# Fungal Metabolites. Part 8.† Primary Structures of Antibiotic Peptides, Hypelcin A-I, A-II, A-III, A-IV, A-V, A-VI, A-VII, A-VIII and A-IX from *Hypocrea peltata*

**Kouji Matsuura,[a] Akgül Yesilada,[a] Akira Iida,[a] Yoshihisa Takaishi,[b] Michiko Kanai[c] and Tetsuro Fujita*[,a]**

[a] *Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan*
[b] *Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima 770, Japan*
[c] *Finnigan MAT Instruments, Inc., Hatsudai Shibuya-ku, Tokyo 151, Japan*

Hypelcin A is a mixture of antibiotic peptides produced by *Hypocrea peltata*. Hypelcin A-I, A-II, A-III, A-IV, A-V, A-VI, A-VII, A-VIII and A-IX are components of this mixture purified by reversed-phase high-performance liquid chromatography. The amino acid sequences of these peptides were determined by fast-atom bombardment mass spectrometry and fast-atom bombardment mass spectrometry/mass spectrometry with the help of NMR spectroscopy. The relative molecular masses of these peptides were all ~2000 and their structures were very similar.

Hypelcin A [1,2] was isolated from *Hypocrea peltata* (Jungh) Sacc. which prevents the growth of *Lentinus edodes*, a Japanese edible mushroom. Hypelcin A belongs to the class of peptaibols [3,4] having the following structural features; a richness in helix-promoting α-aminoisobutyric acid (Aib); the presence of two proline residues; the C-terminal is linked with an amino alcohol, leucinol (Lol); and the compounds are acetylated at the *N*-terminal. Peptaibols, which can also be regarded as alamethicin [5] analogues, show membrane-modifying properties which give rise to various bioactivities, such as formation of voltage-gated ion channels,[6,7] haemolysis,[8] and membrane fusion.[9] Suzukacillins,[10] paraselcins,[11] trichosporins,[12] antiamoebins,[3] trichorzianines,[13-15] trichotoxins,[4,16] emerimicins [17,18] and zervamicins [18] are some peptaibols with similar structures and activities. Hypelcin A, which inhibits growth of various fungi and bacteria,[2] has been shown to uncouple oxidative phosphorylation in rat liver mitochondria [19] and to induce permeability changes in phosphatidylcholine bilayers [20,21] as a result of it being a mixture of components.

In an attempt to separate and identify the components of hypelcin A, we previously reported [22] the isolation and structure investigation of hypelcin A-I, A-II, A-III and A-IV, where hypelcin A-III and A-IV could only be purified partially and the complete structure of hypelcin A-III could not be clarified. In this study, further purification and separation of the above mentioned hypelcins were performed by repeated high-performance liquid chromatography (HPLC) and five new compounds, hypelcin A-V, A-VI, A-VII, A-VIII and A-IX, were isolated. The previously reported structures of hypelcin A-I, A-II, A-III and A-IV were reinvestigated and the structures of the new ones were elucidated by fast-atom bombardment mass spectrometry (FAB-MS), fast-atom bombardment mass spectrometry mass spectrometry (FAB-MS/MS) and 2D NMR techniques.

## Results and Discussion

*Separation of Hypelcin A-I, A-II, A-III, A-IV, A-V, A-VI, A-VII, A-VIII and A-IX.*—Crude hypelcin A, which had previously been subjected to HPLC analysis,[22] gave hypelcin A-I and A-II in a pure state, while hypelcin A-III and A-IV were obtained as a mixture. In this study, further separation of crude hypelcin A by analytical HPLC (Fig. 1) indicated the presence of new, additional hypelcin As V–IX along with minor components. The mixture was subjected to preparative HPLC with a reversed-phase ODS column. The main fractions thus obtained were fractionated on a semi-preparative ODS column using repeated HPLC to yield the above mentioned compounds. The purity of the fractions was checked by analytical HPLC.

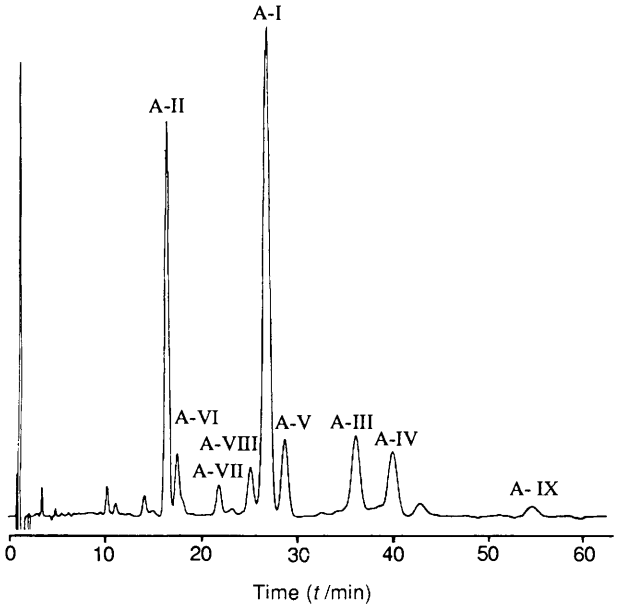



**Fig. 1** HPLC chromatogram of hypelcin A. Analytical conditions: eluent acetonitrile–water (49:51 v/v); column Nacalai Cosmosil 5C18 (4.6 mm i.d. × 150 mm); flow rate 1.2 $cm^3$ $min^{-1}$; UV detector (210 nm); column temperature 40 °C.

