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Studies on Peptides. LXXXVI.[1,2] Application of the Trifluoroacetic Acid-Thioanisole Deprotecting Procedure for the Synthesis of a Wasp Venom, Mastoparan

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A new deprotecting procedure with trifluoroacetic acid–thioanisole–*m*-cresol was employed for the removal of the benzyloxycarbonyl group from the N^{ε}-amino group of lysine. A wasp venom, mastoparan, was synthesized using this procedure to confirm its validity in peptide synthesis.

Keywords——synthesis of wasp venom, mastoparan; thioanisole–*m*-cresol, as scavengers; removal of the benzyloxycarbonyl group by trifluoroacetic acid; acceleration of acidolytic cleavage of benzyloxycarbonyl group by sulfur compounds; purification by partition chromatography on Sephadex G-25

In 1976, we reported that the acidolytic cleavage of aromatic ethers by MSA was accelerated by a sulfur compound, methionine.[4] Independently, Node *et al.*[5,6] reported an efficient procedure for the demethylation of methyl ethers and cleavage of the alcoholic carbon oxygen of lactones using an aluminum halide–thiol system.

Several studies on the effect of nucleophilic sulfur scavenger systems during acidolytic deprotection have appeared in the literature on peptide chemistry. In 1977, Brady *et al.*[7] mentioned briefly that dimethylsulfide enhanced the rate of cleavage of the Z group in TFA. Kiso *et al.*,[8] examining the powerful cation capture properties of sulfur compounds, found that the Z group could be completely cleaved by TFA at room temperature (3 hr) when thioanisole, instead of anisole, was employed as a cation scavenger. In the TFA–anisole system, the Z group is known to remain intact under these conditions. They also stated that removal of the Bzl group from Tyr(Bzl), Thr(Bzl) and Ser(Bzl) by TFA was accelerated by various sulfur compounds, but to different extents. We also found that the N^{im}–Tos or the MBS group of histidine was smoothly cleaved by TFA in the presence of dimethylsulfide,[9] though these groups are known to be acidolytically removed by HF.[10] These findings raise the pos-

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2) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Tos=*p*-toluenesulfonyl, MBS=*p*-methoxybenzenesulfonyl, NP=*p*-nitrophenyl, TCP=2,4,5-trichlorophenyl, TFA=trifluoroacetic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide, THF=tetrahydrofuran, MSA=methanesulfonic acid.
3) Location: *Sakyo-ku, Kyoto, 606 Japan.*
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sibility of an alternative TFA deprotecting procedure in the present peptide synthesis, if suitable sulfur compounds are selected as scavengers.

The role of various cation scavengers during the TFA deprotection of the Boc group was examined in detail by Lundt *et al.*[11] in 1978. It was shown that anisole traps the *tert*-butyl cation at the *p*-position, while thioanisole forms methyl phenyl *tert*-butyl sulfonium trifluoroacetate, which still possesses the ability to alkylate other functional amino acids, such as methionine and tryptophan. Though they suggested that thioanisole should be avoided as a scavenger, we considered the use of an additional scavenger, such as *m*-cresol, to trap all alkylating activity in the TFA deprotecting media in order to apply the TFA–sulfur compounds system for practical peptide synthesis.

In order to evaluate the usefulness of this new deprotecting procedure in practical peptide synthesis, we selected a wasp venom, mastoparan, as a suitable synthetic target. Mastoparan, the structure of which was recently reported by Hirai *et al.*,[12] contains three lysine residues and this is the only amino acid which requires side chain protection for the synthesis. This peptide was synthesized by Yanaihara *et al.*[13] utilizing Lys(Tos) with the sodium in liquid ammonia deprotecting procedure[14] and by Hirai *et al.*[12] using Lys(Z) with the MSA deprotecting procedure.[15] In our synthesis, Lys(Z) was also employed, but the protecting group was

