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Studies on Peptides. LXXXIV.[1,2] Synthesis of the Tetradecapeptide Amide corresponding to the Wasp Venom, Mastoparan X

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The tetradecapeptide amide venom, mastoparan X, isolated from the Japanese hornet (*Vespa xanthoptera*), was synthesized by the methanesulfonic acid deprotecting procedure, employing *m*-cresol as a cation scavenger.

Keywords——wasp venom, mastoparan X; mast cell degranulating activity; suppression of tryptophan; methanesulfonic acid deprotection; *m*-cresol as a scavenger

Nakajima *et al.*[4] isolated a tetradecapeptide amide, named mastoparan X, from the Japanese hornet, *Vespa xanthoptera*. We report here the synthesis of a tetradecapeptide amide corresponding to the entire amino acid sequence of this wasp venom.

Mastoparan X (I) contains three Lys residues, and this is the only amino acid which requires side chain protection. Thus, Lys(Z) was employed in combination with the TFA-labile N^{α}-protecting group, Z(OMe).[5] In addition to Lys, this peptide contains two other functional amino acids, Met and Trp, which, from a synthetic viewpoint, present some difficulty as regards the suppression of side reactions involving their functional groups. We therefore decided to protect Met as Met(O) to prevent partial oxidation and S-alkylation during the synthesis.[6,7] In order to suppress alkylation at the indole moiety of Trp[8,9] during the acidolytic N^{α}-deprotection, the N-terminal tripeptide, Z(OMe)-Ile-Asn-Trp-OH, was selected as one building unit as shown in Fig. 1. This tripeptide was prepared by hydrogenolysis of Z-Asn-Trp-OH followed by condensation with Z(OMe)-Ile-OH by the NP procedure.[10] Besides this tripeptide, five small peptide units were selected as building blocks, *i.e.*, Z(OMe)-Leu-Leu-NH_2 Z(OMe)-Lys(Z)-Lys(Z)-$NHNH_2$, Z(OMe)-Met(O)-Ala-$NHNH_2$, Z(OMe)-Ile-Ala-Ala-$NHNH_2$ and Z(OMe)-Lys(Z)-Gly-$NHNH_2$. Among these, Z(OMe)-Lys(Z)-Lys(Z)-$NHNH_2$ is a known compound[11] which has been prepared by the TCP pro-

1) Part LXXXIII: S. Funakoshi, N. Fujii, K. Akaji, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **27**, 2151 (1979).
2) Amino acids, peptides and their derivatives mentioned here are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, NP=*p*-nitrophenyl, TCP=2,4,5-trichlorophenyl, DCC=dicyclohexylcarbodiimide, HOBT=1-hydroxybenzotriazole, TFA=trifluoroacetic acid, DMSO=dimethylsulfoxide, THF=tetrahydrofuran, MSA=methanesulfonic acid, DMF=dimethylformamide.
3) Location: *a) Sakyo-ku, 606, Kyoto*; *b) 1-2-3 Kasumi, 734, Hiroshima.*
4) Y. Hirai, M. Kuwada, T. Yasuhara, H. Yoshida, and T. Nakajima, *Chem. Pharm. Bull.*, **27**, 1945 (1979).
5) F. Weygand and K. Hunger, *Chem. Ber.*, **98**, 1 (1962).
6) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961).
7) N. Fujii, T. Sasaki, S. Funakoshi, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 650 (1978).
8) E. Wünsch, E. Jaeger, L. Kisfaludy, and M. Low, *Angew. Chem.*, **89**, 330 (1977); E. Jaeger, P. Thamm, S. Knof, E. Wünsch, M. Low, and L. Kisfaludy, *Z. Physiol. Chem.*, **359**, 1617 (1978); E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *ibid.*, **359**, 1629 (1978); M. Low, L. Kisfaludy, E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *ibid.*, **359**, 1637 (1978).
9) H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 3144 (1978).
10) M. Bodanszky and V. du Vigneaud. *J. Am. Chem. Soc.*, **81**, 5688 (1959).
11) H. Yajima, K. Koyama, Y. Kiso, A. Tanaka, and N. Nakamura, *Chem. Pharm. Bull.*, **24**, 492 (1976).

