Iva–OMe Z–Val–OH (20.81 g, 82.8 mmol), HOBt (11.19 g, 1 eq), and DCC (17.08 g, 1 eq) were added successively to a stirred solution of HCl·H–Aib–DL-Iva–OMe (20.93 g, 1 eq) in DMF (140 ml) containing TEA (11.47 ml, 1 eq). After 48 h, the solution was worked up according to procedure A. The residue was triturated with petroleum ether to give Z–Val–Aib–DL-Iva–OMe; yield 27.99 g (75%), mp 100—102 °C, $[\alpha]_D^{26} = -3.6°$ ($c = 1.0$, MeOH), Rf_2 0.76. EI-MS *m/z*: 449 (M^+), 418 ($M^+ - OCH_3$), 319 (418 − Iva), 291 (319 − CO), 234 (319 − Aib), 206 (234 − CO), 91 (C_7H_7). *Anal.* Calcd for $C_{23}H_{35}N_3O_6$: C, 61.45; H, 7.85; N, 9.35. Found: C, 61.35; H, 7.81; N, 9.23.

HCl·H–Val–Aib–DL-Iva–OMe Z–Val–Aib–DL-Iva–OMe (10.0 g, 22.3

mmol) in MeOH (100 ml) containing 1 N HCl (23 ml, 1 eq) was hydrogenated according to precedure D to give HCl·H–Val–Aib–DL-Iva–OMe; yield 7.27 g (93.0%), Rf_2 0.27.

Z–Pro–Val–Aib–DL-Iva–OMe [2] Z–Pro–OH (6.17 g, 24.8 mmol), HOBt (3.35 g, 1 eq) and DCC (5.11 g, 1 eq) were added successively to a stirred solution of HCl·H–Val–Aib–DL-Iva–OMe (8.70 g, 1 eq) in DMF (70 ml) containing TEA (3.43 ml, 1 eq). After 48 h, the solution was worked up according to precedure A, to give the crude tetrapeptide, which was purified by silica gel column chromatography ($CHCl_3$: MeOH = 95 : 5); yield 10.93 g (79%), mp 50—51 °C, $[\alpha]_D^{26} = -49.1°$ ($c = 1$, EtOH), Rf_1 0.50. EI-MS m/z: 546 (M^+), 515 ($M^+ - OCH_3$), 416 (515 − Iva), 331 (416 − Aib), 232 (331 − Val). *Anal.* Calcd for $C_{28}H_{42}O_7N_4 \cdot 1/2H_2O$: C, 60.52; H, 7.80; N, 10.08. Found: C, 60.78; H, 7.84; N, 9.93.

Z–Pro–Val–Aib–DL-Iva–OH The above protected tetrapeptide [2] (1.01 g, 1.90 mmol) in MeOH (10 ml) was saponified according to procedure C to give the tetrapeptide acid; yield 0.79 g (77%), mp 93—95 °C, Rf_3 0.36.

Z–Gly–Aib–Aib–OMe Z–Gly–OH (4.60 g, 22.0 mmol), HOBt (2.97 g, 1 eq), and DCC (4.54 g, 1 eq) were added successively to a stirred solution of HCl·H–Aib–Aib–OMe[6] (5.24 g, 1 eq) in DMF (50 ml) containing TEA (3 ml, 1 eq). After 48 h, the solution was worked up according to procedure A. The residue was recrystallized from EtOAc to give Z–Gly–Aib–Aib–OMe; yield 7.62 g (91.4%), mp 149—151 °C, Rf_2 0.32. EI-MS m/z: 393 (M^+), 362 ($M^+ - OCH_3$), 334 (362 − CO), 249 (334 − C_4H_7NO), 91 (C_7H_7). *Anal.* Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 58.02; H, 7.01; N, 10.65.

Z–Gly–Aib–Aib–OH Z–Gly–Aib–Aib–OMe (5.59 g, 14.2 mmol) was dissolved in MeOH (60 ml) and saponified as described in procedure C. The residue was recrystallized from EtOAc to give Z–Gly–Aib–Aib–OH; yield 4.27 g (76.4%), mp 210—211 °C, Rf_4 0.43.

