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SYNTHESIS OF A NEWLY ISOLATED OPIOID TRIDECAPEPTIDE, RIMORPHIN, FROM PITUITARY USING A TRIFLUOROACETIC ACID - THIOANISOLE DEPROTECTION SYSTEM

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The tridecapeptide corresponding to the amino acid sequence of rimorphin isolated from pituitary was synthesized using a trifluoroacetic acid - thioanisole deprotection system, which could deprotect O-2,6-dichlorobenzyltyrosine, N^G-2-mesitylsulfonylarginine, and N^ε-benzyloxycarbonyllysine. Synthetic rimorphin exhibited an analgesic activity in the tail-pinch test.

KEYWORDS——opioid peptide; rimorphin; dynorphin B; preproenkephalin B; peptide synthesis; thioanisole - trifluoroacetic acid deprotection system; O-2,6-dichlorobenzyltyrosine; analgesia

We describe here the first synthesis of a new opioid peptide, rimorphin,[1,2] from pituitary by the conventional solution method. In the present synthesis, we employed a new trifluoroacetic acid (TFA) - thioanisole deprotection system.[3-5]

Deprotection of O-2,6-dichlorobenzyltyrosine [Tyr(Cl_2Bzl)][6] is accomplished only under drastic conditions such as HF, methanesulfonic acid and trifluoromethanesulfonic acid.[6,7] We previously found that thioanisole had not only a suppressing effect on a side reaction, but also a promoting effect on the cleavage reaction during the acidolysis of protecting groups of tyrosine,[3,4,7,8] and can now report a mild and simple method for cleavage of the 2,6-dichlorobenzyl group at the phenolic oxygen of tyrosine using a thioanisole - TFA system which can deprotect N-benzyloxycarbonyl (Z) group[3,5] and N^G-2-mesitylsulfonylarginine [Arg(Mts)].[3,9]

The complete cleavage of Tyr(Cl_2Bzl) (0.1 mmol) was achieved by thioanisole (5 mmol) in TFA (27 mmol) at 37°C for 48 h without the formation of O-to-C rearrangement products, while in anisole - TFA, the cleavage of Tyr(Cl_2Bzl) was incomplete even after a period of 3 weeks at 37°C.

In 1982, Numa et al.[10] reported amino acid sequence of preproenkephalin B containing β-neo-endorphin, dynorphin and a third Leu-enkephalin sequence with a carboxyl extension deduced from DNA sequence. Very recently, Goldstein et al.[11] isolated from porcine pituitary a dotriacontapeptide named dynorphin-32 containing dynorphin and an unknown Leu-enkephalin-containing peptide called dynorphin B, and

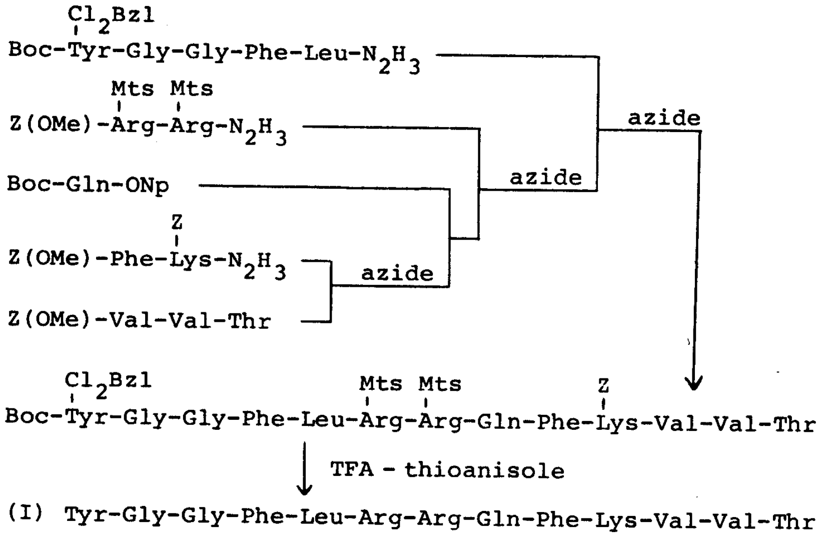

Figure. Synthesis of Rimorphin

independently, Udenfriend et al.[1,2] isolated from bovine posterior pituitary glands a tridecapeptide named rimorphin (I) with sequence identical to that of porcine dynorphin B.