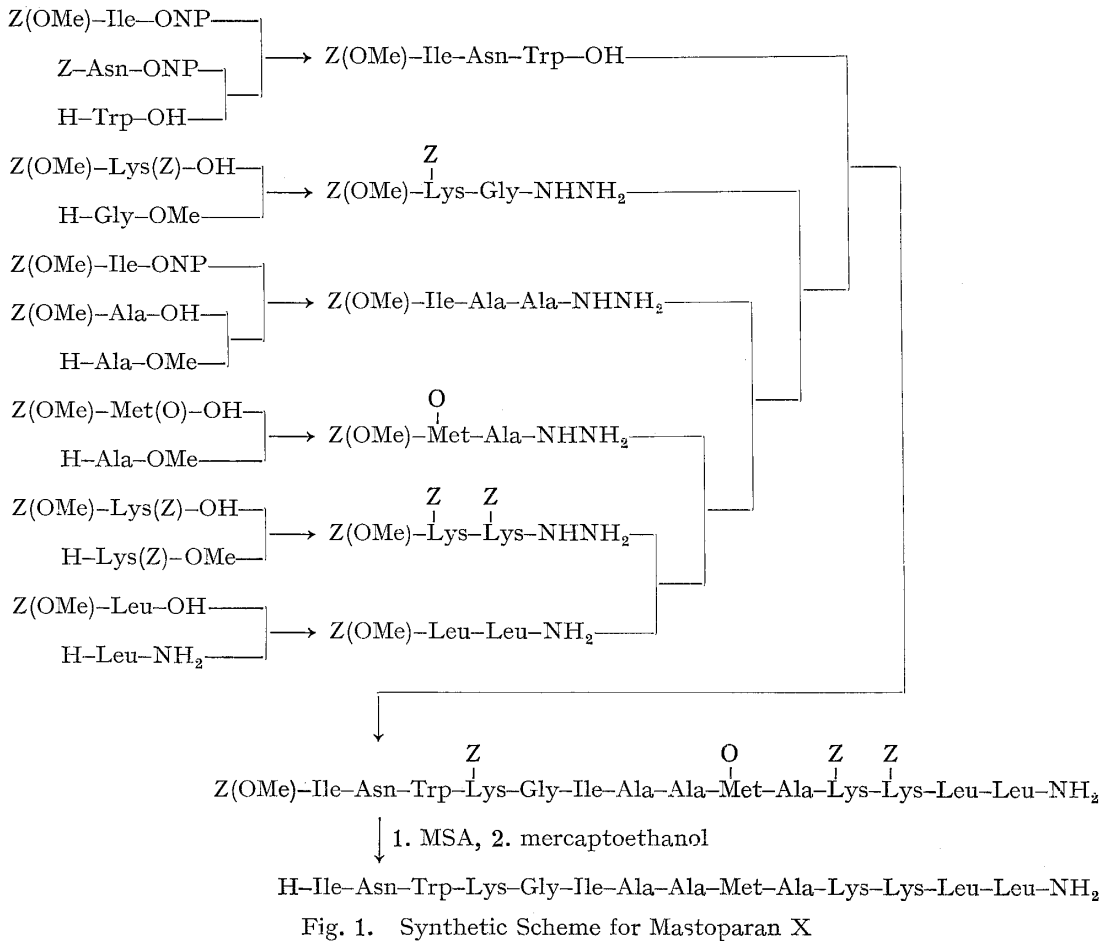

Fig. 1. Synthetic Scheme for Mastoparan X

cedure.[12] The others were prepared by known amide forming reactions, such as the DCC[13] or the mixed anhydride[14] procedure.

Rudinger's azide procedure[15] was employed to condense these units successively, except for the N-terminal tripeptide unit. The final condensation of the TFA-treated sample of the protected undecapeptide amide, Z(OMe)-Lys(Z)-Gly-Ile-Ala-Ala-Met(O)-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2, with the tripeptide was performed using DCC in the presence of HOBT[16] in order to suppress possible racemization. The protected mastoparan X, Z(OMe)-Ile-Asn-Trp-Lys(Z)-Gly-Ile-Ala-Ala-Met(O)-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2, thus obtained, was purified by precipitation from DMF with methanol followed by gel-filtration on Sephadex LH-20 with DMF as an eluent.