*Characterization of Hypelcin A-I, A-II, A-III, A-IV, A-V, A-VI, A-VII, A-VIII and A-IX.*—Amino acid proportions of the peptides were determined by amino acid analysis of the total acidic hydrolysates, except for Aib and isovaline (Iva) residues, because of the low sensitivity for the ninhydrin reaction. The number of those amino acids and the amino alcohols was determined with the help of $^{13}C$ NMR, DEPT spectra where those two amino acids gave quaternary carbon signals at $\delta_C$ 57–59 (Aib) and 60–61 (Iva), and the amino alcohols gave hydroxymethyl carbon signals at $\delta_C$ ~66 (Lol) and ~64 (Iol = isoleucinol), respectively. On the other hand, acetyl groups ($\delta_H$ ~2) were identified by $^1H$ NMR spectroscopy. The absolute

† Part 7, preceding paper.

**Table 1** Characteristics for hypelcin As

| | Formula | $M_r$ (nominal) | M.p. (°C)[a] | Molecular ellipticity [θ] (° cm² dmol⁻¹) 207 nm | 221 nm |
|---|---|---|---|---|---|
| A-I | $C_{89}H_{153}N_{23}O_{24}$ | 1927 | 289–282 | −257 900 | −222 300 |
| A-II | $C_{88}H_{151}N_{23}O_{24}$ | 1913 | 280–282 | −304 200 | −260 100 |
| A-III | $C_{90}H_{155}N_{23}O_{24}$ | 1941 | 266–268 | −260 900 | −229 000 |
| A-IV | $C_{89}H_{153}N_{23}O_{24}$ | 1927 | 269–271 | −277 500 | −243 000 |
| A-V | $C_{89}H_{153}N_{23}O_{24}$ | 1927 | 247–249 | −265 900 | −238 000 |
| A-VI | $C_{88}H_{151}N_{23}O_{24}$ | 1913 | 233–235 | −225 400 | −199 200 |
| A-VII | $C_{89}H_{153}N_{23}O_{24}$ | 1927 | 268–270 | −268 300 | −240 800 |
| A-VIII | $C_{88}H_{151}N_{23}O_{24}$ | 1913 | 245–247 | −305 700 | −268 800 |
| A-IX | $C_{90}H_{155}N_{23}O_{24}$ | 1941 | 255 256 | −293 700 | −264 100 |

[a] Uncorrected.

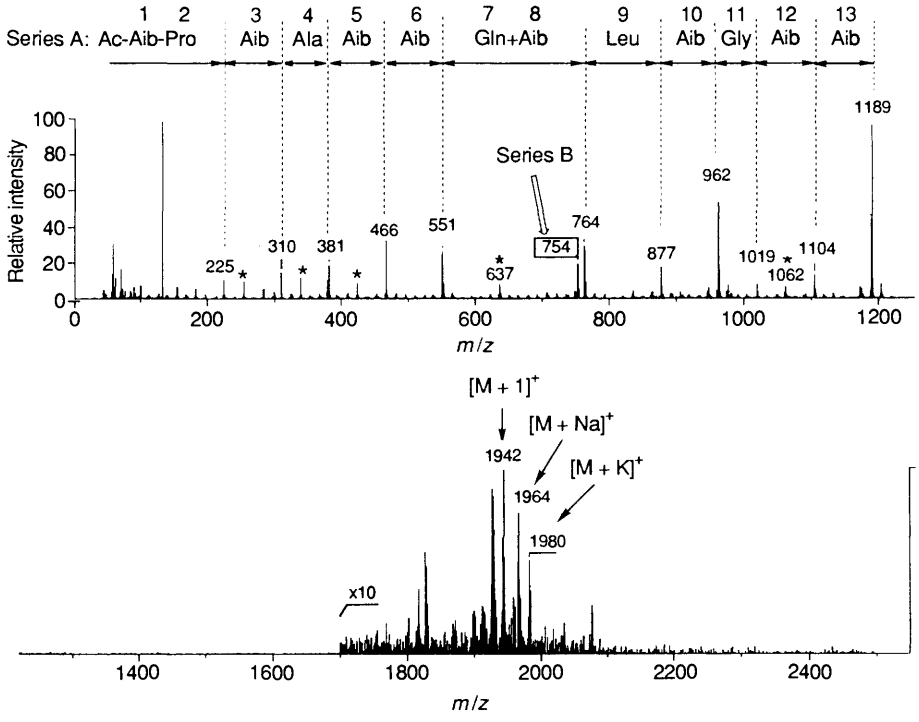
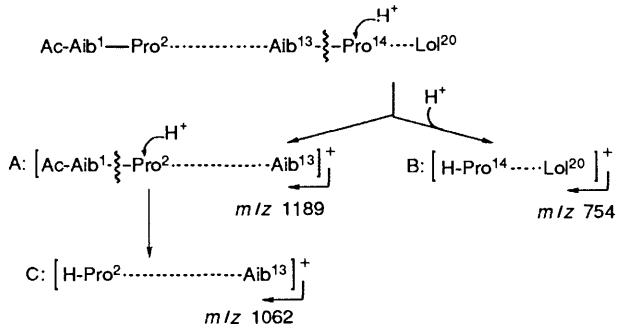



