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Studies on Peptides. CI.[1,2] Synthesis of a Wasp Venom, Mastoparan M[1,2]

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A tetradecapeptide amide, H–Ile–Asn–Leu–Lys–Ala–Ile–Ala–Ala–Leu–Ala–Lys–Lys–Leu–Leu–NH_2, corresponding to the entire amino acid sequence of a wasp venom, mastoparan M, was synthesized using the thioanisole-mediated trifluoroacetic acid deprotecting procedure.

Keywords——wasp venom; mastoparan; mastoparan X; mast cell degranulating activity; histamine release from mast cell; TFA-thioanisole deprotection

Mastoparan M is a tetradecapeptide amide isolated from *Vespa mandarinia*[3] and its amino acid sequence was shown to have a much closer structural homology to those of previously isolated wasp venoms, mastoparan X[4] and mastoparan,[5] than to that of polistes mastoparan[6] (Fig. 1).

Mastoparan M	H–Ile–Asn–Leu–Lys–Ala–Ile–Ala–Ala–Leu–Ala–Lys–Lys–Leu–Leu–NH_2
Mastoparan X	H–Ile–Asn–Trp–Lys–Gly–Ile–Ala–Ala–Met–Ala–Lys–Lys–Leu–Leu–NH_2
Mastparan	H–Ile–Asn–Leu–Lys–Ala–Leu–Ala–Ala–Leu–Ala–Lys–Lys–Ile–Leu–NH_2
P. Mastoparan	H–Val–Asp–Trp–Lys–Lys–Ile–Gly–Gln–His–Ile–Leu–Ser–Val–Leu–NH_2

Fig. 1. Amino Acid Sequences of Wasp Venoms

This new mast cell degranulating peptide has now been synthesized using available peptide fragments from our previous syntheses of mastoparan X[7] and mastoparan,[8] according to the scheme illustrated in Fig. 2.

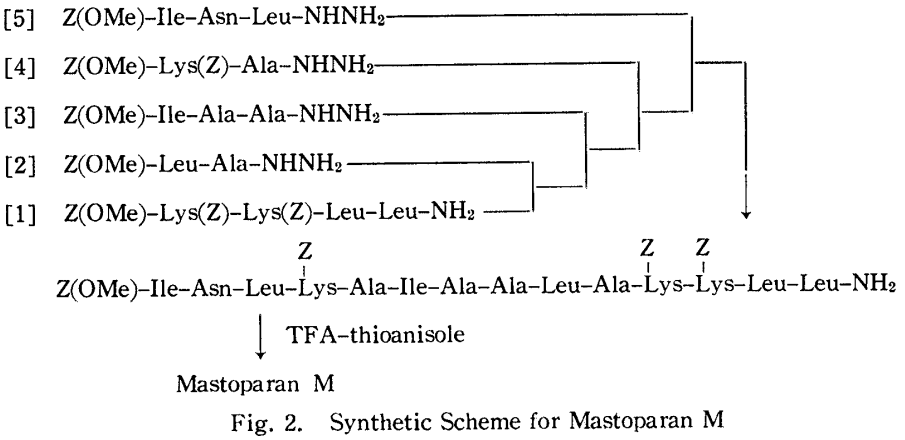

Fig. 2. Synthetic Scheme for Mastoparan M

Two fragments, Z(OMe)–Lys(Z)–Lys(Z)–Leu–Leu–NH_2 [1] and Z(OMe)–Ile–Ala–Ala–$NHNH_2$ [3], are those used for the former synthesis and the other three fragments, Z(OMe)–Leu–Ala–$NHNH_2$ [2], Z(OMe)–Lys(Z)–Ala–$NHNH_2$ [4] and Z(OMe)–Ile–Asn–Leu–$NHNH_2$ [5], are those used for the latter. The azide method[9] was employed exclusively to assemble these fragments according to the scheme, and the protected intermediates were purified by precipitation with methanol from DMF or DMSO, depending on the solubility.

At the final step, the thioanisole-mediated TFA deprotecting procedure[10] was employed, as performed for the synthesis of polistes mastoparan,[11] to remove all protecting groups, Z and Z(OMe). In order to ensure the complete removal of the Z group by this new procedure, the treatment (at room temperature for 4 hr) was repeated. The deprotected peptide, after conversion to the corresponding acetate by Amberlite CG-4B treatment, was purified by partition chromatography[12] on Sephadex G-25 with *n*-BuOH–AcOH–H_2O (4: 1: 5). When monitored by means of the ninhydrin test, the desired compound was eluted as the main fraction in 53% yield. The homogeneity of synthetic mastoparan M was assessed by thin layer chromatography (TLC), elemental analysis, acid hydrolysis and enzymatic digestion.