For deprotection, the protected mastoparan X was exposed to MSA[17] in the presence of *m*-cresol. A preliminary test indicated that when MSA was used as a deprotecting reagent in the final step of the synthesis of Trp-containing peptides, sulfur-containing scavengers, such as thioanisole or mercaptoethanol, had little suppressing effect on side reactions. For the N^{α}-deprotection of Trp-peptides with TFA or dilute MSA,[18] addition of sulfur compounds

12) J. Press and R.A. Boissonnas, *Helv. Chim. Acta*, **46**, 1609, 1625, 1637 (1963).
13) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
14) Th. Wieland and R. Sehring, *Ann. Chem.*, **569**, 122 (1950); J.R. Vaughan, Jr. and R.L. Osato, *J. Am. Chem. Soc.*, **73**, 5553 (1951); R.A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).
15) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, **26**, 2333 (1961).
16) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
17) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, **23**, 1164 (1975).
18) H. Yajima, H. Ogawa, N. Fujii, and S. Funakoshi, *Chem. Pharm. Bull.*, **25**, 740 (1977).

was effective, but in the MSA deprotection, such additional scavengers appeared to give rise to an additional byproduct. In the light of the recent investigations on scavenger systems by Lundt *et al.*,[19] we selected *m*-cresol as a scavenger in the MSA deprotection. The deprotected peptide was converted to the corresponding acetate on Amberlite CG-4B, then the Met(O) residue was reduced by incubation with mercaptoethanol. The progress of the reduction was monitored by thin-layer chromatography (TLC). The reduced peptide was purified by partition chromatography[20] on Sephadex G-25 with *n*-BuOH–AcOH–H_2O (4: 1: 5). Some material eluted by the upper phase of the solvent contained no Trp, when examined by hydrolysis with 4 N MSA,[21] which is known to be useful for the determination of Trp content. The desired compound was eluted with the lower phase and obtained, after lyophilization, as a fluffy white powder. The peptide thus obtained gave a single spot on TLC, and enzymatic digestion gave a satisfactory recovery of Trp.

The dansylated synthetic and natural peptides gave identical *Rf* values on TLC. When the mast cell degranulating activity was compared, the synthetic peptide was found to be as active as natural mastoparan X.

Experimental

General experimental procedures were essentially the same as those described in Part LXII.[22] Aminopeptidase (AP-M, hog kidney) was purchased from the Protein Research Foundation, Osaka (Pierce Co. Lot No. 20214). *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 $CHCl_3$–MeOH–AcOH (9: 1: 0.5), Rf_3 $CHCl_3$–MeOH (10: 0.5), Rf_4 *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2).

Z(OMe)-Leu-Leu-NH_2——The mixed anhydride (prepared from 19.98 g, 67.7 mmol, of Z(OMe)–Leu–OH) in THF (200 ml) was added to an ice-chilled solution of H–Leu–NH_2 (prepared from 14.91 g, 56.4 mmol, of Z–Leu–NH_2[23] by the usual HBr treatment followed by neutralization with Et_3N) in DMF (100 ml) and the mixture was stirred in an ice-bath for 5 hr. The solvent was evaporated off and the residue was treated with 5% citric acid and ether. The resulting powder was washed with 5% citric acid, 5% Na_2CO_3 and H_2O and recrystallized from MeOH and ether; yield 14.43 g (63%), mp 207—210°, $[\alpha]_D^{22}$ −22.0° (c=0.8, DMF), Rf_1 0.65. *Anal.* Calcd for $C_{21}H_{33}N_3O_5$: C, 61.89; H, 8.16; N, 10.31. Found: C, 61.98; H, 8.24; N, 10.48.

