New Peptide Antibiotic, Hypelcin A, from *Hypocrea peltata*

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Summary Two new antibiotics, hypelcins A and B were isolated from the fruit bodies of *Hypocrea peltata* and the structure of hypelcin A was established as (**1**) by chemical degradation and mass spectrometry.

RECENTLY, the peptide antibiotics trichopolyns A and B, which contain α-aminoisobutyric acid (Aib), and which prevent the growth of *Lentinus eddodes*, a Japanese edible mushroom, were reported.[1] We here report the isolation of hypelcins A and B, which also contain Aib, from the fruit bodies of *Hypocrea peltata* (Jungh) Sacc. which inhibits the growth of hymenomycetes; we also report the structure of hypelcin A.

A methanolic extract of the dried fruit bodies (1950 g) was separated into methylene chloride-soluble and -insoluble fractions. Concentration of the soluble fraction gave a crude crystalline mixture, which was chromatographed on Sephadex LH-20 and silica gel columns to give the new antibiotics, hypelcin A (10 g), $C_{89}H_{153}N_{23}O_{24}\cdot 5H_2O$, $[\alpha]_D^{28}$ −9·75° (*c* 1·0, MeOH), and hypelcin B (2 g), $C_{89}H_{152}N_{22}O_{25}\cdot 5H_2O$, $[\alpha]_D^{28}$ −7·25° (*c* 1·0, MeOH). Hypelcins A and B were identified by t.l.c. [R_F 0·35 (A) and 0·18 (B), CH_2Cl_2–acetone–MeOH (4:1:1, v/v), silica gel plates (0·25 mm thickness, E. Merck)]. The hypelcins were found to have a similar inhibitory activity against *L. eddodes* (minimum inhibitory concentration 300 p.p.m.). They showed similar colour reactions (negative ninhydrin and positive Dragendorff's reactions) and also similar u.v. [no maximum above 210 nm (MeOH)], i.r. [ν_{max} (KBr) 3320, 1660, and 1530—1540 cm^{-1}, characteristic of polypeptides], and n.m.r. spectra [δ (C_5D_5N) 2·28 (COMe)].

Hydrolysates obtained from hypelcin A under vigorous conditions (6 N HCl, 110 °C, 24 and 48 h) were subjected to amino-acid analysis. The Aib content was determined[2] by isotachophoresis [constant current: 200 μA; leading electrolyte: 0·05 M 2-amino-2-methylpropanol, 0·5% methylcellulose, HCl, pH 9·0; terminal electrolyte: 0·1 M γ-aminobutyric acid, $Ba(OH)_2$, pH 10·9]. The amino-acid composition of hypelcin A was found to be as follows: (L-Glx)$_3$-(L-Pro)$_2$(Gly)$_1$(L-Ala)$_1$(Aib)$_{9-10}$(L-Val)$_1$(L-Leu)$_1$.† The L-leucinol (Leuol) fraction, m.p. 66—70 °C (lit.,[3] m.p. 44 °C) [*NO*-ditrifluoroacetate *m/e* 240 ($M - CF_3$), 196 ($M - CF_3CO_2$), and 182 ($M - CH_2O_2CCF_3$)], was isolated from the hydrolysates by paper electrophoresis followed by paper

† Absolute L-configuration was determined by a combination of g.l.c. on a glass capillary column coated with a chiral stationary dipeptidic phase (see N. Ôi, H. Takeda, and H. Shimada, *Japan Analyst*, in the press), *N*-L-leucyl amino-acid analysis (see J. M. Manning and S. Moore, *J. Biol. Chem.*, 1968, **243**, 5591), and the use of D-amino-acid oxidase (see P. Boulanger and R. Osteux, in 'Methods of Enzymatic Analysis,' Vol. 4, ed H. U. Bergmeyer, Academic Press, New York, 1974, p. 1648).

partition chromatography and identified by comparison with an authentic sample.[4] Dansylation (dansyl chloride, 0·2 M $NaHCO_3$, acetone, 37 °C, 1 h) and methylation (CH_2N_2, Et_2O–MeOH) recovered the starting material, while acetylation (acetic anhydride–pyridine) gave a monoacetate [ν_{max} (KBr) 3320, 1740, 1660, and 1540 cm^{-1}, δ (C_5D_5N) 2·18 and 2·32, 2 s, 2 × COMe], suggesting that hypelcin A contains neither amino nor carboxy groups but a hydroxy group. Hypelcin A analyses for three $CONH_2$ groups on hydrolysis to ammonia.[5] The presence of an acetyl, an esterifiable hydroxy, and three primary amide groups suggests that (i) hypelcin A is not cyclic but linear; (ii) the terminal N is blocked by an acetyl group; and (iii) a C-terminal group is linked with L-Leuol as an amide.

