Chem. Pharm. Bull.
30(1) 349—353 (1982)

Studies on Peptides. CVII.[1,2] Synthesis of Urotensin II

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(Received July 6, 1981)

Urotensin II, a somatostatin-like fish hormone, was synthesized in a conventional manner using thioanisole-mediated deprotection with trifluoromethanesulfonic acid. The synthetic peptide was indistinguishable from the natural peptide in terms of both biological activity and chromatographic behavior.

Keywords——synthesis of urotensin II; fish hormone; tryptophan side reaction; thioanisole-mediated deprotection; trifluoromethanesulfonic acid deprotection

Urotensin II is a peptide hormone from the caudal neurosecretory system of the toleost, *Gillichthys mirabilis*; its structure was elucidated by Pearson *et al.* in 1980 and its solid phase synthesis was reported.[3] The amino acid sequence, H–Ala–Gly–Thr–Ala–Asp–Cys–Phe–Trp–Lys–Tyr–Cys–Val–OH, is homologous with that of somatostatin[4] in positions 1—2 (Ala–Gly) and 7—9 (Phe–Trp–Lys).

We synthesized this newly found fish hormone in a conventional manner according to the scheme illustrated in Fig. 1. Amino acid derivatives bearing protecting groups removable by TFMSA[5] were employed; *i.e.*, Asp(OBzl), Cys(MBzl) and Lys(Z). This peptide contains

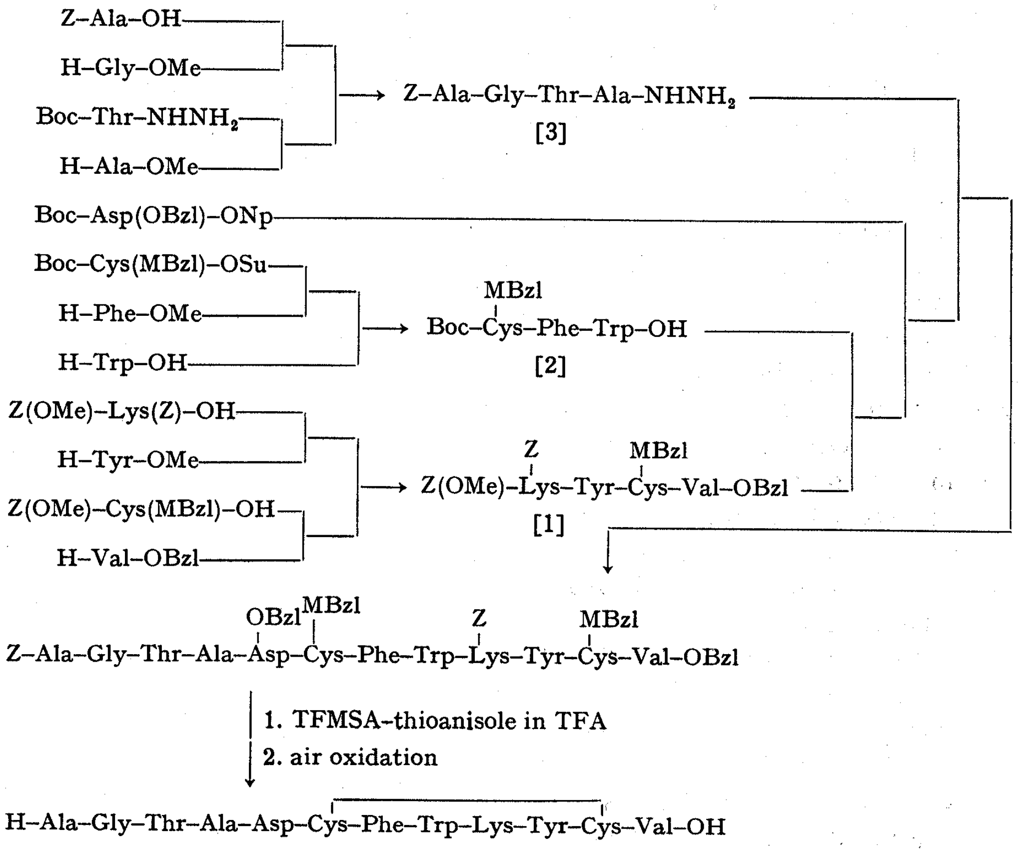

Fig. 1. Synthetic Scheme for Urotensin II

one residue of Trp, which from the synthetic viewpoint involves some difficulty regarding its functional group.6—9) In order to minimize alkylation at the indole moiety of Trp, which occurs mostly during the N^{α}-deprotection with TFA, Boc–Cys(MBzl)–Phe–Trp–OH [2] was selected as one building unit. Since the azide condensation reaction10) seems not to be ideal for Trp-containing peptides, this fragment was terminated with the free carboxyl group. Besides this unit, two peptide fragments were selected as the building blocks, *i.e.*, Z(OMe)–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl [1] (position 9—12) and Z–Ala–Gly–Thr–Ala–NHNH$_2$ [3] (position 1—4). We decided to introduce one amino acid residue, Boc–Asp(OBzl)–OH, in a stepwise manner.