Z(OMe)-Met(O)-Ala-OMe——The mixed anhydride (prepared from 10.04 g, 30.5 mmol, of Z(OMe)–Met(O)–OH[7]) in THF (100 ml) was added to an ice-chilled solution of H–Ala–OMe (prepared from 5.11 g, 36.6 mmol, of the hydrochloride with 5.1 ml, 36.6 mmol, of Et_3N) in DMF (35 ml) and the mixture, after stirring for 5 hr, was concentrated. The residue was extracted with AcOEt. The extract was washed with 5% citric acid, 3% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from MeOH and ether; yield 8.83 g (70%), mp 134—137°, $[\alpha]_D^{22}$ −27.0° (c=0.6, DMF), Rf_1 0.65. *Anal.* Calcd for $C_{18}H_{26}N_2O_7S$: C, 52.16; H, 6.32; N, 6.76. Found: C, 51.90; H, 6.29; N, 6.61.

Z-(OMe)-Met(O)-Ala-NHNH_2——Z(OMe)–Met(O)–Ala–OMe (8.79 g, 21 mmol) dissolved in EtOH (100 ml) was treated with 80% hydrazine hydrate (5.3 ml, 5 equiv.) at room temperature overnight. The resulting mass was collected by filtration and washed with EtOH; yield 6.88 g (78%), mp 203—206°, $[\alpha]_D^{20}$ −18.2° (c=0.7, DMF), Rf_1 0.34. *Anal.* Calcd for $C_{17}H_{26}N_4O_6S$: C, 49.26; H, 6.32; N, 13.52. Found: C, 49.27; H, 6.22; N, 13.55.

Z(OMe)-Ala-Ala-OMe——A mixed anhydride (prepared from 19.0 g, 75 mmol, of Z(OMe)–Ala–OH) in THF (150 ml) was added to an ice-chilled solution of H–Ala–OMe (prepared from 10.0 g, 72 mmol, of the hydrochloride with 10.0 ml, 72 mmol, of Et_3N) in DMF (70 ml) and the mixture was stirred in an ice-bath for 5 hr. The solvent was evaporated off and the residue was treated with ether. The resulting powder was washed with 5% citric acid, 5% Na_2CO_3 and H_2O and recrystallized from MeOH and ether; yield 22.16 g (91%), mp 150—154°, $[\alpha]_D^{20}$ +0.7 (c=1.4, DMF), Rf_1 0.69. *Anal.* Calcd for $C_{16}H_{22}N_2O_6$: C, 56.79; H, 6.55; N, 8.28. Found: C, 56.81; H, 6.54; N, 8.25.

Z(OMe)-Ile-Ala-Ala-OMe——Z(OMe)–Ala–Ala–OMe (5.0 g, 14.8 mmol) was treated with TFA-anisole (10—2.5 ml) in an ice-bath for 60 min, then excess TFA was removed by evaporation. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (20 ml)

19) B.F. Lundt, N.L. Johansen, A. Volund, and J. Markussen, *Int. J. Peptide Protein Res.*, **12**, 258 (1978).
20) D. Yamashiro, *Nature* (London), **201**, 76 (1964).
21) R.J. Simpson, M.R. Neuberger, and T.Y. Liu, *J. Biol. Chem.*, **251**, 1936 (1976).
22) H. Ogawa, M. Kubota, and H. Yajima, *Chem. Pharm. Bull.*, **24**, 2428 (1976).
23) F. Weygand and W. Steglich, *Chem. Ber.*, **93**, 2983 (1960).

together with Et_3N (3.3 ml, 23.6 mmol) and Z(OMe)–Ile–ONP (7.40 g, 17.8 mmol). During stirring for 20 min, the solution solidified. After the addition of DMSO (20 ml), the resulting clear solution was stirred for 24 hr and then concentrated. The residue was treated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid, 3% $NaHCO_3$ and H_2O and precipitated from DMF with MeOH; yield 6.50 g, (97%), mp 220—222°, $[\alpha]_D^{22}$ −4.3° (c=0.7, DMF), Rf_1 0.89. *Anal.* Calcd for $C_{22}H_{33}N_3O_7$: C, 58.52; H, 7.37; N, 9.31. Found: C, 58.40; H, 7.37; N, 9.39.

