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Studies on Peptides. Application of Trifluoromethanesulphonic Acid as a Deblocking Reagent to the Synthesis of Neurotensin[1)]

Neurotensin, a bovine hypothalamic principle, was synthesized in a conventional manner, after removing three different protecting groups employed; tosyl (Tos), benzyl (Bzl) and benzyloxycarbonyl (Z), by trifluoromethanesulphonic acid.

We wish to report that trifluoromethanesulphonic acid (TFMSA)[2)] can be applied as a deprotecting reagent to the synthesis of a new hypotensive peptide named neurotensin. Elucidation of the structure and solid phase synthesis of this hypothalamic principle was recently reported by Carraway and Leeman.[3)]

We have synthesized the tridecapeptide corresponding to the entire amino acid sequence of neurotensin by the conventional method (Fig. 1), in which the Z (OMe) group[4)] removable by TFA played a role as temporary protection for the α-amino function of necessary intermediates and other protecting groups employed, *i.e.*, Z, Tos and Bzl, were removed at the final stage of the synthesis by TFMSA. As stems to construct the entire amino acid sequence of neurotensin, five dipeptide units were selected. Among those, Z(OMe)-Lys(Z)-Pro-OH[5)] and Z(OMe)-Pro-Tyr-$NHNH_2$[6)] are the known compounds.

Z(OMe)-Leu-Tyr-$NHNH_2$ (mp 179—182°, $[\alpha]_D^{27}$ −29.7° in MeOH. *Anal.* Calcd. for $C_{24}H_{32}O_6N_4 \cdot 1/2H_2O$: C, 59.85; H, 6.90; N, 11.63. Found: C, 60.21; H, 7.00; N, 11.65.) and Z(OMe)-

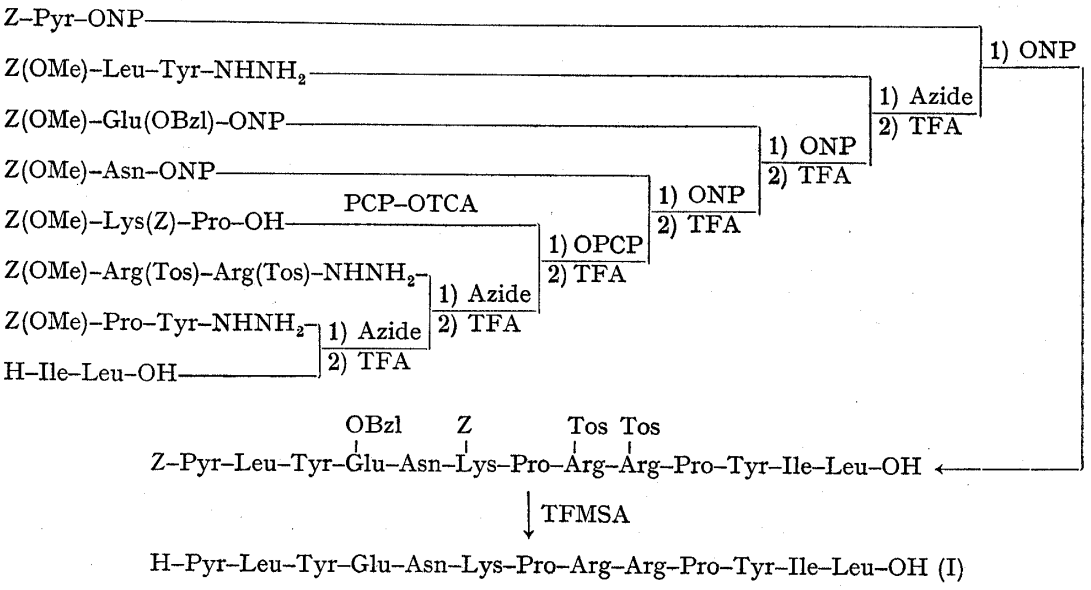

Fig. 1. Synthetic Route to Neurotensin

1) Peptides and peptide derivatives mentioned in this communication are of the L-configuration. Abbreviations: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=tosyl, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, OPCP=pentachlorophenyl ester, PCP-O-TCA=pentachlorophenyl trichloroacetate, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid, DMF=dimethylformamide, Pyr=pyroglutaminyl.

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Arg(Tos)–Arg(Tos)–$NHNH_2$ (mp 113—118°, $[\alpha]_D^{27}$ +1.2° in MeOH. *Anal.* Calcd. for $C_{35}H_{48}O_9N_{10}S_2$: C, 51.45; H, 5.92; N, 17.14. Found: C, 51.65; H, 6.20; N, 16.88.) were derived by the usual hydrazine treatment from the corresponding methyl esters, which were prepared by the DCC condensation[7] of respective amino acid derivatives. Z(OMe)–Ile–Leu–OH (mp 138—140°, $[\alpha]_D^{26}$ −20.0° in MeOH. *Anal.* Calcd. for $C_{21}H_{32}O_6N_2$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.48; H, 8.02; N, 6.75.) was prepared by the *p*-nitrophenyl ester procedure.[8] This, after treatment with TFA in the presence of anisole, was submitted as an amino component to the subsequent chain elongation reaction. In order to minimize racemization, three peptide hydrazides obtained above were condensed successively by the Honzl and Rudinger's azide procedure.[9] The Pro-terminal dipeptide unit, Z(OMe)–Lys(Z)–Pro–OH, was introduced by the pentachlorophenyl trichloroacetate procedure.[10] Z(OMe)–Asn–OH, Z(OMe)–Glu(OBzl)–OH and Z–Pyr–OH were stepwisely introduced by the *p*-nitrophenyl ester procedure.