The fragment [1] was synthesized by the azide condensation of two dipeptide units, Z(OMe)–Lys(Z)–Tyr–NHNH$_2$ and Z(OMe)–Cys(MBzl)–Val–OBzl. The former was prepared by the usual hydrazine treatment of the known compound, Z(OMe)–Lys(Z)–Tyr–OMe.11) The latter was prepared by the Su procedure12) and, after the usual TFA treatment, was subjected to azide condensation with the former dipeptide unit. For the preparation of fragment [2], Boc–Cys(MBzl)–Phe–NHNH$_2$ was first prepared by the Su procedure followed by the usual hydrazine treatment. This was then condensed with Trp by the azide procedure. The product was obtained in an analytically pure form after recrystallization from methanol and ethyl acetate. The Boc group was adopted as the N^{α}-protecting group, since this group is known to give less side reaction at the Trp residue during TFA deprotection, compared to the Z(OMe) group.9) The N-terminal tetrapeptide fragment [3] was prepared by the azide condensation of two dipeptide units, Z–Ala–Gly–NHNH$_2$ and Boc–Thr–Ala–OMe, followed by the usual hydrazine treatment without particular difficulty.

Construction of the peptide backbone was then carried out according to the scheme shown in Fig. 1. The Z(OMe) group was removed by TFA treatment from the fragment [1] and the resulting TFA salt was converted to the free base with Et$_3$N, through the corresponding hydrochloride. The condensation of this free amino component with the fragment [2] was performed using DCC in the presence of HOBT13) in order to suppress possible racemization. Removal of the Boc group from the resulting heptapeptide ester, Boc–Cys(MBzl)–Phe–Trp–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl, by TFA treatment was conducted in the presence of anisole and EDT. The latter is known to be especially effective in suppressing indole alkylation at the Trp residue.14) It seems worthwhile to note that the amount of such side products was greater when the Boc group was removed directly from N^{α} of Trp, and much less when Trp was not at the N-terminus. Next, Boc–Asp(OBzl)–OH was smoothly incorporated into the chain by the Np method.15) Alternatively, it would be possible to introduce this amino acid by preparing the tetrapeptide unit, Boc–Asp(OBzl)–Cys(MBzl)–Phe–Trp–OH, instead of the fragment [2]. In the final step, the azide condensation of fragment [3] proceeded smoothly. However, gel-filtration on Sephadex LH-20 was required to remove the unreacted acyl component which was used in excess during the reaction.

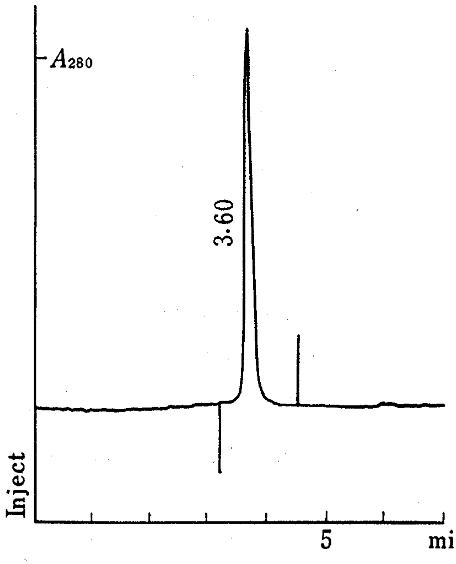

Fig. 2. HPLC of Synthetic Urotensin II

Column; μBondapak C$_{18}$.
Solvent; CH$_3$CN: 0.01 M CH$_3$COONa=40: 60.
Flow rate; 1 ml/min.