**Fig. 2** Positive-ion FAB-MS of hypelcin A-III. * Indicates series C.

configuration of optically active amino acids was determined by HPLC with a chiral ligand-exchange-phase column. The results showed that Iva has the D configuration while the other amino acids have the L configuration. On the other hand, for the identification and configuration analysis of the amino alcohols, Lol and Iol, acid hydrolysates were converted into the *N*,*O*-bis-(3,5-dinitrobenzoates) before analysis and these were applied to the HPLC column with an optically active stationary-phase column. The identity and configuration (L) of the amino alcohols were determined by comparison with standard samples. The circular dichroism (CD) spectra of these peptides showed negative Cotton effects at 208–209 and 223–225 nm, which show that hypelcin molecules have a right-handed helix form.[5,10] The amino acid proportions and other physical constants of hepelcin As are summarized in Tables 1 and 2.

*Structure of Hypelcin A-III.*—The results mentioned above show that hypelcin A-III [M = 1941 (nominal mass),$C_{90}H_{155}N_{23}O_{24}$] is a linear peptide. As can be seen in Fig. 2, the positive-ion FAB-mass spectrum of hypelcin A-III shows the protonated molecular ion $[M + H]^+$ at *m/z* 1942, the sodium adduct $[M + Na]^+$ at *m/z* 1964 and the potassium adduct $[M + K]^+$ at *m/z* 1980. The spectrum gives useful information




**Scheme 1** Proposed fragmentation for three acylium ion series in the FAB-MS of hypelcin A-III

for masses lower than *m/z* 1189. In the higher mass region no significant peak is observed. In the lower mass region of the spectrum, three series of ions, defined as series A, B and C, were formed due to preferential cleavage of $Aib^1$–$Pro^2$ and $Aib^{13}$–$Pro^{14}$ bonds (Scheme 1). Series A peaks, which began at *m/z* 1189, were generated by the cleavage of the $Aib^{13}$–$Pro^{14}$ bond and gave the sequence-specific ions *m/z* 1104, 1019, 962, 877, 764, 551, 466, 381, 310 and 225. The ion at *m/z* 225 is descriptive

**Table 2** Amino acid proportions for hypelcin As

| | Gln | Gly | Ala | Aib[a] | Val | Iva[a] | Ile | Leu | Pro | Lol[b] | Iol[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A-I | 3.04(3) | 1.01(1) | 1.03(1) | 10 | 1.00(1) | | | 0.98(1) | 1.90(2) | 1 | |
| A-II | 3.08(3) | 1.02(1) | 2.04(2) | 9 | 1.00(1) | | | 1.00(1) | 1.88(2) | 1 | |
| A-III | 2.90(3) | 1.00(1) | 0.99(1) | 9 | 1.00(1) | 1 | | 0.95(1) | 1.98(2) | 1 | |
| A-IV | 3.03(3) | 1.02(1) | 1.01(1) | 10 | 1.00(1) | | 0.88(1) | | 2.01(2) | 1 | |
| A-V | 3.02(3) | 1.09(1) | 1.05(1) | 10 | 1.00(1) | | | 0.99(1) | 2.00(2) | | 1 |
| A-VI | 3.02(3) | 1.04(1) | 1.97(2) | 9 | 1.00(1) | | | 0.97(1) | 1.98(2) | | 1 |
| A-VII | 2.90(3) | 1.02(1) | 1.93(2) | 8 | 1.00(1) | 1 | | 0.96(1) | 1.93(2) | 1 | |
| A-VIII | 3.04(3) | 1.03(1) | 2.00(2) | 9 | 1.00(1) | | 0.92(1) | | 2.05(2) | 1 | |
| A-IX | 2.88(3) | 1.00(1) | 0.96(1) | 9 | 1.00(1) | 1 | 0.78(1) | | 1.92(2) | 1 | |

[a] Determined by the NMR spectra. [b] Determined by the NMR spectra and HPLC.

