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Synthesis and Activity of C-Terminal Heptapeptides of Tachykinins and Bombesin-like Peptides[1]

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Five heptapeptide amides related to the C-terminal portion of tachykinins and two heptapeptide amides related to the C-terminal portion of bombesin-like peptides were synthesized and the smooth muscle contractile activity of these peptides was compared with that of the C-terminal heptapeptide of substance P by taking synthetic substance P as a standard.

Keywords——C-terminal heptapeptide of tachykinins; C-terminal heptapeptide of bombesin-like peptides; smooth muscle contractile activity; substance P; Methanesulfonic acid deprotection

In the course of our synthetic studies on a hypothalamic peptide, substance P,[3] it was observed at the first time that the heptapeptide, H–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2, lacking the N-terminal tetrapeptide unit, Arg–Pro–Lys–Pro, exhibited the contractile activity on guinea-pig ileum much higher than that of the parent undecapeptide amide.[4] Similar tendency was later observed by Bergmann *et al.*,[5] and Bury and Mashford.[6]

We now synthesized the octapeptide amide, H–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2, which had hitherto been uncharacterized by us, and reconfirmed our previous observations as shown in Table I. Thus the heptapeptide amide is judged as the shortest peptide, to which the potent smooth muscle contractile activity can be expected. In some instances,[5,6] somewhat higher value was given in the octapeptide, rather than the heptapeptide amide.

Table I. Contractile Activities of Substance P Peptides on Guinea-pig Ileum

Chain length	Relative potency
Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2	1.00
Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2	0.96±0.04
Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2	1.39±0.21
Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2	3.24±0.20
Gln–Phe–Phe–Gly–Leu–Met–NH_2	0.85±0.01
Phe–Phe–Gly–Leu–Met–NH_2	0.01±0.001

1) Amino acids, peptides and their derivatives are of the L-configuration. Abbreviations used are: Z(OMe) = *p*-methoxybenzyloxycarbonyl, Z = benzyloxycarbonyl, Bzl = benzyl, NP = *p*-nitrophenyl, TCP = 2,4,5-trichlorophenyl, DNP = 2,4-dinitrophenyl, Pyr = pyroglutamyl.

2) Location: *a) Sho-machi, Tokushima*; *b) Kasumi-cho, Hiroshima*; *c) Sakyo-ku, Kyoto.*

3) M.M. Chang, S.E. Leeman, and H.D. Niall, *Nature New Biol.*, **232**, 86 (1971); G.W. Tregear, H.D. Niall, J.T. Potts, Jr., S.E. Leeman, and M.M. Chang, *ibid.*, **232**, 87 (1971).

4) H. Yajima and K. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 682 (1973); H. Yajima, K. Kitagawa, and T. Segawa, *ibid.*, **21**, 2500 (1973).

5) J. Bergmann, M. Bienert, H. Niedrich, B. Mehlis, and P. Oehme, *Exp.*, **30**, 401 (1974); J. Bergmann, P. Oehme, M. Bienert, and H. Niedrich, *ibid.*, **30**, 1315 (1974).

6) R.W. Bury and M.L. Mashford, *J. Med. Chem.*, **19**, 854 (1976).

It is interesting to note that, in the nature, peptides structurally related to substance P having the C-terminal Met–NH_2 occur in the skin of certain frogs and the salivary gland of certain octopus. The one is classified as "tachykinins" which include eledoisin,[7] physalaemin,[8] phyllomedusin,[9] uperolein[10] and kassinin.[11] The other is classified as "bombesin-like peptides" which include bombesin,[12] alytesin,[12] litorin[13] and ranatensin.[14] Among these, it has been known that shorter chain peptides of eledoisin and physalaemin possess the *in vitro* contractile activity much higher than that of the parent peptides,[15] as observed in substance P peptides.

[A] Tachykinins		
Eledoisin	Pyr–Pro–Ser–Lys–	–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2
Phyllomedusin	Pyr–Asn–Pro–	–Asn–Arg–Phe–Ile–Gly–Leu–Met–NH_2
Physalaemin	Pyr–Ala–Asp–Pro–	–Asn–Lys–Phe–Tyr–Gly–Leu–Met–NH_2
Uperolein	Pyr–Pro–Asp–Pro–	–Asn–Ala–Phe–Tyr–Gly–Leu–Met–NH_2
Kassinin	Asp–Val–Pro–Lys–Ser–	–Asp–Gln–Phe–Val–Gly–Leu–Met–NH_2
Substance P	Arg–Pro–Lys–Pro–	–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2
[B] Bombesin-like peptides		
Bombesin	Pyr–Gln–Arg–Leu–Gly–Asn–Gln–	–Trp–Ala–Val–Gly–His–Leu–Met–NH_2
Alytesin	Pyr–Gly–Arg–Leu–Gly–Thr–Gln–	–Trp–Ala–Val–Gly–His–Leu–Met–NH_2
Litorin	Pyr–Gln–	–Trp–Ala–Val–Gly–His–Phe–Met–NH_2
Ranatensin	Pyr–Val–Pro–Gln–	–Trp–Ala–Val–Gly–His–Phe–Met–NH_2

Fig. 1. Amino Acid Sequence of Tachykinins and Bombesin-like Peptides

Considering this information, we now compared the activity of the heptapeptide part of these two classes of naturally occurring peptides (Fig. 1). Most of these shortening peptides, but not all, have been synthesized on occations of their structural elucidations using the *tert*-butoxycarbonyl (Boc) group as the N^{α}-amino protecting group, but the activity of these peptides has never been tested in one place. We have synthesized heptapeptide amides related to tachykinins and bombesin-like peptides using the trifluoroacetic acid (TFA) labile Z(OMe) group. In the course of this synthetic studies, we found that the methanesulfonic acid (MSA)-thioanisole deprotecting system can be applied for the synthesis of peptides containing methionine, without protection of its sulfur atom.[16] Previously, it was reported that, when anisole was used as a cation scavenger, methionine had to be protected as its sulfoxide.[17] This deprotecting system was applied in places where it was possible.

[A] Peptides related to Tachykinins

(I) Substance P Octapeptide——Bergmann *et al.*[5] characterized systematically the physical constants and biological activity of deprotected peptides with various chain length related to substance P in 1974. Since we missed to assay only the octapeptide, H–Pro–Gln–

7) V. Erspamer and A. Anastasi, *Exp.*, **18**, 58 (1962).
8) V. Erspamer, A. Anastasi, and G. Bertaccini, J.M. Cei., *Exp.*, **20**, 489 (1964).
9) A. Anastasi and G.F. Erspamer, *Exp.*, **26**, 866 (1970).
10) A. Anastasi, V. Erspamer, and R. Endean, *Exp.*, **31**, 395 (1975).
11) A. Anastasi, P. Montecucchi, V. Erspamer, and J. Visser, *Exp.*, **33**, 857 (1977).
12) A. Anastasi, V. Erspamer, and M. Bucci, *Exp.*, **27**, 166 (1971).
13) A. Anastasi, V. Erspamer, R. Endean, *Exp.*, **31**, 510 (1975).
14) T. Nakajima, T. Tanimura, and J.J. Pisano, *Fed. Proc.*, **29**, 282 (1970).
15) L. Bernardi, G. Bosisio, F. Chillemi, G. de Caro, R. Castiglione, V. Erspamer, A. Glaesser, and O. Goffredo, *Exp.*, **20**, 306 (1964).
16) Y. Kiso, H. Isawa, K. Kitagawa, and T. Akita, *Chem. Pharm. Bull.* (Tokyo), **26**, 2562 (1978).
17) H. Irie, N. Fujii, H. Ogawa, and H. Yajima, *J.C.S. Chem. Commun.*, **1976**, 922; H. Irie, N. Fujii, H. Ogawa, H. Yajima, M. Fujino, and S. Shinagawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 2929 (1977).