Finally, the protected dodecapeptide ester thus obtained was treated with 1 M TFMSA in TFA in the presence of thioanisole and EDT to remove all of the protecting groups employed. Thioanisole was proved to have an ability to accelerate the cleavage of protecting groups.16) The deprotected peptide was next subjected to air-oxidation in a highly diluted solution (peptide concentration 0.1 μmol/l) at pH 7.5 at room temperature. Decrease of the color intensity with Ellman's reagent[17] was used as a guide in this oxidation reaction. After 3 days, the solution was lyophilized and the residue was purified by partition chromatography[18] on Sephadex G-25 with *n*-BuOH–AcOH–H_2O (4: 1: 5). The synthetic urotensin II thus purified exhibited a single spot on TLC and a single peak on high-performance liquid chromatography (HPLC) at a retention time of 3.60 min (Fig. 2). Its homogeneity was confirmed by amino acid analysis after acid hydrolysis and enzymatic digestion.

The synthetic peptide was indistinguishable from the native hormone in terms of both biological activity and chromatographic behavior, when examined at the City of Hope Research Institute.

Experimental

General experimental methods employed in this paper are essentially the same as described in Part 88[19] of this series. *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 (10: 0.5), Rf_3 $CHCl_3$, Rf_4 $CHCl_3$–MeOH–AcOH (9: 1: 0.5), Rf_5 *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2), Rf_6 *n*-BuOH–AcOH–AcOEt–H_2O (1: 1: 1: 1). Papain (Lot. 109C-8015) and LAP (Lot. 79C-8110) were purchased from Sigma Chemical Co.

Z(OMe)-Cys(MBzl)-Val-OBzl——Z(OMe)–Cys(MBzl)–OSu (5.83 g, 11.6 mmol) and Et_3N (1.6 ml, 11.6 mmol) were added to a solution of H–Val–OBzl [prepared from 4.0 g (10 mmol) of the tosylate] in DMF (40 ml) and the mixture was stirred at room temperature for 12 h. The solvent was removed by evaporation and the residue was extracted with AcOEt. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4 and evaporated to dryness. The residue was triturated with ether and recrystallized from MeOH and ether; yield 4.02 g (64%), mp 108—110°C, $[\alpha]^{20}_{D}$ $-41.4°$ ($c=1.4$, DMF), Rf_3 0.74. *Anal.* Calcd for $C_{32}H_{38}N_2O_7S$: C, 64.62; H, 6.44; N, 4.71. Found: C, 64.40; H, 6.45; N, 4.56.

Z(OMe)-Lys(Z)-Tyr-NHNH$_2$——Z(OMe)–Lys(Z)–Tyr–OMe[11] (6.22 g, 10 mmol) in MeOH (30 ml) was treated with 80% hydrazine hydrate (6.3 ml, 10 eq) as usual and the resulting solid was precipitated from DMF with MeOH; yield 5.04 g (81%), mp 225—228°C, $[\alpha]^{30}_{D}$ $-1.2°$ ($c=0.8$, DMSO), $Rf_1=0.54$. *Anal.* Calcd for $C_{32}H_{39}N_5O_8$: C, 61.82; H, 6.32; N, 11.27. Found: C, 61.75; H, 6.13; N, 11.23.

Z(OMe)-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl [1]——Z(OMe)–Cys(MBzl)–Val–OBzl (4.02 g, 6.8 mmol) was treated with TFA–anisole (8.8 ml–2.2 ml) in an ice-bath for 60 min, then excess TFA was evaporated off. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (30 ml) containing Et_3N (0.95 ml, 6.8 mmol). The azide [prepared from 4.62 g (7.4 mmol) of Z(OMe)–Lys(Z)–Tyr–$NHNH_2$] in DMF (30 ml) and Et_3N (1.1 ml, 7.9 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 12 h. The solvent was evaporated off and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid and H_2O and precipitated from DMF with AcOEt; yield 4.01 g (58%), mp 146—148°C, $[\alpha]^{20}_{D}$ $-14.9°$ ($c=1.0$, DMSO), Rf_2 0.35. Amino acid ratios in a 6 N HCl (with phenol) hydrolysate: Lys 1.05, Tyr 0.77, Val 1.00 (recovery of Val 97%). *Anal.* Calcd for $C_{55}H_{65}N_5O_{12}S \cdot H_2O$: C, 63.63; H, 6.51; N, 6.75. Found: C, 63.96; H, 6.62; N, 6.92.