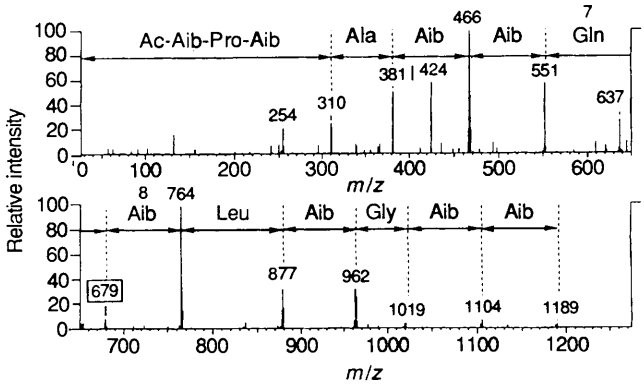



**Fig. 3** CID spectrum of *m/z* 1189 ion

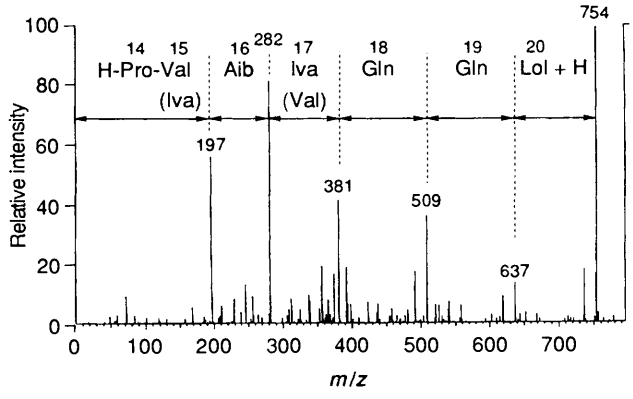



**Fig. 5** CID spectrum of *m/z* 754 ion

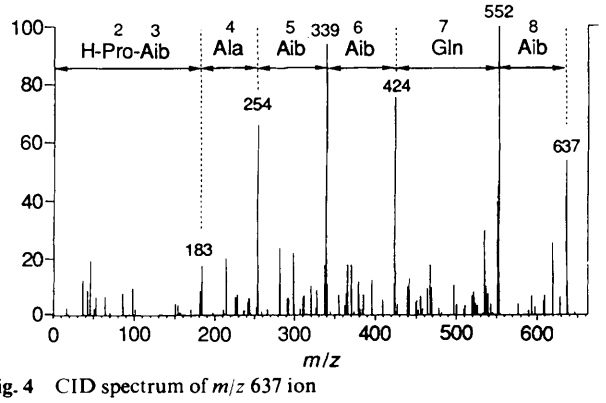



**Fig. 4** CID spectrum of *m/z* 637 ion

of an Ac-Aib-Pro residue indicating that this series originated from the *N*-terminal oligopeptide, but lack of ions belonging to Gln[7] and Aib[8] residues between *m/z* 764 and 551 prevented complete assignment of the sequence of this series. To solve the problem, FAB-MS/MS experiments were carried out by collision-induced dissociation (CID) using a triple-stage quadrupole mass spectrometer.

The CID spectrum of the *m/z* 1189 ion gave ions at *m/z* 679 and 551, corresponding to glutamine and Aib residues, respectively which can lead to the sequence Gln[7]–Aib[8] (Fig. 3). On the other hand, owing to cleavage of the Aib[1]–Pro[2] bond ions of series C beginning at *m/z* 1062 were formed. The cleavage of this fragment at the Aib[8]–Leu[9] bond generated the *m/z* 637 ion, whose CID spectrum showed the protonated Pro[2]–Aib[3] fragment at *m/z* 183 (Fig. 4), which confirmed the existence of the Ac-Aib residue and the sequence of the block 1–3. Therefore, the amino acid sequence of the *N*-terminal oligopeptide of hypelcin A-III was assigned as follows: Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Aib-Aib.

Ions of series B, beginning at *m/z* 754, were expected to give the sequence of block 14–20 corresponding to the *C*-terminal residue. In the CID spectrum (Fig. 5), this acylium ion lost Lol, 2 Gln, Val (or Iva) and Aib successively to give the *m/z* 197 ion, which was descriptive of Pro + Val (or Iva). However, the CID spectrum (data not shown) of the *m/z* 197 ion, did not give the expected proline acylium ion at *m/z* 98, but showed the significantly abundant A-type ion at *m/z* 70, which originated by loss of CO from proline as reported before.[11] This observation showed that Pro was located at the *N*-terminal of the *C*-terminal oligopeptide, and the amino acid sequence was found to be Pro-Val(Iva)-Aib-Iva(Val)-Gln-Gln-Lol.

The complete sequence of hypelcin A-III could be obtained by connecting these two fragments, *N*-terminal and *C*-terminal oligopeptides, but two ambiguities remained: the problem of determining the exact location of the isomeric amino acids Val and Iva, and verification of the unstable Aib–Pro bonds preferentially cleaved in the FAB-MS experiments. At this stage, 2D NMR techniques were helpful such that, in the NH–NH region of the NOESY spectrum (Fig. 6), two separate series of NH–NH connectivities were observed, extending from Aib[3] to Aib[13] and from Val[15] to Lol[20]. The NH–NH connectivities extending from Val[15] to Lol[20] helped to determine the locations of the isomeric residues Val and Iva to positions 15 and 17, respectively. Thus the correct sequence of the *C*-terminal was shown to be Pro-Val-Aib-Iva-Gln-Gln-Lol unambiguously. Furthermore, in the NOESY spectrum (Fig. 7) two couples of Aib NH–Pro $\delta_H$ connectivities were observed which indicated the presence of Aib–Pro bonds at the 1,2 and 13,14 positions. Consequently, the primary structure of hypelcin A-III has been completely elucidated as follows: Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Aib-Aib-Pro-Val-Aib-Iva-Gln-Gln-Lol.

*Structures of other Hypelcin As.*—The sequence analysis by FAB-MS showed that other hypelcin As had very similar structures to that of hypelcin A-III. They all showed $[M + H]^+$, $[M + Na]^+$ and/or $[M + K]^+$ ions between *m/z* 1900 and 2000 (Table 3). According to the FAB-MS and FAB-MS/MS results, the hypelcin As had differences in their amino acid