Partial hydrolyses (12 N HCl, 37 °C, 30 min and 15 h) gave the seven pure peptide fragments (**2**)—(**8**). Their structures were determined by mass spectrometry, Edman analysis, dansylation, and hydrazinolysis (Figure). Dehydration (ethylene chlorophosphite, triethyl phosphite) of fragments (**4**) and (**8**), followed by reduction (Na, NH_3–MeOH), hydrolysis (6 N HCl, 110 °C, 24 h), and amino-acid analysis showed the presence of 1 and 2 mol of ornithine, respectively. This clearly indicated that Glx is present as Gln in hypelcin A. Hence, the structure should be a straight chain and hypelcin A can be represented as (**1**), which contains ten Aib residues. To date, the only reported Aib-containing peptide which contains a phenylalaninol C-terminal linkage is peptaibophol.[6] The first finding of a new peptide antibiotic containing Aib and Leuol is significant.‡

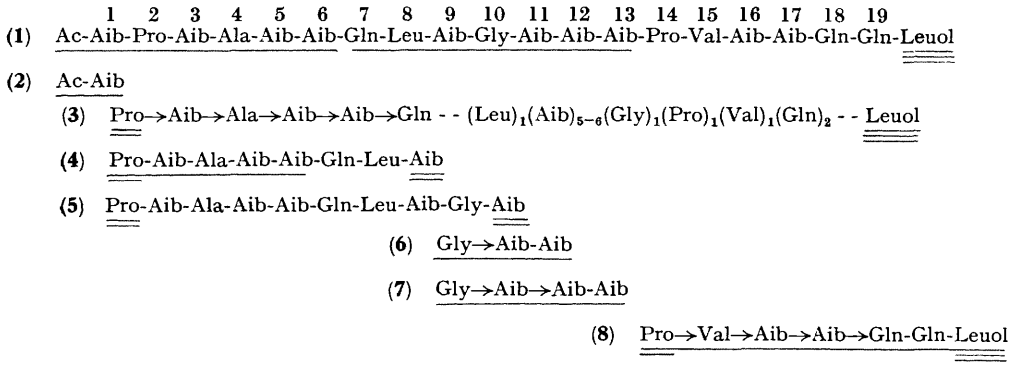

FIGURE. Sequence of hypelcin A (**1**) and the peptides (**2**)—(**8**) obtained from partial hydrolyses of (**1**). The methods of determination are indicated as follows ——: mass spectrometry, →: Edman analysis, ==: dansylation or hydrazinolysis, and ≡≡≡: g.l.c. and paper electrophoresis.

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‡ Recently, the isolation of the peptide antibiotic trichotoxin A-40, which contains Aib but lacks phenylalaninol, from the mycelium of *Trichoderma viride* N.R.R.L. 5242 was reported (see G. Irmscher, G. Bovermann, G. Boheim, and G. Jung, *Biochim. Biophys. Acta*, 1978, **507**, 470).

1 K. Fuji, E. Fujita, Y. Takaishi, T. Fujita, I. Arita, M. Komatsu, and H. Hiratsuka, *Experientia*, 1978, **34**, 237.
2 J. W. Payne, R. Jakes, and B. S. Hartley, *Biochem. J.*, 1970, **117**, 757.
3 H. Adkins and A. A. Pavlic, *J. Amer. Chem. Soc.*, 1947, **69**, 3039.
4 H. Rubinstein, B. Feibush, and E. Gil-Av, *J.C.S. Perkin II*, 1973, 2094.
5 P. E. Wilcox in 'Methods in Enzymology,' vol. 11, ed. C. H. W. Hirs, Academic Press, New York, 1967, p. 63.
6 R. C. Pandey, J. C. Cook, Jr., and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 1977, **99**, 8469 and references therein.