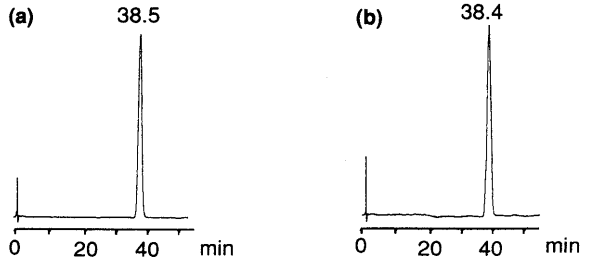

Fig. 4. Analytical HPLC Chromatograms of the Natural Hypelcin A-III (a) and the Synthetic [DL-Iva[17]]Hypelcin A-III (b)

Conditions: mobile phase, acetonitrile–H_2O (49 : 51, v/v); flow rate, 1 ml/min; detector, UV (210 nm); column, Cosmosil 5C18 (4.6 mm i.d. × 150 mm); column temperature, 40 °C.

Z–Gly–Aib–Aib–OPac [3] The above tripeptide acid (4.27 g, 11.3 mmol) and phenacyl bromide (2.24 g, 1 eq) were dissolved in DMF (45 ml), and TEA (1.55 ml, 1 eq) was added with stirring. After 48 h, the solvent was evaporated and the residue was recrystallized from MeOH to give the pure Z–Gly–Aib–Aib–OPac [3]; yield 4.06 g (72.5%), mp 170—172 °C, Rf_2 0.43. EI-MS m/z: 497 (M^+), 362 ($M^+ - OPac$), 334 (362 − CO), 277 (362 − Aib), 249 (277 − CO), 91 (C_7H_7). *Anal.* Calcd for $C_{26}H_{31}N_3O_7$: C, 62.77; H, 6.28; N, 8.45. Found: C, 62.43; H, 6.32; N, 8.64.

HBr·H–Gly–Aib–Aib–OPac Z–Gly–Aib–Aib–OPac [3] (3.6 g, 7.24 mmol) was treated with 30% HBr in AcOH (10 ml) with stirring. After 1.5 h, dry ether was added and the resulting precipitate was filtered off, washed with dry ether and dried over KOH *in vacuo* to give HBr·H–Gly–Aib–Aib–OPac; yield 2.63 g (83.3%), Rf_4 0.44.

Z–Gln–Aib–Leu–Aib–OMe [4] Z–Gln–Aib–OH[6] (4.89 g, 13.38 mmol), HOBt (1.93 g, 1 eq) and DCC (2.80 g, 1 eq) were added successively to a solution of HCl·H–Leu–Aib–OMe[6] (3.56 g, 1 eq) in DMF (20 ml) containing TEA (1.9 ml, 1 eq), and the solution was stirred for 72 h. After filtration to remove DCU, the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 9 : 1); yield 5.86 g (77.7%), mp 80.5—82.5 °C, $[\alpha]_D^{25} = -21.9°$ ($c = 1$, MeOH), Rf_2 0.55. EI-MS m/z: 577 (M^+), 546 (577 − OCH_3), 461 (546 − Aib), 433 (461 − CO), 348 (461 − Leu), 320 (348 − CO), 263 (348 − Aib), 91 (C_7H_7). *Anal.* Calcd for $C_{28}H_{43}N_5O_8$: C, 58.22; H, 7.50; N, 12.10. Found: C, 57.98; H, 7.71; N, 12.10.

Z–Gln–Aib–Leu–Aib–OH The above protected tetrapeptide [4] (3.80 g, 6.58 mmol) was saponified in MeOH (40 ml) according to procedure C, to give the tetrapeptide acid; yield 3.71 g (82.5%), mp 98—100 °C, Rf_4 0.43.