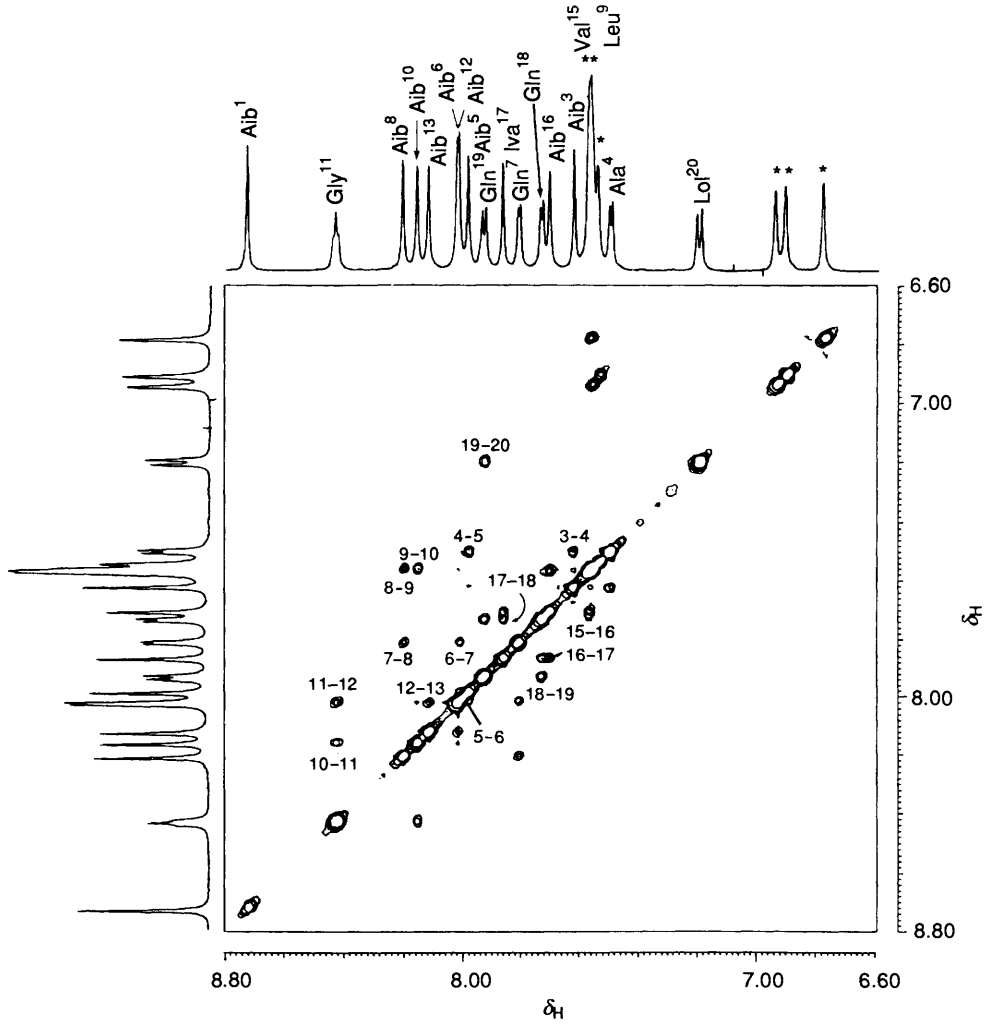



**Fig. 6** NH–NH region of the 600 MHz NOESY spectrum of hypelcin A-III in $CD_3OH$ at 10 °C (60 mmol $dm^{-3}$). The labels are at the $d_{NN}(i, i + 1)$-type cross-peaks. (*) Indicates the ε-carboxamide protons of glutamines.

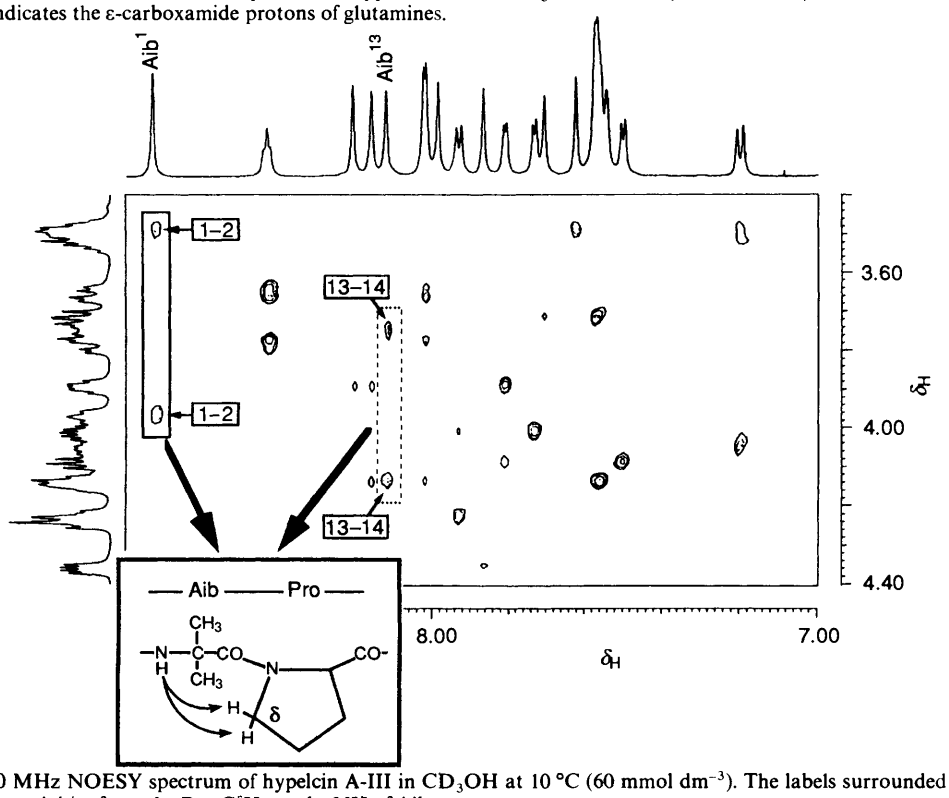



**Fig. 7** Part of the 600 MHz NOESY spectrum of hypelcin A-III in $CD_3OH$ at 10 °C (60 mmol $dm^{-3}$). The labels surrounded by the rectangular frames indicate the connectivities from the Pro $C^{\delta}H_2$ to the NH of Aib.