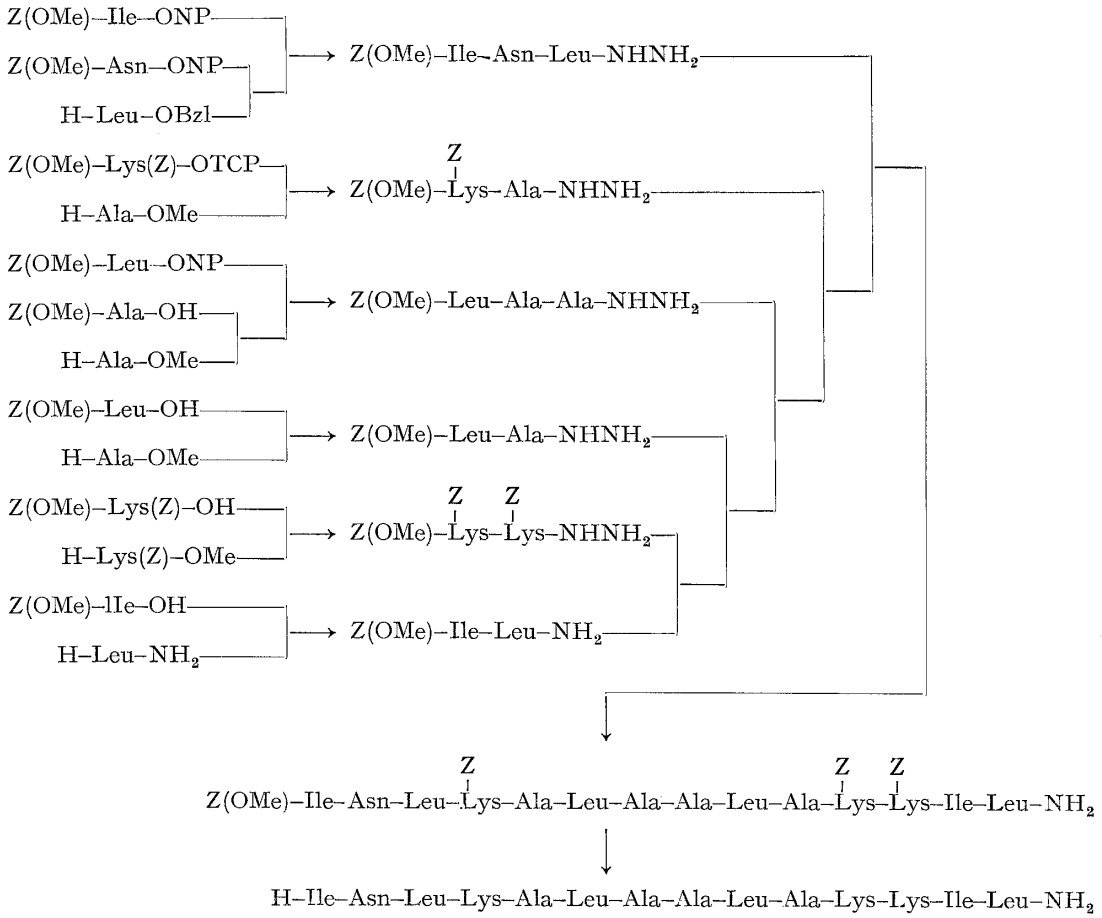

Fig. 1. Synthetic Scheme for Mastoparan

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removed with TFA–thioanisole–*m*-cresol. The identity of our product with that of Hirai *et al.*[12] was confirmed.

As shown in Fig. 1, in combination with Lys(Z), the Z(OMe) group[16] was employed for the temporary protection of the N^{α}-function of intermediates. The entire peptide backbone of mastoparan was built up by the successive azide condensation[17] of 4 dipeptide units and 2 tripeptide units. Among these fragments, Z(OMe)-Lys(Z)-Lys(Z)-$NHNH_2$[18] and Z(OMe)-Leu-Ala-$NHNH_2$[19] are known compounds. The other 4 fragments, Z(OMe)-Ile-Leu-NH_2, Z(OMe)-Leu-Ala-Ala-$NHNH_2$, Z(OMe)-Lys(Z)-Ala-$NHNH_2$ and Z(OMe)-Ile-Asn-Leu-$NHNH_2$, were newly synthesized by known amide forming reactions. Owing to the poor solubility of protected peptide amides in DMF with increasing chain length, the last two condensations were performed with the aid of DMSO. The final protected tetradecapeptide amide, Z(OMe)-Ile-Asn-Leu-Lys(Z)-Ala-Leu-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Ile-Leu-NH_2, was purified by repeated precipitation from DMSO with methanol.