Boc-Cys(MBzl)-Phe-OMe——Boc–Cys(MBzl)–OSu (12.58 g, 28.7 mmol) and Et_3N (4.0 ml, 28.7 mmol) were added to a solution of H–Phe–OMe [prepared from 7.42 g (34.4 mmol) of the hydrochloride] in DMF (100 ml) and the mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation and the residue was treated with 5% citric acid and ether. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and recrystallized from MeOH and ether; yield 9.77 g (68%), mp 128—131°C, $[\alpha]^{20}_{D}$ $-54.5°$ ($c=0.8$, DMF), Rf_2 0.78. *Anal.* Calcd for $C_{26}H_{34}N_2O_6S$: C, 62.13; H, 6.82; N, 5.57. Found: C, 62.27; H, 6.84; N, 5.59.

Boc-Cys(MBzl)-Phe-NHNH$_2$——Boc–Cys(MBzl)–Phe–OMe (9.77 g, 19.4 mmol) dissolved in DMF–MeOH (10 ml–50 ml) was treated with 80% hydrazine hydrate (6.1 ml, 5 eq) at room temperature overnight and the solution was concentrated. The residue was triturated with EtOH and recrystallized from a mixture of MeOH and EtOH; yield 6.89 g (71%), mp 149—152°C, $[\alpha]^{20}_{D}$ $-33.9°$ ($c=1.0$, DMF), Rf_2 0.42. *Anal.* Calcd for $C_{25}H_{34}N_4O_5S$: C, 59.74; H, 6.82; N, 11.15. Found: C, 59.86; H, 6.84; N, 11.15.

Boc-Cys(MBzl)-Phe-Trp-OH (2)——The azide [prepared from 5.03 g (10 mmol) of Boc–Cys(MBzl)–Phe–$NHNH_2$] in DMF (30 ml) and Et_3N (1.5 ml, 11 mmol) were added to an ice-chilled solution of H–Trp–OH (3.06 g, 15 mmol) in H_2O (20 ml) containing Et_3N (2.1 ml, 15 mmol) and the mixture was stirred at 4°C for 12 h. The solvent was evaporated off and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid and H_2O and precipitated from MeOH with AcOEt; yield 6.19 g (92%), mp 148—151°C, $[\alpha]^{20}_{D}$ $-38.2°$ ($c=1.1$, DMF), Rf_4 0.66. Amino acid ratios in a 4 N MSA hydrolysate: Phe 1.00, Trp 0.87 (recovery of Phe 83%). *Anal.* Calcd for $C_{36}H_{42}N_4O_7S \cdot 2H_2O$: C, 60.83; H, 6.52; N, 7.88. Found: C, 60.74; H, 6.05; N, 8.03.

Boc-Thr-Ala-OMe——The azide [prepared from 10.01 g (42.9 mmol) of Boc–Thr–$NHNH_2$] in DMF

(50 ml) and Et_3N (6.0 ml, 42.9 mmol) were added to an ice-chilled solution of H–Ala–OMe [prepared from 8.38 g (60.1 mmol) of the hydrochloride] in DMF (50 ml) and the mixture was stirred at 4°C for 24 h. The solvent was evaporated off and the residue was extracted with AcOEt. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. The oily residue was used for the subsequent reaction: yield 8.07 g (62%), Rf_2 0.52.

Z-Ala-Gly-NHNH$_2$——Z–Ala–Gly–OMe[20] (10.24 g, 34.8 mmol) in MeOH (100 ml) was treated with 80% hydrazine hydrate (10.9 ml, 5 eq) at room temperature overnight, then the solution was concentrated. The residue was triturated with EtOH and recrystallized from MeOH with EtOH: yield 7.34 g (72%), mp 146—148°C, $[\alpha]_D^{20}$ +6.6° (c=1.1, DMF), Rf_1 0.79. *Anal.* Calcd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.17; N, 19.04. Found: C, 53.19; H, 5.95; N, 19.19.

Z-Ala-Gly-Thr-Ala-OMe——Boc–Thr–Ala–OMe (5.21 g, 17.1 mmol) was treated with TFA–anisole (15 ml–5 ml) in an ice-bath for 60 min, then dry n-hexane was added. The resulting oily precipitate was washed with n-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (50 ml) containing Et_3N (2.6 ml, 18.8 mmol). The azide [prepared from 5.03 g (17.1 mmol) of Z–Ala–Gly–$NHNH_2$] in DMF (25 ml) and Et_3N (2.4 ml, 17.1 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 24 h. 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% $NaHCO_3$ and H_2O and recrystallized from a mixture of MeOH and EtOH: yield 4.07 g (51%), mp 198—200°C, $[\alpha]_D^{20}$ −33.0° (c=0.8, DMF), Rf_1 0.69. *Anal.* Calcd for $C_{21}H_{30}N_4O_8$: C, 54.07; H, 6.48; N, 12.01. Found: C, 53.84; H, 6.50; N, 12.12.