Z(OMe)-Ile-Ala-Ala-NHNH$_2$——Z(OMe)–Ile–Ala–Ala–OMe (6.50 g, 14.4 mmol) in DMF (100 ml) was treated with 80% hydrazine hydrate (14.4 ml, 20 equiv.). The gelatinous mass formed on standing overnight was collected by filtration and precipitated from DMSO with MeOH; yield 4.51 g (69%), mp 255—256°, $[\alpha]_D^{23}$ −17.0° (c=0.5, DMSO), Rf_1 0.40. *Anal.* Calcd for $C_{21}H_{33}N_5O_6$: C, 55.86; H, 7.37; N, 15.51. Found: C, 55.72; H, 7.39; N, 15.41.

Z(OMe)-Lys(Z)-Gly-NHNH$_2$——Z(OMe)–Lys(Z)–Gly–OMe[24] (5.0 g, 9.7 mmol) in MeOH (50 ml) was treated with 80% hydrazine hydrate (4.85 ml, 10 equiv.) overnight. The solvent was removed by evaporation and the residue was treated with H_2O. The resulting powder was recrystallized from MeOH and ether; yield 4.52 g (90%), mp 125—126°, $[\alpha]_D^{25}$ −2.1° (c=0.9, DMF), Rf_1 0.52. *Anal.* Calcd for $C_{25}H_{33}N_5O_7$: C, 58.24; H, 6.45; N, 13.59. Found: C, 58.32; H, 6.44; N, 13.44.

Z-Asn-Trp-OH——Z–Asn–ONP (6.32 g, 16.3 mmol) was added to a solution of H–Trp–OH (5.0 g, 24.5 mmol) in DMF (50 ml) containing Et_3N (5.2 ml, 37.1 mmol) and the mixture was stirred at room temperature for 24 hr. The solvent was removed by evaporation and the residue was dissolved in 5% NH_4OH. The aqueous phase was washed with AcOEt and then acidified with 5% citric acid. The resulting solid was washed with H_2O and precipitated from DMF with MeOH; yield 5.60 g (76%), mp 248—251°, $[\alpha]_D^{22}$ +32.1° (c=0.6, DMF), Rf_1 0.18. Rf_2 0.22. *Anal.* Calcd for $C_{23}H_{24}N_4O_6$: C, 61.05; H, 5.35; N, 12.38. Found: C, 61.19; H, 5.46; N, 12.36.

Z(OMe)-Ile-Asn-Trp-OH——Z–Asn–Trp–OH (5.60 g, 12.4 mmol) in DMF (50 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst in the usual manner. After neutralization with Et_3N, the solution was concentrated and the residue was triturated with EtOH. The resulting powder was dissolved in DMF (30 ml) together with Et_3N (3.9 ml, 27.8 mmol) and Z(OMe)–Ile–ONP (5.80 g, 13.9 mmol). After stirring at room temperature for 24 hr, the solution was concentrated and the residue was treated with 5% citric acid and ether. The resulting powder was purified by batchwise washing with 5% citric acid and H_2O followed by precipitation from DMF with MeOH; yield 2.99 g (43%), mp 182—185°, $[\alpha]_D^{22}$ +13.9° (c=0.7, DMF). Amino acid ratios in the 4 N MSA hydrolysate: Asp 1.00, Ile 1.04, Trp 0.81 (average recovery 90%). Rf_1 0.16. *Anal.* Calcd for $C_{30}H_{37}N_5O_8 \cdot H_2O$: C, 58.71; H, 6.40; N, 11.40. Found: C, 59.16; H, 6.40; N, 11.61.

Z(OMe)-Lys(Z)-Lys(Z)-Leu-Leu-NH$_2$——Z(OMe)–Leu–Leu–NH_2 (3.0 g, 7.4 mmol) was treated with TFA-anisole (6—1.5 ml) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and dissolved in DMF (30 ml) containing Et_3N (1.03 ml, 7.36 mmol). The azide (prepared from 5.30 g, 7.36 mmol, of Z(OMe)–Lys(Z)–Lys(Z)–$NHNH_2$) in DMF (25 ml) and Et_3N (1.24 ml, 7.36 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 residue was treated with 5% citric acid and ether. The resulting powder was washed with 5% citric acid and H_2O and precipitated from DMF with MeOH; yield 5.57 g (81%), mp 224—228°, $[\alpha]_D^{22}$ −19.1° (c=0.8, DMF), Rf_1 0.67. Amino acid ratios in an acid hydrolysate: Lys 1.87. Leu 2.00 (average recovery 87%). *Anal.* Calcd for $C_{49}H_{69}N_7O_{11}$: C, 63.14; H, 7.46; N, 10.52. Found: C, 62.90; H, 7.54; N, 10.35.