The protected mastoparan thus obtained was then exposed to TFA at room temperature in the presence of thioanisole and *m*-cresol to remove all protecting groups, Z(OMe) and Z. The progress of the reaction was monitored by TLC. As expected, after 4 hr, the spot corresponding to the starting material was fully transformed to a new spot. The deprotected peptide was converted to the corresponding acetate by treatment with Amberlite CG-4B and the contaminating scavengers were removed by partition chromatography[20] on Sephadex G-25 with *n*-BuOH–AcOH–H_2O (4:1:5). After removing the scavengers with the upper phase of the above solvent system, the desired compound was eluted with the lower phase. The homogeneity of the synthetic tetradecapeptide amide thus obtained in 64% yield was ascertained by TLC, acid hydrolysis and aminopeptidase (AP-M) digestion. Our synthetic peptide exhibited *Rf* values on TLC in two different solvent systems identical with those of synthetic mastoparan obtained by an alternative route.[12]

In this model experiment, we showed that the synthesis of peptides which require the Z protecting group could be successfully achieved by this modified TFA deprotecting procedure. It should be noted that Bodanszky *et al.*[21] exposed protected porcine vasoactive intestinal polypeptide to TFA at room temperature for 2.5 days to remove the Z group. We intend to examine further the applicability of this deprotecting procedure to the synthesis of more complex peptides.

Experimental

The azide reaction was performed according to Honzl and Rudinger[17] with isoamylnitrite. The mixed anhydride was prepared according to Vaughan and Osato[22] with isobutyl chloroformate. Aminopeptidase (AP-M, hog kidney) was purchased from the Protein Res. Foundation, Osaka (Pierce Co. Lot. No. 20214). *Rf* values in thin-layer chromatography 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)-Ile-Leu-NH_2——A mixed anhydride (prepared from 8.39 g, 28.4 mmol of Z(OMe)–Ile–OH) in dry THF (60 ml) was added to an ice-chilled solution of H–Leu–NH_2 (prepared from 5.0 g, 18.9 mmol of Z–Leu–NH_2 by the usual treatment with HBr followed by neutralization with 2.65 ml, 18.9 mmol of Et_3N) in DMF (80 ml). After stirring in an ice-bath for 3 hr, the solution was concentrated and the residue was triturated with ether and 5% citric acid. The resulting powder was purified by washing with 5% citric acid and 5% Na_2CO_3 followed by precipitation from DMF with ether; yield 2.54 g (33%), mp 232—233°,

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$[\alpha]_D^{20}$ −5.6° (c=1.1, DMF), Rf_1 0.69. *Anal.* Calcd for $C_{21}H_{33}N_3O_5$: C, 61.89; H, 8.16; N, 10.31. Found: C, 61.70; H, 8.27; N, 10.51.

Z(OMe)-Leu-Ala-Ala-OMe——Z(OMe)–Ala–Ala–OMe (8.0 g, 23.6 mmol) was treated with TFA–anisole (16 ml–4 ml) in an ice-bath for 60 min and excess TFA was removed by evaporation. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 hr and dissolved in DMF (50 ml) together with Et_3N (6.4 ml, 47.2 mmol) and Z(OMe)–Leu–ONP (9.83 g, 23.6 mmol). After stirring for 24 hr, the solution was concentrated. Treatment of the residue with ether and 5% citric acid afforded a powder, which was purified by washing with 5% citric acid, 5% $NaHCO_3$ and H_2O, followed by recrystallization from MeOH and ether; yield 8.57 g (80%), mp 169—170°, $[\alpha]_D^{22}$ −63.4° (c=0.6, MeOH), Rf_1 0.79. *Anal.* Calcd for $C_{22}H_{33}N_3O_7$: C, 58.52; H, 7.37; N, 9.31. Found: C, 58.61; H, 7.42; N, 9.37.

Z(OMe)-Leu-Ala-Ala-NHNH$_2$——Z(OMe)–Leu–Ala–Ala–OMe (8.58 g, 19 mmol) dissolved in MeOH (100 ml) was treated with 80% hydrazine hydrate (9.5 ml, 10 equiv.) at room temperature overnight. The gelatinous mass formed was precipitated from DMF with MeOH; yield 6.01 g (70%), mp 236—237°, $[\alpha]_D^{22}$ −17.7° (c=0.5, DMF), Rf_1 0.59. Amino acid ratios in an acid hydrolysate: Leu 1.00, Ala 1.92 (recovery 93%). *Anal.* Calcd for $C_{21}H_{33}N_5O_6$: C, 55.86; H, 7.37; N, 15.51. Found: C, 55.65; H, 7.49; N, 15.45.