Z–Pro–Aib–OMe Z–Pro–OH (2.49 g, 10.0 mmol), HOBt (1.35 g, 1 eq) and DCC (2.06 g, 1 eq) were added successively to a solution of HCl·H–Aib–OMe (1.40 g, 1 eq) in DMF (15 ml) containing TEA (1.39 ml, 1 eq) with stirring. After 48 h, the solution was worked up according to procedure A and the residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5); yield 2.34 g (82.0%), mp 85—87 °C, $[\alpha]_D^{25} = -64.6°$ ($c = 1$, MeOH), Rf_1 0.42. EI-MS m/z: 348 (M^+), 317 ($M^+ - OCH_3$), 289 (317 − CO), 232 (317 − Aib), 204 (232 − CO), 91 (C_7H_7). *Anal.* Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.01; H, 7.09; N, 7.96.

Z–Pro–Aib–OH Z–Pro–Aib–OMe (2.94 g, 8.44 mmol) was saponified in MeOH (40 ml) according to procedure C, to afford pure Z–Pro–Aib–OH; yield 2.23 g (79.1%), mp 112—115 °C, Rf_3 0.44.

Z–Pro–Aib–Ala–Aib–Aib–OMe Z–Pro–Aib–OH (2.23 g, 6.67 mmol), HOBt (0.90 g, 1 eq) and DCC were added successively to a solution of H–Ala–Aib–Aib–OMe[6] (1.82 g, 1 eq) in DMF (30 ml). The mixture was stirred for 72 h, filtered to remove DCU, and evaporated to dryness. The residue was triturated with EtOAc and washed with the same solvent. The

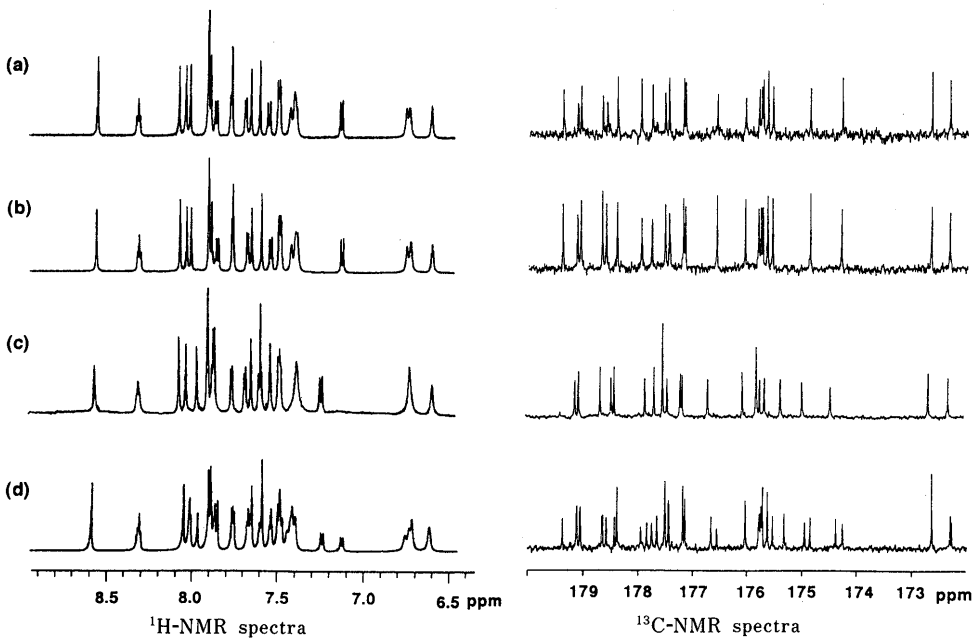

Fig. 5. Amide and Carbonyl Regions of the ^1H- and ^{13}C-NMR Spectra of the Natural and Synthetic Hypelcin A-III Measured in CD_3OH at 27 °C

(a) Natural hypelcin A-III. (b) [D-Iva[17]]Hypelcin A-III. (c) [L-Iva[17]]Hypelcin A-III. (d) [DL-Iva[17]]Hypelcin A-III.

resulting precipitate was collected by filtration, dissolved in MeOH and treated with Amberlite IRA-400. The residue was recrystallized from MeOH to give the protected pentapeptide; yield 1.92 g (48.9%), mp 224—226 °C, $[\alpha]_D^{20} = -11.8°$ ($c=1$, MeOH), Rf_1 0.44. EI-MS m/z: 589 (M^+), 558 ($M^+ - OCH_3$), 473 (558 − Aib), 445 (473 − CO), 388 (473 − Aib), 317 (388 − Ala), 289 (317 − CO), 232 (317 − Aib), 91 (C_7H_7), 70 (C_4H_8N). *Anal.* Calcd for $C_{29}H_{43}N_5O_8 \cdot 1/2H_2O$: C, 58.23; H, 7.33; N, 11.71. Found: C, 58.43; H, 7.33; N, 11.83.