**Table 3** Diagnostic ions[a] observed in the FAB mass and the CID spectra of hypelcin As

| | Acylium ion, *m/z* | | | | | | | | | | | | | | | | | | | 'Molecule ions' (*m/z*) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ← A series[b] → | | | | | | | | | | | | | ← B series[c] → | | | | | | | | |
| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | $[M+1]^+$ | $[M+Na]^+$ | $[M+K]^+$ |
| A-I | **128** | 225 | 310 | 381 | 466 | 551 | **679** | 764 | 877 | 962 | 1019 | 1104 | 1189 | | **197** | **282** | **367** | **495** | **623** | 740 | 1928 | 1950 | 1966 |
| A-II | 128 | 225 | 310 | 381 | 466 | 537 | **665** | 750 | 863 | 948 | 1005 | 1090 | 1175 | | **197** | **282** | **367** | **495** | **623** | 740 | 1914 | 1936 | 1952 |
| A-III | **128** | 225 | 310 | 381 | 466 | 551 | **679** | 764 | 877 | 962 | 1019 | 1104 | 1189 | | **197** | **282** | **381** | **509** | **637** | 754 | 1942 | 1964 | 1980 |
| A-IV | 128 | 225 | 310 | 381 | 466 | 551 | **679** | 764 | 877 | 962 | 1019 | 1104 | 1189 | | **197** | **282** | **367** | **495** | **623** | 740 | 1928 | 1950 | |
| A-V | 128 | 225 | 310 | 381 | 466 | 551 | **679** | 764 | 877 | 962 | 1019 | 1104 | 1189 | | **197** | **282** | **367** | **495** | **623** | 740 | 1928 | 1950 | 1966 |
| A-VI | 128 | 225 | 310 | 381 | 466 | 537 | **665** | 750 | 863 | 948 | 1005 | 1090 | 1175 | | **197** | **282** | **367** | **495** | **623** | 740 | 1914 | 1936 | 1952 |
| A-VII | 128 | 225 | 310 | 381 | 466 | 537 | **665** | 750 | 863 | 948 | 1005 | 1090 | 1175 | | **197** | **282** | **381** | **509** | **637** | 754 | 1928 | 1950 | 1966 |
| A-VIII | 128 | 225 | 310 | 381 | 466 | 537 | **665** | 750 | 863 | 948 | 1005 | 1090 | 1175 | | **197** | **282** | **367** | **495** | **623** | 740 | 1914 | 1936 | 1952 |
| A-IX | 128 | 225 | 310 | 381 | 466 | 551 | **679** | 764 | 877 | 962 | **1019** | 1104 | 1189 | | **197** | **282** | **381** | **509** | **637** | 754 | 1942 | 1964 | 1980 |
| | | ← C series[d] → | | | | | | | | | | | | | | | | | | | | | |
| A-I | | | **183** | 254 | 339 | 424 | **552** | 637 | 750 | 835 | **892** | **977** | 1062 | | | | | | | | | | |
| A-II | | | 183 | 254 | 339 | 410 | **538** | 623 | **736** | 821 | **878** | **963** | 1048 | | | | | | | | | | |
| A-III | | | **183** | 254 | 339 | 424 | **552** | 637 | | 835 | **892** | | 1062 | | | | | | | | | | |
| A-IV | | | 183 | 254 | 339 | 424 | **552** | 637 | **750** | 835 | **892** | **977** | 1062 | | | | | | | | | | |
| A-V | | | 183 | 254 | 339 | 424 | **552** | 637 | **750** | 835 | **892** | **977** | 1062 | | | | | | | | | | |
| A-VI | | | 183 | 254 | 339 | 410 | **538** | 623 | **736** | 821 | **878** | 963 | 1048 | | | | | | | | | | |
| A-VII | | | 183 | 254 | 339 | 410 | **538** | 623 | 736 | 821 | 878 | **963** | 1048 | | | | | | | | | | |
| A-VIII | | | 183 | 254 | 339 | 410 | **538** | 623 | 736 | 821 | **878** | **963** | 1048 | | | | | | | | | | |
| A-IX | | | 183 | 254 | 339 | 424 | **552** | 637 | 750 | **835** | **892** | 977 | 1062 | | | | | | | | | | |

[a] Mass numbers are indicated by nominal units and bold numbers are ions clearly observed in the CID spectra. [b] *N*-Terminal oligopeptide. [c] *C*-Terminal oligopeptide. [d] The ion peak, *m/z* 183 was formulated as H-Pro-Aib residue.

