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A New Mast Cell Degranulating Peptide "Mastoparan" in the Venom of *Vespula lewisii*

The new mast cell degranulating peptide "mastoparan" was isolated from the venom of *Vespula lewisii* (Vespid wasp) and the peptide was synthesized chemically. Mastoparan is tetradecapeptide amide constituted of the restricted amino acid and the repeated sequence.

Keywords——mast cell degranulating peptide; histamine releasing peptide; wasp venom; *Vespula lewisii*; mastoparan

In the past several years, hymenopteran venoms have been characterized on the molecular basis. The presence of various kinds of active amines,[1] peptides such as haemolytic melittin,[2] neurotoxic apamin,[3] and mast cell degranulating peptide(so-called MCD-peptide),[4] and some enzymes including phospholipase A,[5] hyaluronidase[6] *etc.* have been demonstrated in bee venom, similarly various active amines,[7] bradykinin-like peptides,[8] and enzymes[9] in wasp venoms.

We have investigated the new active substances in the venom components of bee and wasps, comparing with these known substances in the venom and differentiating from these by both chromatographical and pharmacological behaviours of the active principles.

This paper describes the isolation, characterization and chemical synthesis of the new mast cell degranulating peptide, mastoparan, in the venom of *Vespula lewisii.*

The rat peritoneal mast cells were prepared according to the method of Bloom *et al.*,[10] and the degranulating activity was assayed under the phasecontrast microscopic observation.

Venom sacs of 5000 pieces were homogenized in 10 ml of 5% trichloroacetic acid, and centrifuged at 7000 rpm for 20 min. The supernatant was chromatographed on a Sephadex G-25 column and the active principle eluted at the 1.5 void volumes of the column was collected and rechromatographed by the similar manner. CM-cellulose column chromatography was employed for the next step of separation with the linear concentration gradient elution from 0.05 M to 1.0 M ammonium bicarbonate (pH 8.5). The activity emerged approximately 0.2 M of the elution buffer, but was still contaminated the rat uterus contracting activity. The principle was finally separated from the contamination by using the preparative thin-layer chromatography on cellulose powder with the solvent system of *n*-butanol: pyridine: acetic acid: water (90: 60: 18: 72). A part of the plate was stained by fluorescamine. The mast cell degranulating activity was shown in a component having the *Rf* value of 0.5 while the uterus contracting activity was 0.2—0.3. The area was scraped, and extracted with 0.1 N hydrochloric acid and the pure material was isolated by Sephadex G-25 column chromatography.

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The active principle was inactivated by chymotrypsin or trypsin digestion and was considered to be a peptide. The amino acid composition of this principle determined by an amino acid analyser after hydrolysis with 6 N hydrochloric acid at 110° for 24 hr. and also with aminopeptidase M, was as follows; Lys_3, Asn_1, Ala_4, Ile_2, Leu_4. The N-terminal amino acid could be determined as isoleucine by the dansyl method. The digestion of the peptide with trypsin gave the 5 peptide fragments (T-1 to T-5), which were separated and purified by thin-layer chromatography of cellulose powder using the same solvent system as that used for the separation of the intact peptide. Amino acid sequences of these fragments were analysed by the dansyl Edman procedure and the following sequences were obtained; T-1, Ala–Lys; T-2, Lys; T-3, Ile–Asn–Leu–Lys; T-4, Ala–Leu–Ala–Ala–Leu; T-5, Ile–Leu–NH_2. The intact peptide was submitted to the subtractive Edman degradation and the dansyl Edman procedure which permitted to deduce the following sequence; Ile–Asx–Leu–Lys–Ala–Leu–Ala–Ala–Leu–Ala–Lys–. The results of the both Edman procedures coincided with that obtained from the trypsin digestion experiment. The structure of the peptide was considered to be