Gln–Phe–Phe–Gly–Leu–Met–NH_2, in 1973, this peptide was now synthesized by condensation of Z(OMe)–Pro–ONP and H–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2 followed by deprotection with TFA.

(II) Eledoisin and Phyllomedusin Heptapeptide Amides——Eledoisin was first synthesized by Sandrin *et al.*[18] and Lübke *et al.*[19] and its C-terminal pentapeptide is identical with that of phyllomedusin. Thus the protected pentapeptide, Z(OMe)–Phe–Ile–Gly–Leu–Met–NH_2, was first prepared. Starting with this peptide, respective two amino acid residues were stepwise introduced by the *p*-nitrophenyl ester procedure[20] as shown in Fig. 2a and b. The MSA-thioanisole deprotecting procedure was applied to the preparation of the eledoisin heptapeptide amide, H–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2 and the HF procedure[21] to the phyllomedusin heptapeptide amide, H–Asn–Arg–Phe–Ile–Gly–Leu–Met–NH_2.

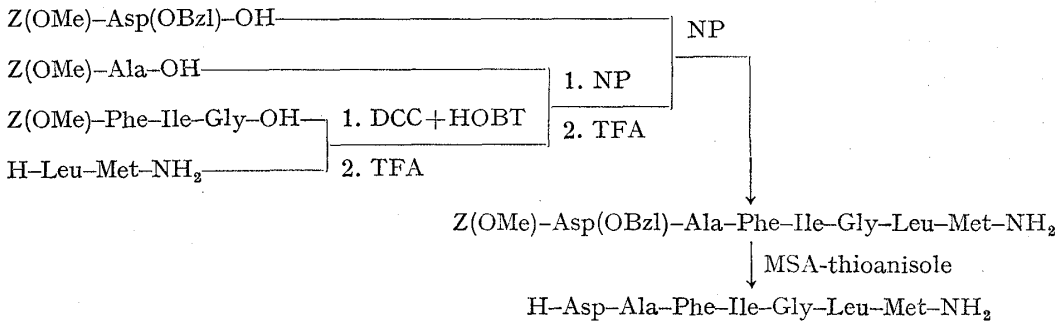

Fig. 2a. Synthetic Scheme of the Eledoisin Heptapeptide Amide

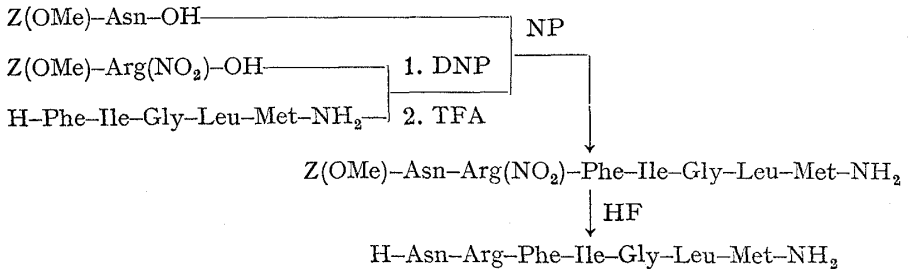

Fig. 2b. Synthetic Scheme of the Phyllomedusin Heptapeptide Amide

(III) Physalaemin and Uperolein Heptapeptide Amides——Physalaemin was synthesized by Bernardi *et al.*,[22] but the synthesis of uperolein has not yet been reported. Their C-terminal pentapeptides are both identical. The physalaemin heptapeptide amide, H–Asn–Lys–Phe–Tyr–Gly–Leu–Met–NH_2, was synthesized by condensation of 5+2 units followed by deprotection with MSA as shown in Fig. 3a. The former pentapeptide unit was prepared by the stepwise chain elongation method starting with the known tripeptide, Z–Phe–Tyr–Gly–OH.[23]

For the synthesis of the uperolein heptapeptide amide, Z(OMe)–Phe–Tyr–Gly–OH prepared similarly to the corresponding Z-derivative was first condensed with H–Leu–Met–NH_2 by dicyclohexylcarbodiimide (DCC) in the presence of N-hydroxybenzotriazole (HOBT)[24] and the alanine and aspargine residues were incorporated stepwise by the *p*-nitrophenyl ester

18) E. Sandrin and R.A. Boissonnas, *Exp.*, **18**, 59 (1962).
19) K. Lübke, E. Schröder, R. Schmieden, and H. Gibian, *Ann. Chem.*, **679**, 195 (1964).
20) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
21) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **46**, 2164 (1967).
22) L. Bernardi, G. Bosisio, O. Goffredo, and R. de Castiglione, *Exp.*, **20**, 490 (1964).
23) H. Yajima, K. Kawasaki, Y. Kinomura, T. Oshima, S. Kimoto, and M. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1342 (1968).
24) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

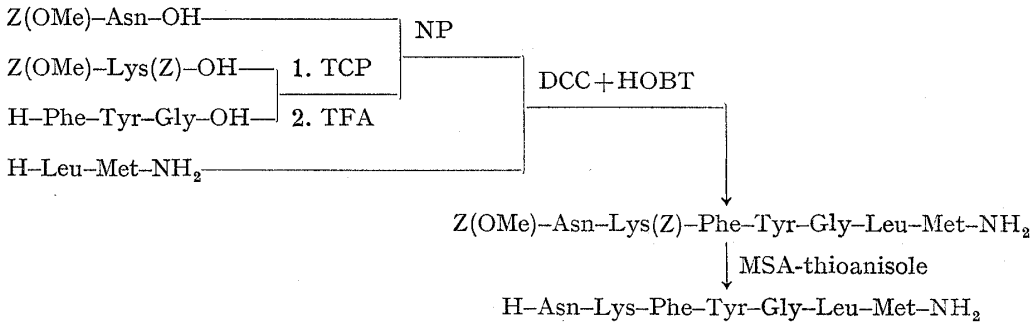

Fig. 3a. Synthetic Scheme of the Physalaemin Heptapeptide Amide

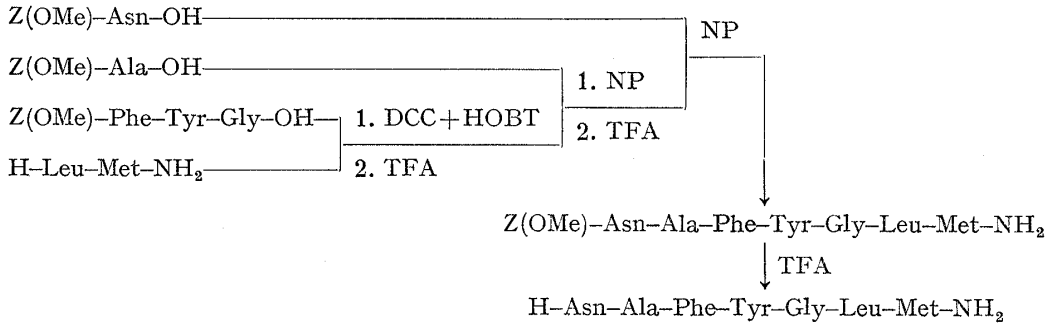

Fig. 3b. Synthetic Scheme of the Uperolein Heptapeptide Amide

method. The final deprotection was achieved by the TFA treatment to give H–Asn–Ala–Phe–Tyr–Gly–Leu–Met–NH_2 as shown in Fig. 3b.