**Table 4** Primary structures of hypelcin As

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A-I | Ac | Aib | Pro | Aib | Ala | Aib | Gln | Aib | Leu | Aib | Gly | Aib | Aib | Pro | Val | Aib | Aib | Gln | Gln | Lol |
| A-II | Ac | Aib | Pro | Aib | Ala | Ala | Gln | Aib | Leu | Aib | Gly | Aib | Aib | Pro | Val | Aib | Aib | Gln | Gln | Lol |
| A-III | Ac | Aib | Pro | Aib | Ala | Aib | Gln | Aib | Leu | Aib | Gly | Aib | Aib | Pro | Val | Aib | Iva | Gln | Gln | Lol |
| A-IV | Ac | Aib | Pro | Aib | Ala | Aib | Gln | Aib | Ile | Aib | Gly | Aib | Aib | Pro | Val | Aib | Aib | Gln | Gln | Lol |
| A-V | Ac | Aib | Pro | Aib | Ala | Aib | Gln | Aib | Leu | Aib | Gly | Aib | Aib | Pro | Val | Aib | Aib | Gln | Gln | Iol |
| A-VI | Ac | Aib | Pro | Aib | Ala | Ala | Gln | Aib | Leu | Aib | Gly | Aib | Aib | Pro | Val | Aib | Aib | Gln | Gln | Iol |
| A-VII | Ac | Aib | Pro | Aib | Ala | Ala | Gln | Aib | Leu | Aib | Gly | Aib | Aib | Pro | Val | Aib | Iva | Gln | Gln | Lol |
| A-VIII | Ac | Aib | Pro | Aib | Ala | Ala | Gln | Aib | Ile | Aib | Gly | Aib | Aib | Pro | Val | Aib | Aib | Gln | Gln | Lol |
| A-IX | Ac | Aib | Pro | Aib | Ala | Aib | Gln | Aib | Ile | Aib | Gly | Aib | Aib | Pro | Val | Aib | Iva | Gln | Gln | Lol |

sequences at positions 6, 9, 17 and 20 as shown in Table 4. Position 6 was occupied either by Aib or Ala as evidenced by the ions observed at *m/z* 537 for Ala in hypelcin A-II, A-VI, A-VII and A-VIII, and *m/z* 551 for Aib in hypelcin A-I, A-III, A-IV, A-V and A-IX. On the other hand the Leu or Ile at position 9 and Lol or Iol at position 20 could not be differentiated by FAB-MS spectroscopy. The ion attributed to Leu or Ile at position 9 in the FAB-MS spectra was assigned to be Ile in hypelcin A-IV, A-VIII and A-IX, and Leu in the others according to the amino acid analysis results. The amino alcohols were identified as Lol or Iol on the basis of comparison with standard samples by HPLC and it was found that hypelcin A-V and A-VI had Iol at position 20 and that the others had Lol. As for position 17, the CID spectrum of series B ions showed prominent ions for Iva or Aib at *m/z* 381 and 367, respectively. Thus it was shown that hypelcin A-III, A-VII and A-IX had Iva residue at position 17, while the others had Aib.

## Experimental

*General Procedures.*— All m.p.s were measured on a Yanagimoto micro melting point apparatus and are uncorrected. All NMR experiments were carried out by using a Bruker AC-300 or a Bruker AM-600 spectrometer at 10 °C or room temperature. Samples were dissolved in $CD_3OH$ (0.45 $cm^3$) containing tetramethylsilane as internal standard. CD spectra were recorded on a JASCO J-720 spectropolarimeter at room temperature. Peptides (0.41–0.82 mg) were dissolved in methanol (1 $cm^3$). For HPLC analysis a Shimadzu LC-8A system was used.

*Reversed-phase HPLC Separation of Hypelcin A-I, A-II, A-III, A-IV, A-V, A-VI, A-VII, A-VIII and A-IX.*—Preliminary separation of the hypelcin A mixture (1.7 g) was performed repeatedly with a YMC-Packed column (50 mm i.d. × 500 mm) [eluent (49:51 v/v) acetonitrile–water; flow rate 80 $cm^3$ $min^{-1}$; UV detector (220 nm); column temperature 40 °C] to give nine main fractions. Further fractionation was carried out repeatedly with a Cosmosil 5C18 column (20 mm i.d. × 250 mm) [eluent (49:51 v/v) acetonitrile–water; flow rate 17 $cm^3$ $min^{-1}$; UV detector (220 nm); column temperature 40 °C] to give pure hypelcin A-I (517.2 mg), A-II (239.8 mg), A-III (102.8 mg), A-IV (95.8 mg), A-V (88.9 mg), A-VI (41.2 mg), A-VII (22.0 mg), A-VIII (36.9 mg) and A-IX (22.1 mg). The purity of the compounds was checked with a Cosmosil 5C18 column (4.6 mm i.d. × 150 mm) [eluent (49:51 v/v) acetonitrile–water; flow rate 1.2 $cm^3$ $min^{-1}$; UV detector (210 nm); column temperature 40 °C].