Z(OMe)-Lys(Z)-Ala-OMe——Z(OMe)–Lys(Z)–OTCP (22.30 g, 36 mmol) and Et_3N (5.0 ml, 36 mmol) were added to a solution of H–Ala–OMe (prepared from 5.0 g, 36 mmol of the hydrochloride with 5.0 ml, 36 mmol of Et_3N) in DMF (150 ml). After stirring for 24 hr, the solution was concentrated and the residue was extracted with AcOEt. The extract was washed with 5% citric acid, 5% Na_2CO_3 and H_2O–NaCl, dried over Na_2SO_4 and then concentrated. The residue was recrystallized from THF and ether; yield 11.44 g (60%), mp 130—131°, $[\alpha]_D^{22}$ −23.5° (c=0.8, DMF), Rf_1 0.87. *Anal.* Calcd for $C_{27}H_{35}N_3O_8$: C, 61.23; H, 6.66; N, 7.94. Found: C, 61.19; H, 6.63; N, 7.90.

Z(OMe)-Lys(Z)-Ala-NHNH$_2$——Z(OMe)–Lys(Z)–Ala–OMe (9.12 g, 17.2 mmol) dissolved in a mixture of DMF–MeOH (80 ml–20 ml) was treated with 80% hydrazine hydrate (8.6 ml, 10 equiv.) at room temperature overnight. The solvent was evaporated off and the resulting mass was precipitated from DMF with EtOH; yield 7.56 g (83%), mp 173—174°, $[\alpha]_D^{20}$ −3.6° (c=0.6, DMF), Rf_1 0.66. *Anal.* Calcd for $C_{26}H_{35}N_5O_7$: C, 58.96; H, 6.66; N, 13.23. Found: C, 59.13; H, 6.60; N, 13.30.

Z(OMe)-Asn-Leu-OBzl——Z(OMe)–Asn–ONP (6.26 g, 15 mmol) and Et_3N (2.1 ml, 15 mmol) were added to a solution of H–Leu–OBzl (prepared from 5.90 g, 15 mmol of the tosylate with 2.1 ml, 15 mmol of Et_3N) in DMF (50 ml). After stirring for 24 hr, the solution was concentrated and the residue was triturated with ether and 5% citric acid. The resulting powder was purified by washing with 5% citric acid, 5% Na_2CO_3 and H_2O, followed by recrystallization from MeOH and ether; yield 5.62 g (75%), mp 146—148°, $[\alpha]_D^{20}$ −1.8° (c=1.1, DMF). *Anal.* Calcd for $C_{26}H_{33}N_3O_7$: C, 62.51; H, 6.66; N, 8.41. Found: C, 62.63; H, 6.70; N, 8.39.

Z(OMe)-Ile-Asn-Leu-OBzl——Z(OMe)–Asn–Leu–OBzl (5.50 g, 11 mmol) was treated with TFA–anisole (11 ml–2.8 ml) as mentioned above and 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 (50 ml) together with Et_3N (3.0 ml, 22 mmol) and Z(OMe)–Ile–ONP (4.60 g, 11 mmol). After stirring for 24 hr, the solution was concentrated and the residue was purified by washing as described above, followed by precipitation from DMF with AcOEt; yield 4.43 g (66%), mp 199—202°, $[\alpha]_D^{20}$ −10.6° (c=0.9, DMF). *Anal.* Calcd for $C_{32}H_{44}N_4O_8$: C, 62.73; H, 7.24; N, 9.15. Found: C, 62.81; H, 7.48; N, 9.12.

Z(OMe)-Ile-Asn-Leu-NHNH$_2$——Z(OMe)–Ile–Asn–Leu–OBzl (4.40 g, 7.2 mmol) dissolved in DMF–MeOH (30 ml–10 ml) was treated with 80% hydrazine hydrate (3.6 ml, 10 equiv.). The mass that formed on standing overnight was washed with EtOH and precipitated from DMSO with MeOH; yield 2.89 g (75%), mp 252—256°, $[\alpha]_D^{22}$ +80.6° (c=0.6, DMSO), Rf_1 0.45. Amino acid ratios in an acid hydrolysate: Ile 0.99, Asp 0.99, Leu 1.00 (average recovery 92%). *Anal.* Calcd for $C_{25}H_{40}N_6O_7 \cdot 1.5H_2O$: C, 53.27; H, 7.69; N, 14.20. Found: C, 53.24; H, 7.61; N, 13.83.