Z(OMe)-Met(O)-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH$_2$——Z(OMe)–Lys(Z)–Lys(Z)–Leu–Leu–NH_2 (5.50 g, 5.90 mmol) was treated with TFA-anisole (11—2.3 ml) as usual and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF (30 ml) containing Et_3N (0.83 ml, 5.90 mmol). The azide (prepared from 2.95 g, 7.10 mmol, of Z(OMe)–Met(O)–Ala–$NHNH_2$) in DMF (15 ml) and Et_3N (1.0 ml, 7.14 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 purified by washing as described above followed by precipitation from DMF with MeOH; yield 6.46 g (95%), mp 244—247°, $[\alpha]_D^{22}$ −26.5° (c=0.4, DMSO), Rf_1 0.67, Rf_2 0.44. Amino acid ratios in an acid hydrolysate: Met+Met(O) 0.89, Ala 0.98, Lys 2.08, Leu 2.00 (average recovery 91%). *Anal.* Calcd for $C_{57}H_{83}N_9O_{14}S$: C, 59.51; H, 7.27; N, 10.96. Found: C, 59.29; H, 7.23; N, 10.79.

Z(OMe)-Ile-Ala-Ala-Met(O)-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH$_2$——The above protected hexapeptide amide (6.0 g, 5.21 mmol) was treated with TFA–anisole (12 ml–3 ml) and the N^{α}-deprotected peptide isolated as described above was then dissolved in DMSO (15 ml) containing Et_3N (0.73 ml, 5.21 mmol). The azide (prepared from 3.06 g, 6.78 mmol of Z(OMe)–Ile–Ala–Ala–$NHNH_2$) in DMF (15 ml) and Et_3N (0.95 ml, 6.78 mmol) were added to the above ice-chilled solution and the mixture, after stirring at 4° for 24 hr, was concentrated. The product was purified by washing as described above, followed by precipitation from DMSO with MeOH; yield 7.25 g (99%), mp 259—261°, $[\alpha]_D^{22}$ −23.8° (c=0.6, DMSO), Rf_1 0.76, Rf_2 0.29. Amino acid ratios in an acid hydrolysate: Ile 1.06, Ala 3.10, Met+Met(O) 0.84, Lys 2.07, Leu 2.00 (average

24) Y. Kai, H. Kawatani and H. Yajima, *Chem. Pharm. Bull.*, **23**, 2339 (1975).

recovery 90%). *Anal.* Calcd for $C_{69}H_{104}N_{12}O_{17}S$: C, 58.95; H, 7.46; N, 11.96. Found: C, 58.69; H,7.50; N, 11.99.

Z(OMe)-Lys(Z)-Gly-Ile-Ala-Ala-Met(O)-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2——The above nonapeptide amide (5.77 g, 4.12 mmol) was treated with TFA-anisole (11.5—2.9 ml). The N^{α}-deprotected peptide isolated as described above was dissolved in DMSO–DMF (25 ml–25 ml) containing Et_3N (0.58 ml, 4.14 mmol). The azide (prepared from 2.54 g, 4.93 mmol, of Z(OMe)–Lys(Z)–Gly–$NHNH_2$) in DMF (12 ml) and Et_3N (0.7 ml, 5.0 mmol) were added to this ice-chilled solution and the mixture, after stirring at 4° for 24 hr, was concentrated. The product was purified by washing as described above, followed by precipitation from DMSO with MeOH; yield 6.54 g (92%), mp 256—258°, $[\alpha]_D^{22}$ −33.8° (c=0.5, DMSO), Rf_1 0.71. Amino acid ratios in an acid hydrolysate: Lys 3.04, Gly 1.01, Ile 1.02, Ala 2.99, Met+Met(O) 0.80, Leu 2.00 (average recovery 90%). *Anal.* Calcd for $C_{85}H_{125}N_{15}O_{21}S$: C, 59.18; H, 7.30; N, 12.18. Found: C, 59.07; H, 7.37; N, 12.25.