Among intermediates, Z(OMe)–Lys(Z)–Pro–Arg(Tos)–Arg(Tos)–Pro–Tyr–Ile–Leu–OH (mp 149—153°, $[\alpha]_D^{27}$ −42.5° in MeOH. *Anal.* Calcd. for $C_{80}H_{109}O_{19}N_{15}S_2 \cdot 3H_2O$: C, 56.41; H, 6.80; N, 12.33. Found: C, 56.45; H, 6.73; N, 12.49.) was purified by column chromatography on silica using the solvent system of chloroform–MeOH–H_2O (8:3:1) and the rest of protected peptides, including the final protected tridecapeptide, Z–Pyr–Leu–Tyr–Glu(OBzl)–Asn–Lys(Z)–Pro–Arg(Tos)–Arg(Tos)–Pro–Tyr–Ile–Leu–OH (mp 148—150°, $[\alpha]_D^{27}$ −8.2° in DMF. Amino acid ratios in an acid hydrolysate: Glu 2.27, Leu 2.07, Tyr 1.76, Asp 1.00, Lys 1.24, Pro 2.22, Arg 2.38, Ile 1.00, average recovery 90%). *Anal.* Calcd. for $C_{115}H_{151}O_{28}N_{21}S_2 \cdot 4H_2O$: C, 57.26; H, 6.64; N, 12.20. Found: C, 56.82; H, 6.74; N, 12.37.) could be purified by batchwise washing with 5% citric acid and H_2O followed by repeated precipitation from MeOH with ethyl acetate or ether. They were characterized by three criteria; thin-layer chromatography, acid hydrolysis and elemental analysis.

The protected tridecapeptide thus synthesized was then treated with TFMSA at 40° for 60 minutes to secure the removal of the Tos group from Arg. Prior to this treatment, the sample was immersed well with anisole and the deblocking reaction was monitored by the positive Sakaguchi test. The deblocked peptide was converted to the corresponding acetate by Amberlite IR-4B (acetate form) and purified by column chromatography on Sephadex G-10 and then by partition chromatography on Sephadex G-25.[11] To elute the desired compound, 10% acetic acid was employed in the former step and *n*-butanol–acetic acid–H_2O (4:1:5) in the latter step.

The tridecapeptide thus purified exhibited, on thin-layer chromatography, a single spot, *Rf* 0.66 (*n*-butanol–pyridine–acetic acid–H_2O=15:10:3:12), identical with that of the product deprotected by hydrogen fluoride[12] (lit.[3] natural neurotensin *Rf* 0.68). Its homogeneity was further assessed by elemental analysis (*Anal.* Calcd. for $C_{78}H_{121}O_{20}N_{21} \cdot 3CH_3COOH \cdot 6H_2O$: C, 51.44; H, 7.45; N, 15.00. Found: C, 51.57; H, 7.71; N, 14.48.) and amino acid analysis (amino acid ratios in an acid hydrolysate: Glu 1.92, Leu 2.09, Tyr 1.86, Asp 0.95, Lys 1.04, Pro 2.18, Arg 2.25, Ile 1.00, average recovery 87%).

In isolated rat uterus, contractility caused by synthetic neurotensin was in a order of bradykinin[13]>neurotensin>substance p.[5]

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Occurrence of Pyr-His-Pro-NH_2 in the Frog Skin

The tripeptide obtained from the skin of *Bombina orientalis* BOULENGER was coincided to mammalian thyrotropin releasing hormone by chemical characterization of the peptide. The amount of the peptide in the skin was much higher in cocentration than in mammalian hypothalamus.

The tripeptide, Pyr–His–Pro–NH_2, has been recognized as the thyrotropin releasing hormone (TRH) in various mammals.[1] Occurrence of this peptide in the brain of many poikilotherms has also been demonstrated.[2] However, administration of synthetic TRH to these animals does not activate thyroid gland function.[3] Recently many kinds of gastropod which do not produce thyroid hormones, have been observed to contain the immunoreactive TRH in the circumesophageal ganglia.[4] Thus it has been proposed that in these animals TRH modulates synaptic transmission rather than releasing thyrotropin. Further supports for a role of TRH in synaptic transmission are the finding that administration of the synthetic TRH leads to an increase in noradrenaline turnover in rat brain,[5] and that hypothermia produced in individual cats by the intravascular injection of TRH is mediated by the release of noradrenaline in the brain.[6]

We have found the occurrence of a lot of amounts of this peptide in the skin of korean frog, *Bombina orientalis* BOULENGER, during the separation of vasoactive peptides in the skin. A role of this peptide in the amphibian skin is not yet clear, but this finding may imply the additional support of another function of TRH.

This report deals with the isolation and chemical characterization of this peptide in the skin of *Bombina orientalis*.

Isolation of TRH in the Skin

The fresh skin of 13 frogs was dripped with 15 ml of 1% trichloroacetic acid and extracted by adding 150 ml of methanol. The skin was extracted further with adding 100 ml of methanol twice. The extracts were combined and evaporated under a reduced pressure. The dilute

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