(IV) Kassinin Heptapeptide Amide Kassinin was synthesized by the MSA-anisole deprotecting procedure.[25] The available synthetic intermediate, Z(OMe)–Asp(OBzl)–Gln–Phe–Val–Gly–Leu–Met(O)–NH_2, was deprotected with MSA-thioanisole and the resulting Met(O)-peptide was reduced by mercaptoethanol in this instance.

[B] Heptapeptide Amides related to Bombesin-like Peptides

(I) Bombesin and Alytesin Heptapeptide Amides——Bombesin was synthesized by Bernardi *et al.*,[26] but the synthesis of alytesin is not in literatures. The C-terminal heptapeptide amides of these two peptides are identical. This heptapeptide amide, H–Trp–Ala–Val–Gly–His–Leu–Met–NH_2, was synthesized by the DCC plus HOBT condensation of Z(OMe)–Trp–Ala–Val–Gly–OH and H–His–Leu–Met–NH_2 followed by deprotection with dilute hydrochloric acid in dimethylformamide (DMF). Anisole containing 2% ethanedithiol

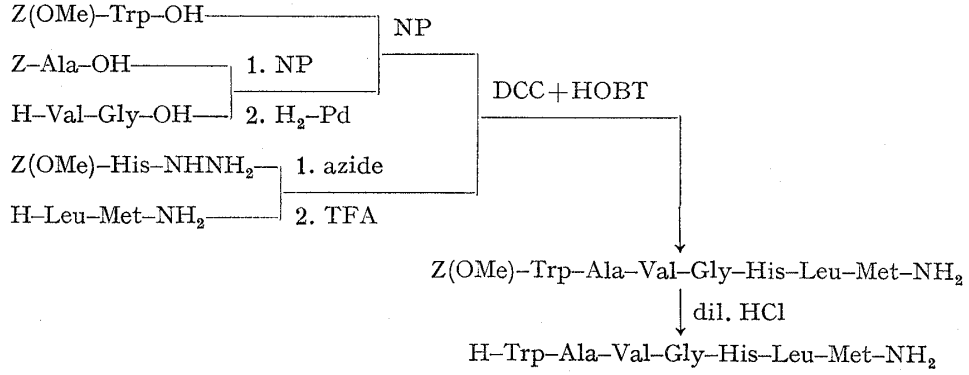

Fig. 4. Synthetic Scheme of the Bombesin (Alytesin) Heptapeptide Amide

25) H. Yajima, T. Sasaki, H. Ogawa, N. Fujii, T. Segawa, and Y. Nakata, *Chem. Pharm. Bull.* (Tokyo), **26**, 1231 (1978).
26) L. Bernardi, R. de Castiglione, O. Goffredo, and F. Angelucci, *Exp.*, **27**, 873 (1971).

was used as a cation scavenger, since usual TFA or dilute acid treatment of tryptophan-peptides gives colored substances. A small amount of the by-product was removed by repeated precipitation from DMF with ethanol. The above tripeptide amide was prepared by the azide condensation[27] of Z(OMe)–His–$NHNH_2$ with H–Leu–Met–NH_2 and the former tetrapeptide was prepared by the stepwise manner starting with H–Val–Gly–OH[28] by the *p*-nitrophenyl ester procedure as shown in Fig. 4.

(II) Litrin and Ranatensin Heptapeptide Amides——Litrin was synthesized by Angelucci and Castiglione,[29] but ranatensin is not. Their *C*-terminal heptapeptides are identical. This heptapeptide amide, H–Trp–Ala–Val–Gly–His–Phe–Met–NH_2, is different from that of alytesin and bombesin at the 2nd portion of the C-terminus. The phenylalanine residue locates in litrin and ranatensin, while leucine in the latters. Synthesis of the heptapeptide amide was performed by the 4 plus 3 condensation as stated above. The latter was prepared by the azide condensation of Z(OMe)–His–$NHNH_2$ with H–Phe–Met–NH_2 which was synthesized as shown in Fig. 5.

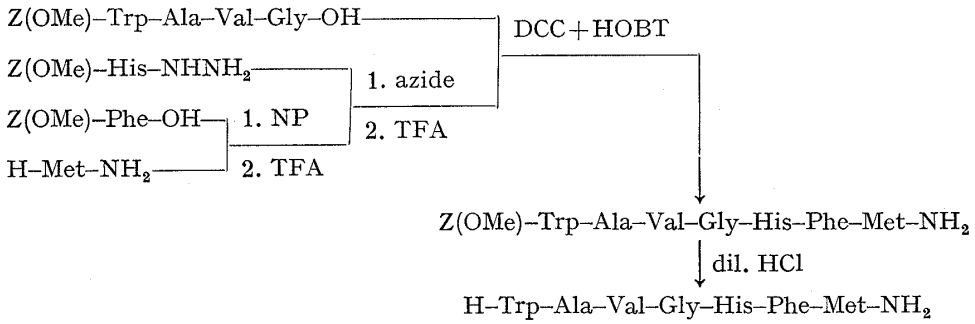

Fig. 5. Synthetic Scheme of the Litrin (Ranatensin) Heptapeptide Amide

Seven heptapeptide amides obtained here together with the substance P heptapeptide amide were assayed using isolated guinea-pig ileum and their contractile activities (in molar basis) relative to that of substance P were listed in Table II. As far as this assay system

Table II. Relative Contractile Activities of Heptapeptides related to Tachykinins and Bombesin-like Peptides on Guinea-pig Ileum

Peptides	Relative potency (in mol basis)	Heptapeptides related to
Substance P	1.0	
Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2	3.2± 0.2	Substance P
Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2	55.3±10.2	Eledoisin
Asn–Arg–Phe–Ile–Gly–Leu–Met–NH_2	9.6± 0.4	Phyllomedusin
Asn–Lys–Phe–Tyr–Gly–Leu–Met–NH_2	20.3± 1.4	Physalaemin
Asn–Ala–Phe–Tyr–Gly–Leu–Met–NH_2	38.3± 5.2	Uperolein
Asp–Gln–Phe–Val–Gly–Leu–Met–NH_2	10.8± 0.3	Kassinin
Trp–Ala–Val–Gly–His–Leu–Met–NH_2	0.007	Bombesin
Trp–Ala–Val–Gly–His–Phe–Met–NH_2	0.012	Litorin

is concerned, heptapeptides related to tachykinins exhibited much higher activity than those of bombesin-like peptides. Within a series of tachykinin peptides, eledoisin and uperolein heptapeptides were still the strong contractile agents as predicted from the activities of parent

27) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
28) K. Hofmann, E. Stutz, G. Spühler, H. Yajima, and E.T. Schwartz, *J. Am. Chem. Soc.*, **82**, 3727 (1960).
29) F. Angelucci and R. de Castiglione, *Exp.*, **31**, 507 (1975).
30) J. Szeli, E. Molina, L. Zappia, and G. Bertaccini, *Eur. J. Pharmacol.*, **43**, 285 (1977).

molecules.[30] The observable activities of heptapeptides were in orders of eledoisin>uperolein >physalaemin>phyllomedusin ≒ kassinin>substance P.