*Identification and Absolute Configuration of Amino Acids and Amino Alcohols.*—For amino acid analyses, samples (0.1 mg) were hydrolysed in 6 mol $dm^{-3}$ HCl at 110 °C for 24 h. The hydrolysate was separated into three portions, one of which was analysed by an Hitachi amino acid analyser (model 835). For the chiral separation of amino acids but not the amino alcohols the second portion was subjected to HPLC analysis with a Sumichiral OA-5000 column (4.6 mm i.d. × 150 mm) [eluent 2 mmol $dm^{-3}$ aq. $CuSO_4$; flow rate 1.2 $cm^3$ $min^{-1}$; UV detector (254 nm); column temperature 40 °C]. $t_R$-Values from standard equimolar mixtures of DL isomers (min): Ala, 4.85; D-Ala, 6.67; Aib, 7.78; Pro, 9.35; Iva, 11.19; Val, 13.03; D-Iva, 13.64; D-Pro, 19.31; D-Val, 22.35; Ile, 32.87; Leu, 39.35; Glu, 55.77; D-Ile, 59.64; D-Leu, 67.51; D-Glu, 68.6. The third portion of the hydrolysate was used to determine the absolute configuration of the amino alcohol of each peptide, in the following manner. The acid hydrolysate was refluxed in absolute methanol–thionyl dichloride (10:1; 2 $cm^3$) for 3 h to give the methyl esters of the amino acids. After removal of the solvent and reagent under reduced pressure, the residues were treated with a solution of 3,5-dinitrobenzoyl chloride (~1 mg) and triethylamine (one drop) in ethyl acetate (2 $cm^3$) while being stirred at room temperature overnight. The resulting *N,O*-bis(3,5-dinitrobenzoates) were analysed by HPLC with a Sumipax OA-4100 column (4 mm i.d. × 250 mm) [eluent (85:15: 3 v/v) hexane–1,2-dichloroethane–ethanol; flow rate 0.8 $cm^3$ $min^{-1}$; UV detector (254 nm); column temperature 35 °C]. $t_R$-Values from standard equimolar mixtures of DL isomers (min): Lol, 45.16; D-Lol, 37.18; Iol, 43.16; D-Iol, 35.41.

*FAB-MS and FAB-MS/MS Spectra.*—FAB-MS and FAB-MS/MS spectra were obtained by using a Finnigan MAT 700 triple-stage quadrupole mass spectrometer. Samples were bombarded with 8 keV xenon atoms. Argon was used as the collision gas at a pressure of 1.0–1.5 × $10^{-3}$ Torr.* The collision energy was −10 to −20 eV. For FAB-MS and FAB-MS/MS experiments glycerol-thioglycerol (1:1) was used as a matrix. Samples were dissolved in methanol–water (1:1 v/v) containing 0.1% trifluoroacetic acid, and mixed with the matrix on the target.

## Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (03303013 and 04671292).

* 1 Torr = 133.322 Pa.

## References

1 T. Fujita, Y. Takaishi and T. Shiromoto, *J. Chem. Soc., Chem. Commun.*, 1979, 413.
2 T. Fujita, Y. Takaishi, H. Moritoki, T. Ogawa and K. Tokimoto, *Chem. Pharm. Bull.*, 1984, **32**, 1822.
3 R. C. Pandey, H. Meng, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, 1977, **99**, 5203.
4 H. Brückner and M. Przybylski, *J. Chromatogr.*, 1984, **296**, 263.
5 R. C. Pandey, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, 1977, **99**, 8469.

6 P. Mueller and D. O. Rudin, *Nature*, 1968, **217**, 713.
7 M. S. P. Sansom, *Prog. Biophys. Mol. Biol.*, 1991, **55**, 139.
8 G. Irmscher and G. Jung, *Eur. J. Biochem.*, 1977, **80**, 165.
9 A. Y. L. Lau and S. I. Chan, *Biochemistry*, 1974, **13**, 4942.
10 E. Katz, M. Aydin, N. Lucht, W. A. König, T. Ooka and G. Jung, *Liebigs Ann. Chem.*, 1985, 1041.
11 H. Brückner and H. Graf, *Experientia*, 1983, **39**, 528.
12 A. Iida, M. Okuda, S. Uesato, Y. Takaishi, T. Shingu, M. Morita and T. Fujita, *J. Chem. Soc., Perkin Trans. 1*, 1990, 3249.
13 B. Bodo, S. Rebuffat, M. E. Hajji and D. Davoust, *J. Am. Chem. Soc.*, 1985, **107**, 6011.
14 M. E. Hajji, S. Rebuffat, D. Lecommandeur and B. Bodo, *Int. J. Peptide Protein Res.*, 1987, **29**, 207.
15 S. Rebuffat, M. E. Hajji, P. Hennig, D. Davoust and B. Bodo, *Int. J. Peptide Protein Res.*, 1989, **34**, 200.
16 H. Brückner, W. A. König, M. Greiner and G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 476.
17 R. C. Pandey, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, 1977, **99**, 5205.
18 K. L. Rinehart, Jr., L. A. Gaudioso, M. L. Moore, R. C. Pandey and J. C. Cook, Jr., *J. Am. Chem. Soc.*, 1981, **103**, 6517.
19 Y. Takaishi, H. Terada and T. Fujita, *Experientia*, 1980, **36**, 550.
20 K. Matsuzaki, S. Nakai, T. Handa, Y. Takaishi, T. Fujita and K. Miyajima, *Biochemistry*, 1989, **28**, 9392.
21 K. Matsuzaki, Y. Takaishi, T. Fujita and K. Miyajima, *Colloid Polym. Sci.*, 1991, **269**, 604.
22 T. Fujita, Y. Takaishi, K. Matsuura, T. Takeda, Y. Yoshioka and H. Brückner, *Chem. Pharm. Bull.*, 1984, **32**, 2870.

*Paper* 2/04597D
*Received* 26*th August* 1992
*Accepted* 24*th September* 1992