The synthetic peptide exhibited *Rf* values identical with those of the natural peptide and was as active as the natural peptide in terms of histamine releasing activity from mast cells.

Experimental

General experimental procedures were essentially the same as those described in Part LXXXIV.[7] Leucine aminopeptidase (Lot. 79C-8110) was purchased from Sigma Chemical Co. *Rf* values in TLC performed on silica gel (Kieselgel G, Merck) refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8: 3: 1), Rf_2 *n*-BuOH–AcOH–AcOEt–H_2O (1: 1: 1: 1), Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (30: 6: 20: 24), Rf_4 *n*-BuOH–AcOH–pyridine–H_2O (90: 18: 60: 72), Rf_5 CH_2Cl_2–AcOMe–EtOH (20: 20: 4).

Z(OMe)-Leu-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2——Z(OMe)–Lys(Z)–Lys(Z)–Leu–Leu–NH_2 (3.25 g, 3.49 mmol) was treated with TFA-anisole (9 ml—3 ml) in an ice-bath for 60 min, then *n*-hexane was added. Trituration of an oily residue with dry ether afforded a powder, which was dried over KOH pellets *in vacuo* for 3 hr and dissolved in DMF (12.0 ml) containing Et_3N (0.5 ml, 3.8 mmol). The azide [prepared from 1.99 g (5.22 mmol) of Z(OMe)–Leu–Ala–$NHNH_2$][8] in DMF (20 ml) and Et_3N (0.8 ml, 5.80 mmol) were added to the above ice-chilled solution and the mixture, after being stirred at 4° for 18 hr, was concentrated. Treatment of the residue with 5% citric acid and AcOEt afforded a powder, which was washed with 5% citric acid and H_2O and then precipitated from DMF with MeOH; yield 3.02 g (77%), mp 260—261°, $[\alpha]_D^{20}$ −33.7° (c=1.0, DMSO), Rf_1 0.78. Amino acid ratios in 6 N HCl hydrolysate: Leu 3.00, Lys 2.00, Ala 1.01 (recovery of Leu 87%). *Anal.* Calcd for $C_{58}H_{85}N_9O_{13}$: C, 62.40; H, 7.68; N, 11.29. Found: C, 62.46; H, 7.73; N, 11.19.

Z(OMe)-Ile-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2——The above protected hexapeptide amide (1.50 g, 1.34 mmol) was treated with TFA-anisole (2.2 ml—0.7 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (6.0 ml) containing Et_3N (0.2 ml, 1.47 mmol). The azide [prepared from 0.91 g (2.02 mmol) of Z(OMe)–Ile–Ala–Ala–$NHNH_2$] in DMF (9.0 ml) and Et_3N (0.41 ml, 2.94 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4° for 24 hr. Additional azide [prepared from 0.46 g (1.02 mmol) of the hydrazide] in DMF (3.5 ml) and Et_3N (0.15 ml, 1.12 mmol) were added and stirring was continued for an additional 12 hr. The solvent was evaporated off, the residue was treated with 5% citric acid and AcOEt and the resulting powder was purified as described above; yield 1.40 g (76%), mp 279—281°, $[\alpha]_D^{20}$ −16.8° (c=1.0, DMSO), Rf_1 0.75. Amino acid ratios in 6 N HCl hydrolysate: Leu 3.00, Ile 1.00, Ala 2.90, Lys 1.99 (recovery of Leu 87%). *Anal.* Calcd for $C_{70}H_{106}N_{12}O_{16}$: C, 61.29; H, 7.79; N, 12.26. Found: C, 61.48; H, 7.64; N, 12.39.