Experimental

Thin-layer chromatography was performed on silica (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8: 3: 1), Rf_2 *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2), Rf_3 $CHCl_3$–MeOH–AcOH (9: 1: 0.5). Ether stored over $FeSO_4$ was employed.

[A] Peptides related to Tachykinins

(I) Substance P Octapeptide——Z(OMe)–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2: Z(OMe)–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2[4] (1.55 g) was treated with TFA (3 ml) in the presence of anisole (0.8 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder was then dissolved in DMF (30 ml) together with Et_3N (0.4 ml) and Z(OMe)–Pro–ONP (0.92 g). The mixture was stirred at room temperature for 48 hr, the solvent was evaporated and the residue was treated with ether. The resulting powder was washed batchwise with 10% citric acid, 5% Na_2CO_3 and H_2O and then precipitated from DMF with AcOEt; yield 1.29 g (76%), mp 243—248°, $[\alpha]_D^{23}$ −28.6° (c=0.8, dimethylsulfoxide), Rf_1 0.51. Amino acid ratios in an acid hydrolysate: Pro 0.84, Glu 1.82, Phe 1.98, Gly 1.00, Leu 0.81, Met 0.74 (average recovery 80%). *Anal.* Calcd. for $C_{55}H_{75}N_{11}O_{13}S$: C, 58.44; H, 6.68; N, 13.63. Found: C, 58.17; H, 6.96; N, 13.36.

H–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2: The above protected octapeptide (300 mg) was treated with TFA (0.6 ml)–anisole (0.15 ml) as stated above and dry ether was added. The resulting powder was then dissolved in a small amount of 90% MeOH. The solution, after treatment with Amberlite IR-4B (acetate form, approximately 1 g) for 30 min, was filtered and the filtrate was condensed. Treatment of the residue with 1% NH_4OH gave a gelatinous mass, which was precipitated from MeOH with H_2O; yield 176 mg (71%), mp 248—250°, $[\alpha]_D^{23}$ −58.3° (c=0.5, 50% AcOH). (lit.[5] mp 234—238°, $[\alpha]_D^{25}$ −32.1° in DMF). Rf_1 0.12. Amino acid ratios in an acid hydrolysate: Pro 1.16, Glu 2.04; Phe 2.18, Gly 1.00, Leu 1.00, Met 0.70 (average recovery 87%). *Anal.* Calcd. for $C_{46}H_{67}N_{11}O_{10}S \cdot 1/2H_2O$: C, 56.65; H, 7.03; N, 15.80. Found: C, 56.76; H, 7.07; N, 15.91.

(II) Eledoisin and Phyllomedusin Heptapeptide Amides——Z(OMe)–Ile–Gly–OEt: DCC (20.6 g) was added to a solution of Z(OMe)–Ile–OH (29.5 g) and H–Gly–OEt (prepared from 14.0 g of the hydrochloride with 14.0 ml of Et_3N) in DMF–AcOEt (100: 50 ml) and the mixture, after stirring for 48 hr, was filtered. The filtrate was condensed and the residue was extracted with AcOEt. The organic phase was washed with 10% citric acid, 5% Na_2CO_3 and H_2O and then evaporated. The residue was triturated with ether and then recrystallized from AcOEt; yield 26.52 g (70%), mp 165—167°, $[\alpha]_D^{25}$ −1.8° (c=0.6, DMF). Rf_1 0.88, Rf_3 0.50. *Anal.* Calcd. for $C_{19}H_{28}N_2O_6$: C, 59.98; H, 7.42; N, 7.36. Found: C, 59.92; H, 7.49; N, 7.39.

Z(OMe)–Phe–Ile–Gly–OEt: Z(OMe)–Ile–Gly–OEt (21.0 g) was treated with TFA (40 ml)–anisole (10 ml) as usual and dry *n*-hexane was added. An oily precipitate was dried over KOH pellets *in vacuo* and then dissolved in DMF (250 ml), to which Et_3N (14 ml) and Z(OMe)–Phe–ONP (22.5 g) were added. After stirring at room temperature for 48 hr, the solution was evaporated and the residue was treated with ether. The resulting powder was washed batchwise with 5% citric acid, 5% Na_2CO_3 and H_2O and then recrystallized from MeOH; yield 22.50 g (85%), mp 187—193°, $[\alpha]_D^{25}$ −11.6° (c=0.7, DMF). Rf_1 0.93, Rf_3 0.54. *Anal.* Calcd. for $C_{28}H_{37}N_3O_7$: C, 63.77; H, 7.07; N, 7.97. Found: C, 63.70; H, 7.02; N, 7.84.

Z(OMe)–Phe–Ile–Gly–OH: To an ice-chilled solution of Z(OMe)–Phe–Ile–Gly–OEt (5.28 g) in dioxane (50 ml), 1 N NaOH (10 ml) was added and the mixture was stirred for 60 min. The solution, after acidification with AcOH, was condensed and the residue was crystallized from MeOH; yield 4.85 g (97%), mp 231—233°, $[\alpha]_D^{25}$ −7.6° (c=0.5, DMF). Rf_1 0.40. *Anal.* Calcd. for $C_{26}H_{33}N_3O_7 \cdot 1.5H_2O$: C, 59.30; H, 6.89; N, 7.98. Found: C, 59.57; H, 7.24; N, 8.43.

Z(OMe)–Phe–Ile–Gly–Leu–Met–NH_2: DCC (1.03 g) and HOBT (0.62 g) were added to a mixture of Z(OMe)–Phe–Ile–Gly–OH (2.50 g) and H–Leu–Met–NH_2 (derived from 4.25 g of the Z(OMe)-derivative)[4] in DMF (20 ml) and the mixture was stirred at room temperature for 48 hr. The solution was filtered, the filtrate was condensed and the residue was treated with ether. The resulting powder was washed batchwise as mentioned above and then precipitated from DMF with AcOEt; yield 2.50 g (67%), mp 240—245°, $[\alpha]_D^{25}$ −22.4° (c=0.8, DMF). Rf_1 0.54. *Anal.* Calcd. for $C_{37}H_{54}N_6O_8S$: C, 59.81; H, 7.33; N, 11.31. Found: C, 59.51; H, 7.39; N, 11.38.