It now became clear that preproenkephalin B was a precursor protein of the three Leu-enkephalin-containing peptides β-neo-endorphin, dynorphin and rimorphin (dynorphin B). This new and interesting opioid rimorphin was a major enkephalin-containing peptide in the bovine tissues, and we therefore synthesized it applying the new simple method for deprotection of Tyr(Cl_2Bzl) by thioanisole - TFA.

Amino acid derivatives bearing protecting groups removable by thioanisole - TFA were employed, i.e., Tyr(Cl_2Bzl),[6] Arg(Mts),[12] and Lys(Z).[13] The acid-labile p-methoxybenzyloxycarbonyl [Z(OMe)] or t-butoxycarbonyl (Boc) groups served as temporary protection for the α-amino function of the various peptide segments. Prior to each condensation reaction, the Z(OMe) or Boc group was cleaved under mild conditions using 20% TFA in methylene chloride containing anisole so as not to impair the protecting groups of side-chain functions.

The necessary peptide segments were prepared by conventional methods and purified using silica gel column chromatography when necessary. Those segments obtained were assembled successively using Rudinger's modified azide procedure[14] as a main tool except for Boc-Gln-ONp (ONp = p-nitrophenyl ester) as shown in the Figure. Using excess of acyl components (1.5 - 2 eq) each coupling reaction proceeded smoothly in dimethylformamide. The resulting protected tridecapeptide and protected intermediates were purified by washing with 5% citric acid and water followed by repeated precipitation from dimethylformamide with appropriate solvents, such as ethyl acetate, ethanol, or methanol; and their purities were assessed by t.l.c., acid hydrolysis, and elemental analysis.

The protected tridecapeptide was deblocked with thioanisole - TFA in the presence of o-cresol (an additional cation scavenger)[12] at 37°C for 48 h. After evaporation of the bulk of TFA, the deprotected peptide was precipitated as a powder with ether and converted into the corresponding acetate form with Amberlite IRA-400 (acetate cycle). This material was purified by partition chromatography on Sephadex G-25 using the solvent system n-butanol - acetic acid - water (4:1:5), and then by ion-exchange chromatography on CM-cellulose with gradient elution using ammonium acetate buffer (0.6 M). After gel filtration on Sephadex G-15 (3% acetic acid), the homogeneous peptide was obtained (overall yield 52% in the deprotection and purification steps). The presence of the Val-Val sequence gave low yields of valine on 24 h acid hydrolysis of synthetic rimorphin [$[\alpha]_D^{20}$ -35.71° (c=0.8, water); t.l.c. (silica), Rf (Bu^nOH-AcOH-pyridine-H_2O, 4:1:1:2) 0.41, Rf (Bu^nOH-AcOH-pyridine-H_2O, 30:20:6:24) 0.68; high performance liquid chromatography (μ-Bondapak C_{18} 0.39 x 30 cm, 0.1 M KH_2PO_4-CH_3CN = 75:25, 0.5 ml/min) retention time 8.2 min; satisfactory elemental analysis was obtained for $C_{74}H_{115}N_{21}O_{17} \cdot 4\,CH_3CO_2H \cdot 7\,H_2O$; amino acid ratios in 6 N HCl hydrolysate (110°C, 24 h), Tyr 0.97, Gly 2.00, Phe 1.99, Leu 0.99, Arg 1.96, Glu 1.02, Lys 0.99, Val (1.81 after 72 h), Thr 0.96 (average recovery, 93%)] in agreement with the result of natural rimorphin,[2)] and this bond is known[15)] to be hydrolysed slowly due to steric hindrance. A reasonable value of valine was obtained on 72 h acid hydrolysate.

Synthetic rimorphin exhibited a potent in vitro opioid activity ($ED_{50} = 3.85 \times 10^{-9}$M) on the inhibitory assay of electrically evoked contraction of the isolated guinea pig ileum, in agreement with the literature value of natural rimorphin.[1,2)] It is noteworthy that synthetic rimorphin exhibited an analgesic activity [ED_{50} = 32.97 (95% confidence limits 14.2-76.1) nmol/mouse] in the tail-pinch analgesic test after intracerebroventricular administration in mice.

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