H–Pro–Aib–Ala–Aib–Aib–OMe The above protected pentapeptide (1.21 g, 2.05 mmol) in MeOH (50 ml) was hydrogenated according to procedure D to give the deprotected pentapeptide ester; yield 1.16 g (95.9%), Rf_4 0.3.

Ac–Aib–Pro–Aib–Ala–Aib–Aib–OMe [5] Ac–Aib–OH was prepared by refluxing H–Aib–OH and Ac_2O in AcOH. Ac–Aib–OH (92 mg, 0.63 mmol), HOBt (85 mg, 1 eq) and DCC (130 mg, 1 eq) were added successively to a solution of H–Pro–Aib–Ala–Aib–Aib–OMe (287 mg, 1 eq) in DMF (10 ml). The mixture was stirred for 72 h, filtered to remove DCU and evaporated to dryness. The residue was dissolved in MeOH and treated with Amberlite IR-120 and Amberlite IRA-400. The solvent was evaporated off *in vacuo* and the residue was recrystallized from MeOH and ether; yield 328.7 mg (89.8%), mp 108—111 °C, $[\alpha]_D^{25} = 16.4°$ ($c=1$, MeOH), Rf_3 0.52. EI-MS m/z: 583 (MH^+), 551 ($M^+ - OCH_3$), 466 (551 − Aib), 438 (466 − CO), 381 (466 − Aib), 339 (H–Pro–Aib–Ala–Aib), 310 (381 − Ala), 254 (H–Pro–Aib–Ala), 225 (310 − Aib), 183 (H–Pro–Aib), 128 (Ac–Aib), 70 (C_4H_8N). *Anal.* Calcd for $C_{27}H_{46}N_6O_8$: C, 55.65; H, 7.96; N, 14.42. Found: C, 55.81; H, 8.23; N, 14.26.

Ac–Aib–Pro–Aib–Ala–Aib–Aib–OH The above protected hexapeptide [5] (150 mg, 0.26 mmol) was saponified and the solution was neutralized with Amberlite IR-120. The solvent was evaporated off *in vacuo* to give the pure hexapeptide acid; yield 143.3 mg (93.2%), mp 246—249 °C, Rf_4 0.47.

Z–Pro–Val–Aib–DL-Iva–Gln–Gln–Lol The tetrapeptide acid (194 mg, 0.36 mmol) obtained by deprotection of fragment [2], HOBt (49.2 mg, 1.1 eq), and DCC (75.2 mg, 1.1 eq) were dissolved successively in a solution of H–Gln–Gln–Lol (136 mg, 1 eq) in DMF (5 ml). After stirring for 24 h, the solution was worked up according to procedure B. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 9 : 1), to give the heptapeptide (positions 14—20); yield 180.2 mg (61%), mp 118—120 °C, $[\alpha]_D^{26} = -45.6°$ ($c=1$, MeOH), Rf_3 0.21. Positive FAB-MS m/z: 888 (MH^+), 754 ([$MH^+ - Z$] + H), 657 (754 − Pro), 643 (M^+ − Gln–Lol), 558 (657 − Val), 515 (643 − Gln), 473 (558 − Aib), 416 (515 − Iva), 372 (473 − Iva), 331 (416 − Aib), 232 (331 − Val). *Anal.* Calcd for $C_{43}H_{69}O_{11}N_9 \cdot H_2O$: C, 57.00; H, 7.90; N, 13.91. Found: C, 57.05; H, 7.82; N, 13.91.