Z(OMe)–Ala–Phe–Ile–Gly–Leu–Met–NH_2: Z(OMe)–Phe–Ile–Gly–Leu–Met–NH_2 (1.86 g) was treated with TFA (3.8 ml)–anisole (1.0 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (20 ml) together with Et_3N (0.7 ml) and Z(OMe)–Ala–ONP (0.94 g). After stirring for 48 hr, the solution was condensed and the residue was treated with AcOEt. The resulting powder was washed batchwise as mentioned above and precipitated from DMF with AcOEt; yield 1.65 g (81%), mp 262—266°, $[\alpha]_D^{25}$ −23.0° (c=0.4, DMF), Rf_1 0.57. *Anal.* Calcd. for $C_{40}H_{59}N_7O_9S \cdot 1/2H_2O$: C, 58.37; H, 7.35; N, 11.91. Found: C, 58.33; H, 7.39; N, 11.78.

Z(OMe)–Asp(OBzl)–Ala–Phe–Ile–Gly–Leu–Met–NH_2: The above protected hexapeptide amide (0.81 g) was treated with TFA (1.6 ml)–anisole (0.4 ml) as usual and dry ether was added. The resulting powder

was dissolved in DMF (20 ml) together with Et_3N (0.14 ml) and Z(OMe)–Asp(OBzl)–ONP (0.51 g). After stirring for 48 hr, the solution was condensed and the residue was treated with AcOEt. The resulting powder was washed batchwise as mentioned above and then precipitated from DMF with AcOEt; yield 0.75 g (74%), mp 235—238°, $[\alpha]_D^{25}$ −37.2° (c=0.5, dimethylsulfoxide). Rf_1 0.66. Amino acid ratios in an acid hydrolysate: Asp 0.94, Ala 0.95, Phe 1.00, Ile 0.97, Gly 1.00, Leu 1.14, Met 0.70 (average recovery 88%). *Anal.* Calcd. for $C_{51}H_{70}N_8O_{12}S \cdot H_2O$: C, 59.05; H, 7.00; N, 10.80. Found: C, 58.88; H, 6.96; N, 10.87.

H–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2 (Eledoisin Heptapeptide Amide): The above protected heptapeptide amide (300 mg) was treated with MSA (0.5 ml)–TFA (1 ml) in the presence of thioanisole (0.5 ml) in an ice-bath for 45 min. As a trial, dimethylsulfide (0.5 ml) was used as an additional scavenger. The deprotected peptide precipitated by addition of dry ether as a powder was dissolved in a small amount of H_2O and treated with Amberlite CG-4B (acetate form, approximately 1 g) for 30 min. The filtered solution was washed with AcOEt and then condensed. The residue was treated with ether and the resulting powder was precipitated from DMF with ether; yield 120 mg (53%), mp 260—263°, $[\alpha]_D^{25}$ −49.3° (c=0.5, dimethylsulfoxide), (lit.[31] mp 250°, $[\alpha]_D$ −34.0° in AcOH; lit.[15] mp 230°, $[\alpha]_D$ −17° in 95% AcOH), Rf_2 0.68, Rf_1 0.11. Amino acid ratios in an acid hydrolysate: Asp 0.93; Ala 0.92, Phe 0.74, Ile 0.88, Gly 0.97, Leu 1.00, Met 0.84 (average recovery 87%). *Anal.* Calcd. for $C_{35}H_{56}N_8O_9S \cdot CH_3COOH \cdot 3H_2O$: C, 50.55; H, 7.57; N, 12.75. Found: C, 50.37; H, 7.06; N, 12.51.

Z(OMe)–Arg(NO_2)–Phe–Ile–Gly–Leu–Met–NH_2: Z(OMe)–Phe–Ile–Gly–Leu–Met–NH_2 (1.20 g) was treated with TFA (2.4 ml)-anisole (0.6 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (20 ml) containing Et_3N (0.23 ml). This solution was combined with a solution containing the active ester (prepared from 1.15 g of Z(OMe)–Arg(NO_2)–OH with 0.61 g of 2,4-dinitrophenol and 0.70 g of DCC) in tetrahydrofuran (30 ml). After stirring at room temperature for 48 hr, the solution was condensed. Treatment of the residue with AcOEt afforded a powder, which was washed batchwise as mentioned above and precipitated from DMF with AcOEt; yield 1.0 g (66%), mp 224—228°, $[\alpha]_D^{25}$ −25.2° (c=0.8, DMF), Rf_1 0.52. *Anal.* Calcd. for $C_{43}H_{65}N_{11}O_{11}S \cdot 3H_2O$: C, 51.74; H, 7.17; N, 15.44. Found: C, 51.67; H, 7.03; N, 15.13.

Z(OMe)–Asn–Arg(NO_2)–Phe–Ile–Gly–Leu–Met–NH_2: Z(OMe)–Arg(NO_2)–Phe–Ile–Gly–Leu–Met–NH_2 (1.90 g) was treated with TFA (3.8 ml)-anisole (1.0 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (20 ml) together with Et_3N (0.8 ml) and Z(OMe)–Asn–ONP (0.84 g). After stirring at room temperature for 48 hr, the solution was condensed. Treatment of the residue with ether afforded a powder, which was washed batchwise as mentioned above and then precipitated from DMF with ether; yield 1.21 g (57%), mp 236—240°, $[\alpha]_D^{25}$ −22.2° (c=1.0, dimethylsulfoxide). Rf_1 0.39. *Anal.* Calcd. for $C_{47}H_{71}N_{13}O_{13}S \cdot 1.5H_2O$: C, 52.01; H, 6.87; N, 16.78. Found: C, 52.29; H, 7.06; N, 16.37.

H–Asn–Arg–Phe–Ile–Gly–Leu–Met–NH_2 (Phyllomedusin Heptapeptide Amide): The above protected heptapeptide amide (300 mg) was treated with HF (approximately 5 ml) in the presence of anisole (0.2 ml) in an ice-bath for 30 min. The excess HF was removed by evaporation, the residue 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, the filtrate was condensed and the residue was treated with ether. The resulting powder was precipitated twice from DMF with ether; yield 195 mg (71%), mp 262—265°, $[\alpha]_D^{25}$ −25.8° (c=0.2, DMF), Rf_2 0.48, Rf_1 0.04. Amino acid ratios in an acid hydrolysate: Asp 0.91, Arg 0.94, Phe 0.80, Ile 0.89, Gly 1.02, Leu 1.00, Met, 0.86 (average recovery 91%). *Anal.* Calcd. for $C_{38}H_{64}N_{12}O_8S \cdot 2CH_3COOH \cdot 4H_2O$: C, 48.45; H, 7.74; N, 16.14. Found: C, 48.22; H, 7.02; N, 16.12.