Z(OMe)-Lys(Z)-Lys(Z)-Ile-Leu-NH$_2$——Z(OMe)–Ile–Leu–NH_2 (3.0 g, 7.36 mmol) was treated with TFA–anisole (6.0 ml–1.5 ml) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and dissolved in DMF (20 ml) containing Et_3N (1.03 ml, 7.36 mmol). To this ice-chilled solution were added the azide (prepared from 5.31 g, 7.36 mmol of Z(OMe)–Lys(Z)–Lys(Z)–$NHNH_2$) in DMF (25 ml) and Et_3N (1.24 ml, 8.86 mmol), and the mixture was stirred at 4° for 24 hr. The solvent was evaporated off and the residue was treated with ether and 5% citric acid. The residue was purified by washing with 5% citric acid and H_2O, followed by precipitation from DMSO with MeOH; yield 6.31 g (92%), mp 247—249°, $[\alpha]_D^{20}$ −14.6° (c=0.8, DMSO), Rf_1 0.83. Amino acid ratios in an acid hydrolysate: Ile 1.00, Leu 1.06, Lys 2.05 (average recovery 94%). *Anal.* Calcd for $C_{49}H_{69}N_7O_{11}$: C, 63.14; H, 7.46; N, 10.52. Found: C, 63.12; H, 7.46; N, 10.42.

Z(OMe)-Leu-Ala-Lys(Z)-Lys(Z)-Ile-Leu-NH$_2$——Z(OMe)–Lys(Z)–Lys(Z)–Ile–Leu–NH_2 (2.0 g, 2.15 mmol) was treated with TFA–anisole (4 ml–1 ml) and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF (15 ml) containing Et_3N (0.30 ml, 2.15 mmol). To this ice-chilled solution were added the azide (prepared from 0.98 g, 2.57 mmol of Z(OMe)–Leu–Ala–$NHNH_2$) in DMF (8 ml) and Et_3N (0.3 ml, 2.57 mmol), and the mixture was stirred at 4° for 24 hr. The solvent was evaporated off and the residue was treated with ether and 5% citric acid. The product was purified by washing as described above, followed by precipitation from DMSO with MeOH; yield 2.02 g (83%), mp 246—248°, $[\alpha]_D^{20}$ −15.2°

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(c=0.5, DMSO), Rf_1 0.77. Amino acid ratios in an acid hydrolysate: Ala 1.11, Ile 1.00, Leu 2.01, Lys 2.20 (average recovery 89%). *Anal.* Calcd for $C_{58}H_{85}N_9O_{13}$: C, 62.40; H, 7.68; N, 11.29. Found: C, 62.18; H, 7.69; N, 11.43.

Z(OMe)-Leu-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Ile-Leu-NH_2——The above protected hexapeptide amide (2.0 g, 1.79 mmol) was treated with TFA–anisole (4 ml–1 ml) and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF (15 ml) containing Et_3N (0.25 ml, 1.79 mmol). To this ice-chilled solution were added the azide (prepared from 1.0 g, 2.15 mmol of Z(OMe)–Leu–Ala–Ala–$NHNH_2$) in DMF (8 ml) and Et_3N (0.25 ml, 1.79 mmol), 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 DMSO with MeOH; yield 2.07 g (81%), mp 274—275°, $[\alpha]_D^{20}$ −32.0° (c=0.4, DMSO), Rf_1 0.66. Amino acid ratios in an acid hydrolysate: Leu 3.13, Ala 3.11, Lys 2.01, Ile 1.00 (average recovery 90%). *Anal.* Calcd for $C_{70}H_{106}N_{12}O_{16}\cdot H_2O$: C, 60.50; H, 7.83; N, 12.10. Found: C, 60.67; H, 7.80; N, 12.03.