H–Pro–Val–Aib–DL-Iva–Gln–Gln–Lol The above protected heptapeptide (590 mg, 0.66 mmol) in MeOH (5 ml) was hydrogenated according to precedure D to give the deprotected heptapeptide; yield 472.4 mg (94.3%), Rf_4 0.22.

Z–Gln–Aib–Leu–Aib–Gly–Aib–Aib–OPac [Positions 7—13] Z–Gln–Aib–Leu–Aib–OH (1.95 g, 3.46 mmol), HOBt (468 mg, 1 eq), and DCC (715 mg, 1 eq) were added successively to a stirred solution of HBr · H–Gly–Aib–Aib–OPac (1.54 g, 1 eq) in DMF (20 ml) containing TEA (0.48 ml, 1 eq). After 72 h, the mixture was worked up according to procedure A. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 9 : 1); yield 1.84 g (58.5%), mp 106—108 °C, $[\alpha]_D^{25} = -9.2°$ ($c=1$, MeOH), Rf_2 0.34. ISP-MS m/z: 909.5 (MH^+), MS/MS (daughter ions from MH^+) m/z: 772.6 (MH^+ − OPac), 687.5 (772.6 − Aib), 602.8 (687.5 − Aib), 545.5 (602.8 − Gly), 460.9 (545.5 − Aib), 347.7 (460.9 − Leu). *Anal.* Calcd for $C_{45}H_{64}N_8O_{12} \cdot H_2O$: C, 58.30; H, 7.18; N, 12.09. Found: C, 58.48; H, 7.16; N, 12.24.

Z–Gln–Aib–Leu–Aib–Gly–Aib–Aib–OH The above heptapeptide (positions 7—13) (521.4 mg, 0.57 mmol) was dissolved in 90% AcOH (15 ml), and Zn powder (1.68 g) was added to the solution at 0 °C. Stirring was continued at the same temperature for 1 h and at room temperature for 2 h. After filtration to remove the Zn powder, the solvent was evaporated off *in vacuo*. The residue was taken up in 5% citric acid and the aqueous layer was extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was triturated with ether and washed with the same solvent; yield 426 mg (94.0%), mp 119—121 °C, Rf_4 0.47.

Z–Gln–Aib–Leu–Aib–Gly–Aib–Aib–Pro–Val–Aib–DL-Iva–Gln–Gln–Lol [Positions 7—20] The above heptapeptide acid (positions 7—13) (498.5 mg, 0.63 mmol), HOBt (85.2 mg, 1 eq), and DCC (130.1 mg, 1 eq) were added successively to a stirred solution of the deprotected heptapeptide (positions 14—20) (475.2 mg, 1 eq) in DMF (10 ml). After 72 h, the mixture was worked up according to precedure B. The residue was purified by preparative HPLC [conditions: mobile phase, acetonitrile–H_2O (47 : 53, v/v); flow rate, 8 ml/min; detector, UV (220 nm); column, Cosmosil 5C18 (20 mm i.d. × 250 mm); column temperature, 40 °C); yield 331.7 mg (34.5%), mp 106—108 °C, Rf_3 0.3. ISP-MS m/z: 1527.9 (MH^+), 773.3 (Z–Gln–Aib–Leu–Aib–Gly–Aib–Aib), 764.2 ([$M+2H]^{2+}$), 754.4 (H–Pro–Val–Aib–Iva–Gln–Gln–Lol); MS/MS (granddaughter ions from m/z 773.3) m/z: 687.8 (773.3 − Aib), 603.2 (687.8 − Aib), 546.1 (603.2 − Gly), 461.0 (546.1 − Aib), 348.1 (461.0 − Leu); MS/MS (granddaughter ions from m/z 754.4) m/z: 637.2 (754.4 − Lol), 508.8 (637.2 − Gln), 381.2 (508.8 − Gln), 282.0 (381.2 − Iva), 196.8 (282.0 − Aib). Amino acid ratios (6N HCl, 24 h): observed (Calcd); Glu 3.12 (3), Val 1.00 (1), Leu 1.01 (1), Pro 0.95 (1). *Anal.* Calcd for $C_{45}H_{64}N_8O_{12} \cdot 3H_2O$: C, 58.30; H, 7.18; N, 12.09. Found: C, 58.48; H, 7.16; N, 12.24.