(III) Physalaemin and Uperolein Heptapeptide Amides——Z(OMe)–Lys(Z)–Phe–Tyr–Gly–OH: Z–Phe–Tyr–Gly–OH[23] (20.8 g) in 80% aqueous MeOH (120 ml) was hydrogenated over a Pd catalyst in the presence of a few drops of AcOH. The solution was filtered, the filtrate was condensed and the residue was treated with EtOH. The resulting powder was then dissolved in DMF (200 ml) together with Et_3N (11.2 ml) and Z(OMe)–Lys(Z)–OTCP (25.0 g). After stirring at room temperature for 48 hr, the solution was condensed and the residue was treated with ether. The resulting powder was washed batchwise as mentioned above and then dissolved in the solvent consisting of $CHCl_3$–MeOH–H_2O (8: 3: 1) and the solution was applied to a column of silica (4×24 cm), which was eluted with the same solvent system. Fractions containing the substance of Rf_1 0.39 were combined and the solvent was evaporated. Treatment of the residue with H_2O afforded a fine powder, which was recrystallized from AcOEt; yield 20.5 g (63%), mp 197—201°, $[\alpha]_D^{22}$ −29.2° (c=1.0, DMF). Rf_1 0.39. *Anal.* Calcd. for $C_{43}H_{49}N_5O_{11} \cdot 1.5H_2O$: C, 61.56; H, 6.25; N, 8.35. Found: C, 61.62; H, 5.93; N, 8.30.

Z(OMe)–Asn–Lys(Z)–Phe–Tyr–Gly–OH: Z(OMe)–Lys(Z)–Phe–Tyr–Gly–OH (4.10 g) was treated with TFA (8.0 ml)-anisole (2.0 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (30 ml) together with Et_3N (2.1 ml) and Z(OMe)–Asn–ONP (2.10 g). After stirring at room temperature for 48 hr, the mixture was condensed. Treatment of the residue with ether afforded a powder, which was washed batchwise as mentioned above and then precipitated from DMF with AcOEt; yield 3.20 g (69%),

31) E. Stürmer, E. Sandrin, and R.A. Boissonnas, *Exp.*, **20**, 303 (1964). The hydrochloride was reported by E. Schröder and K. Lübke, *Exp.*, **20**, 19 (1964).

mp 205—207°, $[\alpha]_D^{25}$ −20.5° (c=0.7, dimethylsulfoxide). Rf_1 0.34. *Anal.* Calcd. for $C_{47}H_{55}N_7O_{13}$: C, 60.96; H, 5.98; N, 10.58. Found: C, 60.66; H, 5.92; N, 10.48.

Z(OMe)–Asn–Lys(Z)–Phe–Tyr–Gly–Leu–Met–NH_2: DCC (0.21 g) was added to a mixture of Z(OMe)–Asn–Lys(Z)–Phe–Tyr–Gly–OH (0.93 g), HOBT (0.13 g) and H–Leu–Met–NH_2 (prepared from 0.85 g of the Z(OMe)-derivative)[4] in DMF (20 ml) and the mixture was stirred at room temperature for 48 hr. The solution was filtered, the filtrate was condensed and the residue was treated with ether. The resulting powder was washed batchwise as mentioned above and then precipitated from DMF with AcOEt; yield 1.05 g (90%), mp 225—228°, $[\alpha]_D^{25}$ −22.6° (c=0.9, dimethylsulfoxide), Rf_1 0.63. *Anal.* Calcd. for $C_{58}H_{76}N_{10}O_{14}S$: C, 59.57; H, 6.55; N, 11.97. Found: C, 59.87; H, 6.45; N, 11.77.

H–Asn–Lys–Phe–Tyr–Gly–Leu–Met–NH_2 (Physalaemin Heptapeptide Amide): The above protected heptapeptide amide (300 mg) was treated with MSA (0.5 ml)–TFA (1 ml) in the presence of thioanisole (0.5 ml) and dimethylsulfide (0.5 ml) and the product was isolated in essentially the same manner as described in the preparation of the eledoisin heptapeptide amide; yield 115 mg (45%), mp 255—260°, $[\alpha]_D^{25}$ −44.5° (c=0.4, DMF). (lit.[32] mp 234—235°, $[\alpha]_D$ −21.4° in 95% AcOH). Rf_2 0.42, Rf_1 0.04. Amino acid ratios in an acid hydrolysate: Asp 0.95, Lys 1.12, Phe 0.96, Tyr 0.72, Gly 1.10, Leu 1.00, Met 0.82 (average recovery 91%). *Anal.* Calcd. for $C_{41}H_{62}N_{10}O_9S\cdot 2CH_3COOH\cdot 5H_2O$: C, 49.98; H, 7.46; N, 12.96. Found: C, 49.81; H, 6.94; N, 13.21.

Z(OMe)–Phe–Tyr–OMe: DCC (7.51 g) was added to a stirred mixture of Z(OMe)–Phe–OH (10.01 g) and H–Tyr–OMe (prepared from 6.62 g of the hydrochloride with 4.7 ml of Et_3N) in DMF (110 ml). After 24 hr, the solution was filtered, the filtrate was condensed and the residue was dissolved in AcOEt. An organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and then condensed. The residue was recrystallized from MeOH and ether; yield 9.35 g (61%), mp 120—123°, $[\alpha]_D^{22}$ −12.5° (c=1.0, MeOH), Rf_1 0.88. *Anal.* Calcd. for $C_{28}H_{30}N_2O_7\cdot 1/2H_2O$: C, 65.23; H, 6.06; N, 5.43. Found: C, 65.54; H, 6.12; N, 5.69.

Z(OMe)–Phe–Tyr–$NHNH_2$: Z(OMe)–Phe–Tyr–OMe (9.35 g) in MeOH (50 ml) was treated with 80% hydrazine hydrate (4.6 ml) at room temperature overnight. The resulting mass was filtered and purified by precipitation from DMF with MeOH; yield 8.10 g (87%), mp 222—223°, $[\alpha]_D^{22}$ −22.2° (c=1.0, DMF), Rf_1 0.55. *Anal.* Calcd. for $C_{27}H_{30}N_4O_6$: C, 64.02; H, 5.97; N, 11.06. Found: C, 64.02; H, 5.97; N, 10.95.

Z(OMe)–Phe–Tyr–Gly–OH: The azide (prepared from 18.44 g of Z(OMe)–Phe–Tyr–$NHNH_2$ with 33 ml of 2.6 N HCl–DMF, 5.8 ml of isoamyl nitrite and 12.2 ml of Et_3N) in DMF (100 ml) was added to a solution of H–Gly–OH (5.47 g) in H_2O (30 ml) containing Et_3N (20.4 ml). After stirring at 4° for 24 hr, the solution was condensed and the residue was dissolved in 10% NH_4OH. An aqueous phase was washed with AcOEt and acidified with citric acid. The resulting solid was washed with H_2O and then recrystallized from tetrahydrofuran and ether; yield 12.26 g (61%), mp 154—161°, $[\alpha]_D^{25}$ −36.5° (c=1.0, DMF), Rf_1 0.38. *Anal.* Calcd. for $C_{29}H_{31}N_3O_8$: C, 63.38; H, 5.69; N, 7.65. Found: C, 63.24; H, 5.81; N, 7.39.