Z(OMe)-Lys(Z)-Ala-Leu-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Ile-Leu-NH_2——The above protected nonapeptide amide (1.50 g, 1.09 mmol) was treated with TFA–anisole (3 ml–0.75 ml) and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF–DMSO (10 ml–5 ml) containing Et_3N (0.15 ml, 1.09 mmol). To this ice-chilled solution were added the azide (prepared from 0.69 g, 1.31 mmol of Z(OMe)–Lys(Z)–Ala–$NHNH_2$) in DMF (5 ml) and Et_3N (0.15 ml, 1.09 mmol) and the mixture, after stirring at 4° for 24 hr, was concentrated. The product was purified by washing as described above, followed by precipitation twice from DMSO with MeOH; yield 1.78 g (96%), mp 260—261°, $[\alpha]_D^{20}$ −43.6° (c=0.3, DMSO), Rf_1 0.74. Amino acid ratios in an acid hydrolysate: Ala 4.28; Leu 3.23, Lys 3.18; Ile 1.00 (average recovery 82%). *Anal.* Calcd for $C_{87}H_{129}N_{15}O_{20}\cdot 4H_2O$: C, 58.80; H, 7.77; N, 11.82. Found: C, 58.98; H, 7.54; N, 11.64.

Z(OMe)-Ile-Asn-Leu-Lys(Z)-Ala-Leu-Ala-Ala-Leu-Ala-Lys(Z)-Lys(Z)-Ile-Leu-NH_2——The above protected undecapeptide amide (0.80 g, 0.47 mmol) was treated with TFA–anisole (2.4 ml–0.4 ml) and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF–DMSO (1: 1, 8 ml) containing Et_3N (0.06 ml, 0.47 mmol). To this ice-chilled solution, the azide (prepared from 0.30 g, 0.56 mmol of Z(OMe)–Ile–Asn–Leu–$NHNH_2$) in DMF (3 ml) and Et_3N (0.06 ml, 0.47 mmol) were added. After stirring at 4° for 48 hr, the solution was concentrated and the product was purified by washing as described above, followed by precipitation twice from DMSO with MeOH; yield 0.58 g (60%), mp 261° dec., $[\alpha]_D^{20}$ −52.5° (c=0.7, DMSO), Rf_1 0.71. Amino acid ratios in an acid hydrolysate: Ala 4.00, Asp 1.18, Ile 2.12, Leu 3.93, Lys 2.92 (average recovery 82%). *Anal.* Calcd for $C_{103}H_{157}N_{19}O_{24}\cdot 3.5H_2O$: C, 58.67; H, 7.84; N, 12.62. Found: C, 58.78; H, 7.82; N, 12.55.

H-Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2 (Mastoparan)——The above protected tetradecapeptide amide (214 mg, 0.1 mmol) was treated with TFA (5 ml) in the presence of thioanisole (0.49 ml, 40 equiv.) and *m*-cresol (0.44 ml, 40 equiv.) at room temperature for 4 hr, then dry ether was added. The resulting gummy precipitate was washed with ether and dissolved in H_2O (7 ml). This solution was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min, 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 (3.2×133 cm) equilibrated with the lower phase of the above solvent system. The column was developed with the upper phase (2000 ml) and then with the lower phase (1500 ml). Individual fractions (15.5 ml each) were collected and examined by means of the ninhydrin test. Fractions eluted with the lower phase (tube No. 145—190, positive to ninhydrin) were collected and the solvent was removed by evaporation. Lyophilization of the residue afforded a fluffy white powder; yield 119 mg (67%), $[\alpha]_D^{22}$ −71.4° (c=0.1, 3% AcOH), (lit.[12] −77.1° in 1 N AcOH), Rf_4 0.38. Amino acid ratios in acid hydrolysate and AP–M digest (numbers in parentheses): Asp 1.01, Asn (not determined), Ala 4.06 (3.93), Ile 2.00 (2.00), Leu 4.10 (4.09), Lys 3.14 (3.17), average recovery 84% (80%). *Anal.* Calcd for $C_{70}H_{131}N_{19}O_{15}\cdot 4CH_3COOH\cdot H_2O$: C, 53.93; H, 8.65; N, 15.32. Found: C, 53.67; H, 8.37; N, 15.73.

The sample exhibited *Rf* values identical with those of synthetic mastoparan[12] prepared by MSA deprotection: *Rf* 0.53 in *n*-BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24), *Rf* 0.60 in *n*-BuOH–AcOEt–AcOH–H_2O (1: 1: 1: 1).

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