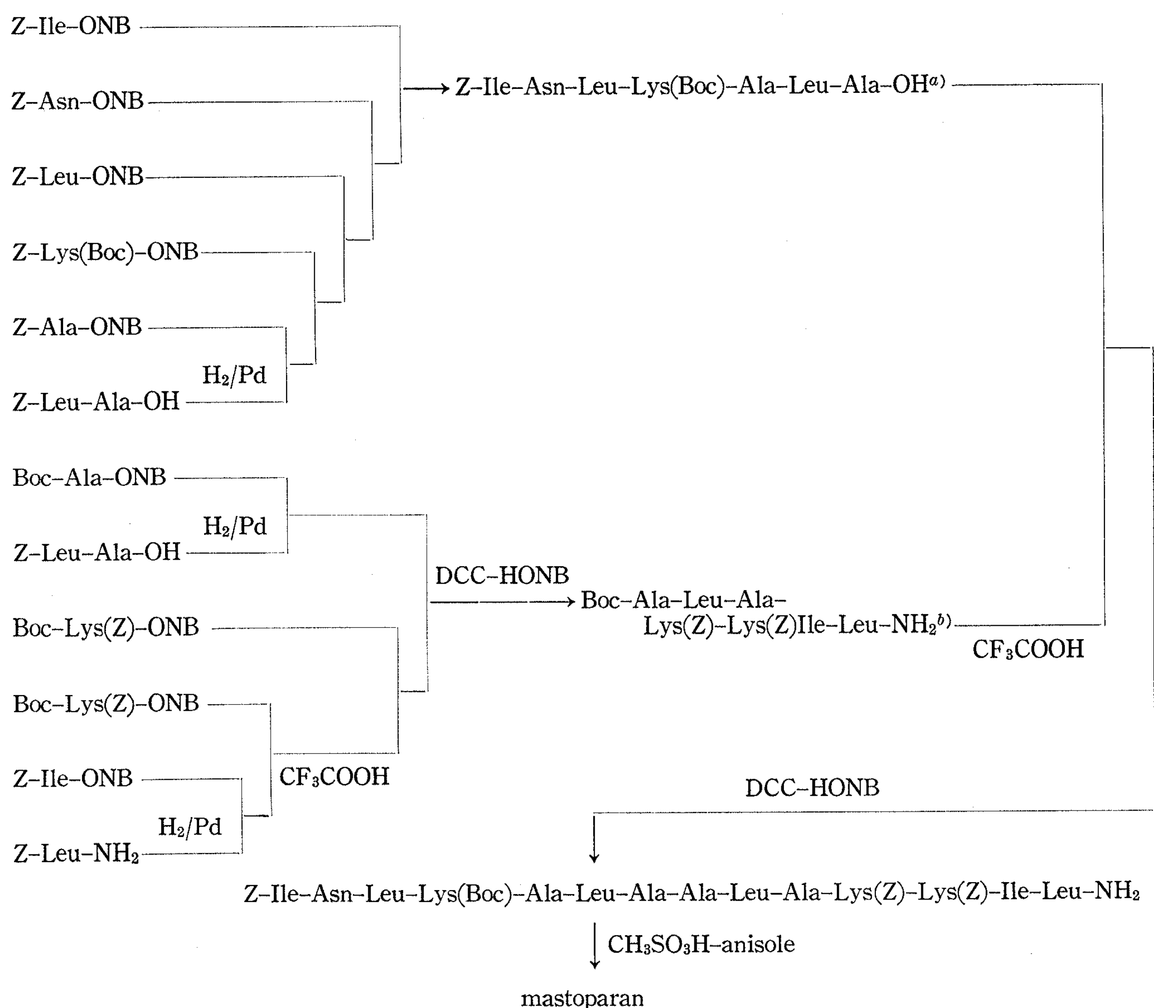

Fig. 1. Synthetic Route to Mastoparan

Z=benzyloxycarbonyl, Boc=*t*-butoxycarbonyl, DCC=dicyclohexylcarbodiimide, HONB=N-hydroxy-4-norbornene-2,3-dicarboximide, -ONB=HONB ester.

a) $[\alpha]_D^{23}=-30.0°$ (*c*, 0.23 in DMF), *Rf* (silica gel, $CHCl_3$-MeOH-AcOH, 9:1:0.5)=0.22. *Anal.* Calcd. for $C_{47}H_{77}N_9O_{13}$: C, 57.82; H, 7.96; N, 12.91. Found: C, 57.72; H, 7.94; N, 12.82.

b) $[\alpha]_D^{23}=-24.6°$ (*c*, 0.2 in DMF), *Rf* (silica gel, $CHCl_3$-MeOH-AcOH, 9:1:0.5)=0.43. *Anal.* Calcd. for $C_{57}H_{90}N_{10}O_{13}$: C, 60.94; H, 8.08; N, 12.47. Found: C, 60.92; H, 8.16; N, 12.36.

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The peptide is the first example of the mast cell degranulating peptide isolated from the wasp venom, and composed of the restricted amino acid such as lysine and mainly hydrophobic amino acids. The structure is quite unique as being repeated sequence. The peptide was named "mastoparan."

Mastoparan degranulates mast cells in the concentration of 0.5 μg/ml (0.3 nmol/ml) and releases histamine from the cells in the same concentration.

The peptide was synthesized by the conventional solution method. The synthetic scheme is outlined in Fig. 1. The partially protected N-terminal heptapeptide and the partially protected C-terminal heptapeptide amide were finally coupled by DCC–HONB method to yield the corresponding fully protected tetradecapeptide amide, which was deblocked with methanesulfonic acid in the presence of anisole to give crude mastoparan. The product was then purified by a column chromatography on Sephadex LH-20 (elution solvent, 1 N acetic acid and 5 N acetic acid): $[\alpha]_D^{21}$ $-77.1°$ (c=0.31 in 1 N acetic acid), TLC (cellulose) *Rf* (*n*-butanol: pyridine: acetic acid: water, 90: 60: 18: 72)=0.53, *Rf* (*n*-butanol: ethyl acetate: acetic acid: water, 1: 1: 1: 1)=0.60; Paper electrophoresis (pH 6.5, pyridine acetate buffer) Mobility= 0.91×Arg (Arg=4.7 cm); Amino acid ratios in acid hydrolysate, Lys 3.31(3), Asp 0.99(1), Ala 4.01(4), Ile 2.00(2), Leu 4.00(4).

The synthetic peptide was consistent chromatographically with natural mastoparan. The degranulating activity of the synthetic peptide in the rat peritoneal mast cells was found equivalent to that of the natural peptide.

In addition, the C-terminal heptapeptide amide revealed 1/10 of mastoparan activity, while the N-terminal heptapeptide did not in the same concentration.

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