Z(OMe)–Phe–Tyr–Gly–Leu–Met–NH_2: DCC (2.06 g) was added to a mixture of Z(OMe)–Phe–Tyr–Gly–OH (4.80 g), HOBT (1.35 g) and H–Leu–Met–NH_2 (derived from 4.30 g of the Z(OMe)-derivative) in DMF (50 ml) and the mixture was stirred at room temperature for 24 hr. After filtration, the solvent was evaporated and the residue was treated with ether. The resulting powder was washed batchwise as mentioned above and recrystallized from tetrahydrofuran and ether; yield 7.20 g (90%), mp 207—211°, $[\alpha]_D^{25}$ −35.9° (c=0.9, DMF). Rf_1 0.64. *Anal.* Calcd. for $C_{40}H_{52}N_6O_9S$: C, 60.58; H, 6.61; N, 10.60. Found: C, 60.82; H, 6.83; N, 10.25.

Z(OMe)–Ala–Phe–Tyr–Gly–Leu–Met–NH_2: The above protected pentapeptide amide (1.50 g) was treated with TFA (3 ml)–anisole (0.75 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (20 ml) together with Et_3N (0.28 ml) and Z(OMe)–Ala–ONP (0.74 g). After stirring for 24 hr, the solution was condensed and the residue was treated with ether. The resulting powder was washed as mentioned above and then precipitated from tetrahydrofuran and ether; yield 0.90 g (52%), mp 213—217°, $[\alpha]_D^{25}$ −33.3° (c=0.5, DMF), Rf_1 0.67. *Anal.* Calcd. for $C_{43}H_{57}N_7O_{10}S\cdot H_2O$: C, 58.55; H, 6.74; N, 11.12. Found: C, 58.83; H, 6.66; N, 10.55.

Z(OMe)–Asn–Ala–Phe–Tyr–Gly–Leu–Met–NH_2: The above protected hexapeptide amide (1.73 g) was treated with TFA (3.4 ml)–anisole (0.9 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (20 ml) together with Et_3N (0.56 ml) and Z(OMe)–Asn–ONP (0.83 g). After stirring for 24 hr, the solution was condensed and the residue was treated with ether. The resulting powder was purified by batchwise washing as mentioned above followed by precipitation from DMF with ether; yield 1.20 g (62%), mp 231—236°, $[\alpha]_D^{25}$ −22.4° (c=0.8, dimethylsulfoxide). Rf_1 0.80. *Anal.* Calcd. for $C_{47}H_{63}N_9O_{12}S\cdot H_2O$: C, 56.67; H, 6.58; N, 12.66. Found: C, 56.40; H, 6.57; N, 12.75.

H–Asn–Ala–Phe–Tyr–Gly–Leu–Met–NH_2 (Uperolein Heptapeptide Amide): The above protected heptapeptide amide (300 mg) was deprotected by TFA (0.6 ml)–anisole (0.15 ml) and the product was isolated as described in the preparation of the substance P octapeptide amide; yield 120 mg (48%), mp 245—251°,

32) L. Bernardi, G. Bosisio, F. Chillemi, G. de Caro, R. de Castiglione, V. Erspamer, and O. Goffredo, *Exp.*, 22, 29 (1966).

$[\alpha]_D^{25}$ −46.5° (c=0.5, DMF), Rf_2 0.56, Rf_1 0.11. Amino acid ratios in an acid hydrolysate: Asp 0.97, Ala 0.95, Phe 0.98, Tyr 0.88, Gly 1.00, Leu 1.07, Met 0.96 (average recovery 86%). *Anal.* Calcd. for $C_{38}H_{55}N_9O_9S \cdot 1.5H_2O$: C, 54.27; H, 6.95; N, 14.99. Found: C, 54.43; H, 6.85; N, 14.69.

(IV) Kassinin Heptapeptide Amide——H–Asp–Gln–Phe–Val–Gly–Leu–Met–NH_2: Z(OMe)–Asp(OBzl)–Gln–Phe–Val–Gly–Leu–Met(O)–NH_2 (300 mg) was treated with MSA (0.5 ml)–TFA (1 ml) in the presence of thioanisole (0.5 ml) and dimethylsulfide (0.5 ml) and the product was isolated in essentially the same manner as mentioned in the preparation of kassinin;[25] yield 145 mg (59%), mp 249—252°, $[\alpha]_D^{25}$ −52.3° (c=0.4, dimethylsulfoxide), Rf_2 0.53, Rf_1 0.05. Amino acid ratios in an acid hydrolysate: Asp 0.82, Glu 0.94; Phe 0.88, Val 0.97, Gly 0.97, Leu 1.00, Met 0.90 (average recovery 93%). *Anal.* Calcd. for $C_{36}H_{57}N_9O_{10}S \cdot CH_3COOH \cdot 3H_2O$: C, 49.50; H, 7.32; N, 13.67. Found: C, 48.99; H, 6.97; N, 13.71.

[B] Heptapeptide Amides related to Bombesin-Like Peptides

(I) Bombesin (Alytesin) Heptapeptide Amide——Z(OMe)–His–Leu–Met–NH_2: The azide (prepared from 5.0 g of Z(OMe)–His–$NHNH_2$ according to Honzl and Rudinger[27]) was added to a solution of H–Leu–Met–NH_2 (prepared from 4.26 g of the Z(OMe)-derivative)[4] in DMF (20 ml) and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was dissolved in AcOEt. The organic phase was washed with 5% Na_2CO_3 and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and then recrystallized from AcOEt; yield 3.54 g (63%), mp 182—185°, $[\alpha]_D^{25}$ −30.0° (c=1.0, DMF). Rf_1 0.63. *Anal.* Calcd. for $C_{26}H_{38}N_6O_6S$: C, 55.49; H, 6.80; N, 14.93. Found: C, 55.25; H, 6.95; N, 14.63.

Z–Ala–Val–Gly–OH: Z–Val–Gly–OH[28] (21.6 g) in 80% aqueous tetrahydrofuran (70 ml) was hydrogenated over a Pd catalyst in the usual manner. The catalyst was removed by filtration, the filtrate was condensed and the residue was treated with EtOH. The resulting powder was then dissolved in 80% aqueous tetrahydrofuran (100 ml) together with Et_3N (19.6 ml) and Z–Ala–ONP (24.1 g). After stirring at room temperature for 72 hr, the solution was condensed and the residue was dissolved in 3% NH_4OH. An aqueous phase, after washing with AcOEt, was acidified with citric acid and the resulting crystalline mass was recrystallized from AcOEt; yield 20.5 g (77%), mp 205—207°, $[\alpha]_D^{25}$ −1.7° (c=0.6, DMF), Rf_1 0.15. *Anal.* Calcd. for $C_{18}H_{25}N_3O_6 \cdot 1/2H_2O$: C, 55.65; H, 6.74; N, 10.81. Found: C, 55.32; H, 6.65; N, 10.55.

Z(OMe)–Trp–Ala–Val–Gly–OH: Z–Ala–Val–Gly–OH (11.4 g) in 75% aqueous MeOH (70 ml) was hydrogenated as stated above. The catalyst was removed by filtration, the filtrate was condensed and the residue was treated with EtOH. The resulting powder was dissolved in DMF (50 ml) together with Et_3N (8.4 ml) and Z(OMe)–Trp–ONP (14.7 g). After stirring for 48 hr, the solution was condensed and the residue was dissolved in 3% NH_4OH. An aqueous phase was washed with ether and acidified with citric acid. The resulting oily precipitate turned to the solid on cooling with ice, which was recrystallized from MeOH; yield 12.5 g (70%), mp 187—188°, $[\alpha]_D^{25}$ −16.3° (c=0.8, DMF), Rf_1 0.14. *Anal.* Calcd. for $C_{30}H_{37}N_5O_8 \cdot H_2O$: C, 58.71; H, 6.41; N, 11.41. Found: C, 58.52; H, 6.43; N, 11.44.