H–Gln–Aib–Leu–Aib–Gly–Aib–Aib–Pro–Val–Aib–DL-Iva–Gln–Gln–Lol The above protected tetradecapeptide (124 mg, 78.7 mmol) in MeOH (4 ml) was hydrogenated according to procedure D to give the deprotected tetradecapeptide; yield 108 mg (96%), Rf_4 0.60.

Ac–Aib–Pro–Aib–Ala–Aib–Aib–Gln–Aib–Leu–Aib–Gly–Aib–Aib–Pro–Val–Aib–DL-Iva–Gln–Gln–Lol ([DL-Iva^{17}]Hypelcin A-III) The hexapeptide acid derived from [5] (114.0 mg, 2 eq), HOBt (27.1 mg, 2 eq), and DCC (41.4 mg, 2 eq) were added successively to a stirred solution of the above amine component (positions 7—20) (139.6 mg, 0.10 mmol) in DMF (3 ml). After 72 h, the mixture was worked up according to procedure B. The residue was recrystallized from $CHCl_3$; yield 139.7 mg (71.7%), mp 256—258 °C, $[\alpha]_D^{26} = -10.8°$ ($c=1$, MeOH), Rf_4 0.31. ISP-MS: see Fig. 3. Amino acid ratios (6N HCl, 24 h): observed (Calcd); Glu 3.33 (3), Ala 1.02 (1), Val 1.03 (1), Gly 1.00 (1), Leu 1.02 (1), Pro 1.96 (2). *Anal.* Calcd for $C_{90}H_{155}N_{23}O_{24} \cdot 3H_2O$: C, 54.12; H, 8.12; N, 16.13. Found: C, 54.36; H, 7.85; N, 16.22.

Separation of [D-Iva^{17}] and [L-Iva^{17}]Hypelcin A-III [DL-Iva^{17}]-Hypelcin A-III (100 mg) was subjected to recycle HPLC [conditions: mobile phase, acetonitrile–H_2O (1 : 1, v/v); flow rate, 12 ml/min; detector, UV (210 nm); column, Cosmosil 5C18 (20 mm i.d. × 150 mm + 20 mm i.d. × 250 mm); column temperature, 40 °C]. After 6 cycles, the peak was divided into two parts. Each part was repeatedly chromatographed and divided in the same manner. The first part gave [D-Iva^{17}]hypelcin A-III, mp 263—265 °C, $[\alpha]_D^{26} = -10.4°$ ($c=1$, MeOH) [natural, mp 266—268 °C,[1] $[\alpha]_D^{30} = -10.1°$ ($c=1$, MeOH)]. The later part gave [L-Iva^{17}]hypelcin A-III, mp 260—264 °C, $[\alpha]_D^{26} = -14.3°$ ($c=1$, MeOH).

Identification and Absolute Configuration of Isovaline in [D-Iva^{17}] and [L-Iva^{17}]Hypelcin A-III Each sample was hydrolyzed in 6 N HCl at 110 °C for 24 h and the hydrolysate was analyzed by HPLC using a Shimadzu LC-6A system [conditions: mobile phase, 2 mM copper (II) sulfate in water; flow rate, 1 ml/min; detector, UV (254 nm); column, Sumichiral OA 5000 (4.6 mm i.d. × 150 mm); column temperature, 40 °C]. Iva in [D-Iva^{17}]hypelcin A-III: t_R 14.83 (standard D-Iva, 15.05), Iva in [L-Iva^{17}]hypelcin A-III: t_R 12.33 (standard L-Iva, 12.38).

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2) The following abbreviations are used: AcOH = acetic acid, Boc = *tert*-butyloxycarbonyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMF = dimethylformamide, DCU = *N*,*N*′-dicyclohexylurea, EtOAc = ethyl acetate, HOBt = 1-hydroxybenzotriazole, Lol = leucinol, Pac = phenacyl, TEA = triethylamine, Su = succinimidyl, Z = benzyloxycarbonyl.
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