Z-Ala-Gly-Thr-Ala-NHNH$_2$ [3]——Z–Ala–Gly–Thr–Ala–OMe (3.07 g, 6.6 mmol) dissolved in DMF–MeOH (6 ml–24 ml) was treated with 80% hydrazine hydrate (2.1 ml, 5 eq) at room temperature overnight. The resulting solid was precipitated from DMF with MeOH; yield 2.15 g (70%), mp 218—220°C, $[\alpha]_D^{20}$ −5.1° (c=1.0, DMSO), Rf_1 0.48. Amino acid ratios in a 6 N HCl hydrolysate: Ala 1.93, Gly 1.00, Thr 1.04 (recovery of Gly 90%). *Anal.* Calcd for $C_{20}H_{30}N_6O_7$: C, 51.49; H, 6.48; N, 18.02. Found: C, 51.41; H, 6.36; N, 17.97.

Boc-Cys(MBzl)-Phe-Trp-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl——Z(OMe)–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl (1.02 g, 1 mmol) was treated with TFA–anisole (2.0 ml–0.4 ml), and the N^{α}-deprotected peptide, precipitated with n-hexane as stated above, was treated with 4.5 N HCl–dioxane (2.2 ml, 10 eq). After 10 min at 0°C, n-hexane was added and the resulting gummy precipitate was washed with n-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (5 ml) together with Et_3N (0.14 ml, 1 mmol), Boc–Cys(MBzl)–Phe–Trp–OH (0.88 g, 1.3 mmol) and HOBT (0.20 g, 1.3 mmol). DCC (0.33 g, 1.6 mmol) was added to the above ice-chilled solution and the mixture was stirred at room temperature for 24 h. The solution was filtered, the filtrate was concentrated and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and precipitated from DMF with EtOH; yield 1.11 g (73%), mp 208—210°C, $[\alpha]_D^{20}$ −6.8° (c=1.1, DMSO), Rf_2 0.30. Amino acid ratios in a 4 N MSA hydrolysate: Val 1.00, Tyr 0.94, Phe 0.92, Trp 0.88, Lys 0.93 (recovery of Val 85%). *Anal.* Calcd for $C_{82}H_{97}N_9O_{15}S_2 \cdot H_2O$: C, 64.33; H, 6.52; N, 8.24. Found: C, 64.42; H, 6.68; N, 8.09.

Boc-Asp(OBzl)-Cys(MBzl)-Phe-Trp-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl——The above protected heptapeptide ester (1.08 g, 0.71 mmol) was treated with TFA–anisole–EDT (3.0 ml–0.6 ml–0.12 ml) in an ice-bath for 60 min, then dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (5 ml) together with Et_3N (0.1 ml, 0.71 mmol), Boc–Asp(OBzl)–ONp (0.38 g, 0.85 mmol) and N-methylmorpholine (0.09 ml, 0.85 mmol). After being stirred at room temperature for 24 h, the solution was concentrated and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and precipitated from DMF with MeOH; yield 1.02 g (83%), mp 210—212°C, $[\alpha]_D^{20}$ −14.3° (c=0.7, DMSO), Rf_2 0.24. Amino acid ratios in a 4 N MSA hydrolysate: Asp 1.27, Val 1.00, Tyr 1.00, Phe 0.93, Trp 0.99, Lys 0.95 (recovery of Val 86%). *Anal.* Calcd for $C_{93}H_{108}N_{10}O_{18}S_2$: C, 65.01; H, 6.34; N, 8.15. Found: C, 64.99; H, 6.38; N, 8.02.