Z(OMe)–Trp–Ala–Val–Gly–His–Leu–Met–NH_2: Z(OMe)–His–Leu–Met–NH_2 (2.81 g) was treated with TFA (5.5 ml)–anisole (1.4 ml) as usual and dry ether was added. The resulting powder was dissolved in 1.33 N HCl–DMF (3.1 ml) and again dry ether was added. The resulting powder was dissolved in DMF (10 ml) containing Et_3N (0.56 ml). Z(OMe)–Trp–Ala–Val–Gly–OH (2.38 g), HOBT (0.54 g) and DCC (0.85 g) were successively added and the mixture, after stirring at room temperature for 48 hr, was filtered. The filtrate was condensed and the residue was treated with ether. The resulting powder was washed batchwise with 5% citric acid, 5% $NaHCO_3$ and H_2O and then precipitated from DMF with AcOEt; yield 2.40 g (49%), mp 230—233°, $[\alpha]_D^{25}$ −28.5° (c=0.8, dimethylsulfoxide), Rf_1 0.62. *Anal.* Calcd. for $C_{47}H_{65}N_{11}O_{10}S \cdot 2H_2O$: C, 55.88; H, 6.89; N, 15.25. Found: C, 55.79; H, 6.76; N, 14.81.

H–Trp–Ala–Val–Gly–His–Leu–Met–NH_2 (Bombesin, Alytesin heptapeptide amide): The above protected heptapeptide amide (300 mg) was exposed to 3.57 N HCl–DMF (0.6 ml) in the presence of anisole (0.15 ml) containing 2% ethanedithiol in an ice-bath for 60 min. The product precipitated with dry ether was dissolved in a small amount of H_2O and the solution was neutralized with Et_3N. The resulting gelatinous mass was precipitated three times from DMF with EtOH to remove a trace of impurity; yield 105 mg (42%), mp 190—195°, $[\alpha]_D^{25}$ −40.7° (c=0.2, DMF), Rf_2 0.58, Rf_1 0.09. Amino acid ratios in 4 N Tos·OH hydrolysate:[33] Trp 0.76, Ala 0.94, Val 0.92, Gly 1.00, His 1.19, Leu 1.06, Met 0.93 (average recovery 89%). *Anal.* Calcd. for $C_{38}H_{57}N_{11}O_7S \cdot 3.5H_2O$: C, 52.16; H, 7.37; N, 17.60. Found: C, 52.93; H, 7.00; N, 16.80. This peptide was not characterized by Bernardi *et al.*[26]

(II) Litrin (Ranatensin) Heptapeptide Amide——Z(OMe)–Phe–Met–NH_2: Z(OMe)–Met–NH_2 (9.36 g) was treated with TFA (20 ml)–anisole (5.0 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (50 ml) together with Et_3N (12.6 ml) and Z(OMe)–Phe–ONP (13.50 g). After stirring for 48 hr, the solution was condensed and the residue was treated with ether. The resulting powder was recrystallized twice from DMF and MeOH; yield 10.32 g (75%), mp 214—217°, $[\alpha]_D^{25}$ −18.4° (c=1.0, DMF), Rf_1 0.65. *Anal.* Calcd. for $C_{23}H_{29}N_3O_5S$: C, 60.11; H, 6.36; N, 9.14. Found: C, 59.98; H, 6.43; N, 9.13.

33) T.Y. Liu and Y.H. Chang, *J. Biol. Chem.*, **216**, 2842 (1971).

Z(OMe)–His–Phe–Met–NH_2: Z(OMe)–Phe–Met–NH_2 (4.60 g) was treated with TFA (9.2 ml)–anisole (2.3 ml) as usual and dry ether was added. The resulting powder was then dissolved in DMF (15 ml) containing Et_3N (1.4 ml). To this ice-chilled solution was added the azide (prepared from 5.0 g of Z(OMe)–His–$NHNH_2$ according to Honzl and Rudinger[27]). After stirring at 4° for 48 hr, the solution was condensed and the residue was treated with ether. The resulting powder was washed batchwise with 5% Na_2CO_3 and H_2O and then recrystallized from MeOH and ether; yield 4.76 g (80%), mp 160—163°, $[\alpha]_D^{25}$ 41.2° (c= 1.0, DMF), Rf_1 0.35. *Anal.* Calcd. for $C_{29}H_{36}N_6O_6S \cdot H_2O$: C, 56.66; H, 6.23; N, 13.67. Found: C, 56.84; H, 6.34; N, 13.40.

Z(OMe)–Trp–Ala–Val–Gly–His–Phe–Met–NH_2: Z(OMe)–His–Phe–Met–NH_2 (2.38 g) was treated with TFA (4.8 ml)–anisole (1.2 ml) as usual and dry ether was added. The resulting powder was dissolved in 1.33 N HCl–DMF (3.1 ml) and dry ether was again added. The resulting powder was then dissolved in DMF (10 ml) together with Et_3N (0.56 ml), Z(OMe)–Trp–Ala–Val–Gly–OH (2.38 g) and HOBT (0.54 g). After addition of DCC (0.85 g), the solution was stirred at room temperature for 48 hr. The filtered solution was condensed and the residue was treated with ether and H_2O. The resulting powder was washed batchwise as stated above and precipitated from DMF with AcOEt; yield 2.51 g (62%), mp 160—162°, $[\alpha]_D^{25}$ −19.2° (c=0.9, dimethylsulfoxide), Rf_1 0.41. *Anal.* Calcd. for $C_{50}H_{63}N_{11}O_{10}S \cdot 3H_2O$: C, 56.43; H, 6.54; N, 14.48. Found: C, 56.77; H, 6.59; N, 14.34.

H–Trp–Ala–Val–Gly–His–Phe–Met–NH_2 (Litrin, Ranatensin Heptapeptide Amide): The above protected heptapeptide amide (300 mg) was treated with 3.57 N HCl–DMF (0.6 ml) in the presence of anisole (0.15 ml) containing 2% ethanedithiol and the product was isolated as stated in the preparation of the bombesin heptapeptide amide; yield 120 mg (48%), mp 218—223°, $[\alpha]_D^{25}$ −26.5° (c=0.8, DMF), Rf_2 0.55, Rf_1 0.18. Amino acid ratios in 4 N Tos–OH hydrolysate: Trp 0.81, Ala 0.98, Val 1.04, Gly 1.00, His 1.01, Phe 1.23, Met 0.75 (average recovery 88%). *Anal.* Calcd. for $C_{41}H_{55}N_{11}O_7S \cdot 3H_2O$: C, 54.71; H, 6.83; N, 17.12. Found: C, 55.05; H, 6.55; N, 16.32. Characterization of this peptide was not performed by Angelucci *et al.*[29]

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