Z(OMe)-Ile-Asn-Trp-Lys(Z)-Gly-Ile-Ala-Ala-Met(O)-Ala-Lys(Z)-Lys(Z)-Leu-Leu-NH_2——The above protected undecapeptide amide (2.0 g, 1.16 mmol) was treated with TFA-anisole (4.0 ml–1.0 ml) as usual and a mixture of ether and 5% Na_2CO_3 was added to provide a powder, which was collected by filtration, dried over P_2O_5 *in vacuo* for 3 hr and then dissolved in DMSO–DMF (10 ml–10 ml). To this solution, HOBT (0.36 g, 2.32 mmol), Z(OMe)–Ile–Asn–Trp–OH (1.04 g, 1.74 mmol) and DCC (0.48 g, 2.32 mmol) were successively added. The mixture was stirred at room temperature for 12 hr and further DCC (0.24 g, 1.16 mmol) was added. After stirring for 24 hr, the solution was filtered, the filtrate was concentrated and the residue was treated with AcOEt and 5% citric acid. The resulting powder was washed with 5% citric acid, 3% $NaHCO_3$ and H_2O and then precipitated twice from DMF with MeOH; yield 1.80 g (73%), mp 257—261°, $[\alpha]_D^{20}$ −30.4° (c=0.2, DMSO), Rf_1 0.60. Amino acid ratios in 4 N MSA hydrolysate: Ile 2.12, Asp 1.00, Trp 0.85, Lys 3.17, Gly 1.00, Ala 3.08, Met+Met(O) 0.99, Leu 2.00 (average recovery 88%). *Anal.* Calcd for $C_{106}H_{152}N_{20}O_{25}S$: C, 59.53; H, 7.16; N, 13.10. Found: C, 59.83; H, 7.45; N, 12.89.

H-Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH_2 (Mastoparan X)——The above protected tetradecapeptide amide (88 mg, 41 μmol) was treated with MSA (3.5 ml) in the presence of *m*-cresol (0.3 ml) in an ice-bath for 30 min and at room temperature for 60 min, then dry ether was added. The precipitate was dissolved in a small amount of H_2O and treated with Amberlite CG-4B (acetate form, approximately 1 g) for 30 min. The resin was removed by filtration and the filtered solution was lyophilized. The residue was dissolved in H_2O (3 ml) and incubated with 2-mercaptoethanol (0.3 ml) at 70° for 20 hr. This solution was applied to a column of Sephadex G-25 (3×130 cm), equilibrated with the lower phase of *n*-BuOH–AcOH–H_2O (4: 1: 5), and the column was eluted with the upper phase of this solvent system (440 ml) at a flow rate of 27 ml/hr. The absorption of each fraction (5.5 ml) was monitored at 280 nm. Tow peaks were detected: F-1 (tube No. 41—49, *m*-cresol and mercaptoethanol) and F-2 (tube No. 52—79). Lyophilization of F-2 afforded a colored powder; yield 3.0 mg. Amino acid ratios in the 4 N MSA hydrolysate: Ile 1.87, Asp 0.81, Trp 0, Lys 3.07, Gly 1.02, Ala 2.99, Met 0.89, Leu 2.00 (recovery of Leu, 81%). When the column was further eluted with the lower phase, the main peak, F-3 (tube No. 212—226), appeared. Removal of the solvent of F-3 followed by lyophilization gave a white fluffy powder; yield 47 mg (64%), $[\alpha]_D^{22}$ +39.0° (c=0.2, 3% AcOH), Rf_4 0.45. Amino acid ratios in the 4 N MSA hydrolysate and AP-M digest (numbers in brackets): Ile 1.87 (1.85), Asp 0.81, Asn (not determined), Lys 3.07 (3.04), Trp 0.75 (0.88), Gly 1.02 (0.92), Ala 2.99 (2.93), Met 0.89 (0.89), Leu 2.00 (2.00), average recovery 81% (78%). *Anal.* Calcd for $C_{73}H_{126}H_{20}O_{15}\cdot 4CH_3COOH$: C, 54.16; H, 7.97; N, 15.60. Found: C, 54.85; H, 7.96; N, 15.74.

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