Z-Ala-Gly-Thr-Ala-Asp(OBzl)-Cys(MBzl)-Phe-Trp-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl——The above protected octapeptide ester (1.02 g, 0.60 mmol) was treated with TFA–anisole–EDT (2.5 ml–0.5 ml–0.1 ml) as described above, then dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (3 ml) containing Et_3N (0.08 ml, 0.60 mmol). The azide [prepared from 0.33 g (0.71 mmol) of Z–Ala–Gly–Thr–Ala–$NHNH_2$] in DMF (3 ml) and Et_3N (0.11 ml, 0.79 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 12 h. The solvent was evaporated off, the residue was treated with H_2O and the resulting powder was precipitated from DMF with MeOH to afford a crude product contaminated slightly with the unreacted acyl component; yield 1.08 g. This product (300 mg) was next purified by gel-filtration on Sephadex LH-20 (3×143 cm), eluted with DMF. Individual fractions (9 ml each) were collected and the absorption at 280 nm was determined. The fractions corresponding to the main peak (tube Nos. 43—70) were combined and the solvent was evaporated off. The residue was treated with MeOH to give a powder; yield 213 mg (71%). The rest of the crude product was similarly purified; total yield 0.775 g (63%), mp 262—264°C, $[\alpha]_D^{20}$ −26.5° (c=0.5, DMSO). Rf_1=Rf_5=Rf_6=0. Amino acid ratios in a 6 N HCl (with phenol) hydrolysate: Asp 0.98, Thr 1.02, Gly 0.98, Ala 1.97, Val 1.00, Tyr 0.90, Phe 0.97, Lys 1.00 (recovery of Val 89%). *Anal.* Calcd for $C_{108}H_{126}N_{14}O_{23}S_2 \cdot H_2O$: C, 62.65; H, 6.23; N, 9.47. Found: C, 62.79; H, 6.12; N, 9.45.

H-Ala-Gly-Thr-Ala-Asp-$\overline{\text{Cys-Phe-Trp-Lys-Tyr-Cys}}$-Val-OH (Urotensin II)——The above protected dodecapeptide ester (60 mg, 2.92×10^{-5} mol) was treated with 1 M TFMSA–thioanisole in TFA (0.88 ml) in the presence of EDT (0.037 ml, 15 eq) in an ice-bath for 90 min, then dry ether (stored over $FeSO_4$) was added and the resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 60 min and dissolved in a small amount of H_2O. The solution was diluted with 0.01 M $AcONH_4$ (300 ml) and the pH was adjusted with 5% NH_4OH to 7.5 (peptide concentration 0.1 μmol/l). This solution was kept standing at room temperature for 3 d, while the absorbancy at 412 nm with Ellman's reagent decreased from 0.303 to a constant value, 0.060. The solution, after adjustment of the pH to 5.0 with 5% AcOH, was lyophilized. The residue (broad band on TLC) was dissolved in a small amount of the upper phase of *n*-BuOH–AcOH–H_2O (4:1:5) and applied to a column of Sephadex G-25 (1.8 × 141 cm) equilibrated with the lower phase of the above solvent system. The column was then eluted with the upper phase of the above solvent system. Individual fractions (5 ml each) were collected and the absorbancy at 280 nm was determined. After elution of a large peak (tube Nos. 27—32) consisting of scavengers and polymers, two peaks were detected. The fractions corresponding to a middle peak (tube Nos. 40—44) were pooled and the solvent was removed by evaporation. The residue was lyophilized: yield 7 mg (16%), Rf_5 0.56, Rf_6 0.58. $[\alpha]^{30}_{D}$ −60.4° (c=0.6, 1% AcOH), Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.02, Thr 0.92, Gly 1.01, Ala 1.96, Cys 0.86, Val 1.00, Tyr 1.00, Phe 1.02, Lys 1.05 (recovery of Val 78%). Amino acid ratios in papain+LAP digest: Asp 0.80, Thr 1.01, Gly 1.00, Ala 1.66, Val 1.00, Tyr 0.92, Phe 0.81, Trp 0.77, Lys 0.91 (recovery of Val 98%) *Anal.* Calcd for $C_{62}H_{84}N_{14}O_{17}S_2 \cdot 8H_2O$: C, 49.45; H, 6.69; N, 13.02. Found: C, 49.19; H, 6.45; N, 12.46. HPLC exhibited a single peak at a retention time of 3.60 min (Fig. 2).

When assayed by using the trout hindgut,[21] the activity of synthetic urotensin II was 564 units/mg and that of the native hormone was 557 units/mg.

References and Notes

1) Part CVI: *Chem. Pharm. Bull.*, **30**, 344 (1982).
2) Amino acids, peptides and their derivatives mentioned in this report are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, MBzl=*p*-methoxybenzyl, Boc=*tert*-butoxycarbonyl, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid, DCC=dicyclohexylcarbodiimide, HOBT=*N*-hydroxybenzotriazole, EDT=ethanedithiol, DMF=dimethylformamide, Np=*p*-nitrophenyl, Su=*N*-hydroxysuccinimide.
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