Z(OMe)-Lys(Z)-Ala-Ile-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2——The above protected nonapeptide amide (2.80 g, 2.04 mmol) was treated with TFA-anisole (3.6 ml—0.9 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (6.0 ml) containing Et_3N (0.31 ml, 2.24 mmol). The azide [prepared from 1.60 g (3.02 mmol) of Z(OMe)–Lys(Z)–Ala–$NHNH_2$] in DMF (3 ml) and Et_3N (0.47 ml, 3.37 mmol) were added and the mixture was stirred at 4° for 20 hr. Additional azide [prepared from 0.80 g (1.51 mmol) of the hydrazide] in DMF (1.5 ml) and Et_3N (0.23 ml, 1.68 mmol) were added and stirring was continued for an additional 18 hr. The solvent was removed by evaporation and the product was isolated as described above; yield 2.10 g (50%), mp 283° dec., $[\alpha]_D^{20}$ −31.9° (c=1.0, DMSO), Rf_1 0.75. Amino acid ratios in 6 N HCl hydrolysate: Ala 3.99, Ile 0.98, Leu 3.00, Lys 3.16 (recovery of Leu 88%). *Anal.* Calcd for $C_{87}H_{129}N_{15}O_{20}\cdot 2H_2O$: C, 60.01; H, 7.70; N, 12.07. Found: C, 59.81; H, 7.33; N, 12.13.

Z(OMe)-Ile-Asn-Leu-Lys(Z)-Ala-Ile-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2——The above pro-

tected undecapeptide amide (2.05 g, 1.20 mmol) was treated with TFA-anisole (2.0 ml—0.5 ml) and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF (7.0 ml) containing Et_3N (0.13 ml, 1.32 mmol). The azide [prepared from 0.81 g (1.50 mmol) of Z(OMe)–Ile–Asn–Leu–$NHNH_2$] in DMF (7 ml) and Et_3N (0.23 ml, 1.70 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4° for 24 hr. The solvent was evaporated off and the product was isolated as stated above, then precipitated twice from DMSO with MeOH; yield 1.20 g (49%), mp 286° dec., $[\alpha]_D^{20}$ −15.4° (c=0.7, DMSO), Rf_1 0.70. Amino acid ratios in 6 N HCl hydrolysate: Asp 0.94, Ala 3.91, Ile 1.96, Leu 4.00, Lys 2.94 (recovery of Leu 87%). *Anal.* Calcd for $C_{103}H_{157}N_{19}O_{24}\cdot 3H_2O$: C, 58.92; H, 7.82; N, 12.67. Found: C, 59.06; H, 7.59; N, 12.88.

H-Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Ala-Lys-Lys-Leu-Leu-NH_2 (mastoparan M)——The above protected tetradecapeptide amide (105 mg, 50 μmol) was treated with TFA (2.0 ml) in the presence of thioanisole (0.24 ml, 40 equiv.) and *m*-cresol (0.21 ml, 40 equiv.) at room temperature for 4 hr, then *n*-hexane was added. The residue was treated with ether and the resulting powder was treated again with the same reagent system as above for 4 hr. The deprotected peptide precipitated by ether was dissolved in H_2O (7 ml) and treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min. The solution was filtered, and the filtrate was lyophilized. The residue was dissolved in a small amount of the upper phase of *n*-BuOH–AcOH–H_2O (4: 1: 5) and the solution was applied to a column of Sephadex G-25 (3.0 × 112 cm) previously equilibrated with the lower phase of the above solvent system. The column was then developed with the upper phase of the above solvent and individual fractions (7.0 ml each) were examined by ninhydrin. The desired fractions (tube Nos. 90—110) were combined and the solvent was removed by lyophilization to give a fluffy white powder; yield 47 mg (53%), Rf_2 0.45, Rf_3 0.56, $[\alpha]_D^{20}$ −96.5° (c=0.1, 3% AcOH). Amino acid ratios in 6 N HCl hydrolysate: Asp 0.94, Ala 3.92; Ile 1.86, Leu 4.00, Lys 3.05 (recovery of Leu 91%). Amino acid ratios in leucine aminopeptidase digest: Asn 0.96, Ala 4.03, Ile 1.93, Leu 4.00, Lys 3.12 (recovery of Leu 89%). *Anal.* Calcd for $C_{70}H_{131}N_{19}O_{15}\cdot 4CH_3COOH\cdot 2H_2O$: C, 53.40; H, 8.62; N, 15.17. Found: C, 53.46; H, 8.51; N, 15.86.

The sample and its DNS derivative exhibited *Rf* values identical with those of natural mastoparan M: Rf_4 0.70 (fluorescamine stain) and Rf_5 0.50 (DNS-derivative).

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References and Notes

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2) Amino acids, peptides and their derivatives mentioned here are of the L-configuration. Abbreviations used: Z(OMe), *p*-methoxybenzyloxycarbonyl; Z, benzyloxycarbonyl; TFA, trifluoroacetic acid; DMF, dimethylformamide; DMSO, dimethylsulfoxide.
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