SYNTHESIS AND CHARACTERISATION OF A DECAPEPTIDE HAVING LH-RH/FSH-RH ACTIVITY

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Summary: A decapeptide with the proposed structure of LH-RH/FSH-RH was synthesized, characterized and compared with LH-RH active preparations from porcine hypothalamus.

According to the procedure published by Schally and coworkers (1) for the purification of several releasing hormones from hypothalamic tissue, we attempted to isolate LH-RH from 20.000 porcine hypothalami. Acetic acid extracts were subjected to gel filtration on Sephadex G-25, phenol extraction, chromatography on CMC, and were then further purified by several TLC systems. During this purification we obtained two well separated preparations with LH-RH activity (2).

In the meantime Schally and his group (3) succeeded in determining the complete amino acid sequence from less than 200 nmoles of a highly purified LH-RH/FSH-RH preparation isolated from porcine hypothalami. The following decapeptide sequence for porcine LH-RH/FSH-RH was proposed:

Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (I)

Since for a comparison of I with our highly purified extracts

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Abbreviations: DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; Mbh, 4.4'-dimethoxybenzhydryl; Pyr, pyroglutamic acid; But = t-butyl

no substance was available and no synthesis of LH-RH/FSH-RH was published yet, we synthesized the decapeptide I.

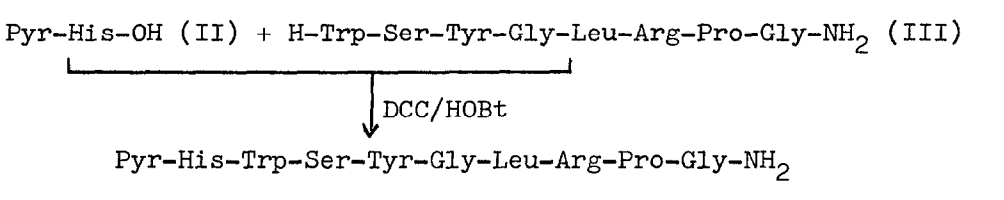
Only synthesis could provide enough material for further chemical and physical characterisation and especially for a broad biological testing of the hormone, which is indicated because of its possibly far-reaching significance in clinical medicine.

Due to the presence of numerous complicated amino acids, some difficulties for the synthesis of the decapeptide had to be expected. We therefore applied intermediates which supposedly could be purified easily. For these intermediates we partially employed newer protecting groups and condensation methods especially developed to avoid those difficulties. Schemes 1,2 and 3 line out the methods of the synthesis.

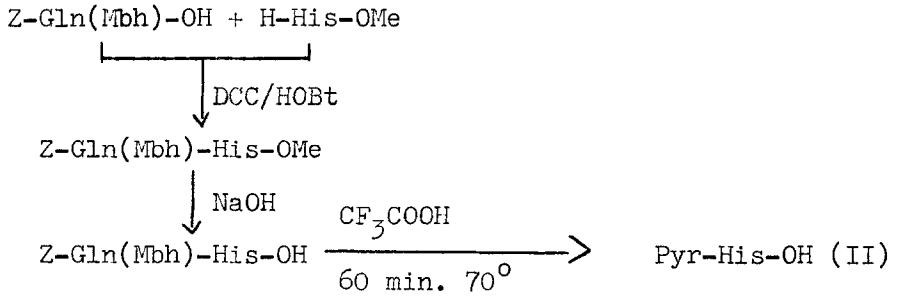
The DCC/HOBt method (4) was applied for all condensation procedures with the exception of the synthesis of the pentapeptide derivative Z-Trp-Ser-Tyr-Gly-Leu-OBut, which was obtained in higher yield following the azide method.

For preparing Pyr-His-OH we chose Z-Gln(Mbh)-OH (5) as an intermediate. Under cleavage of the protecting groups and simultaneous cyclisation the crystalline dipeptide Z-Gln(Mbh)-

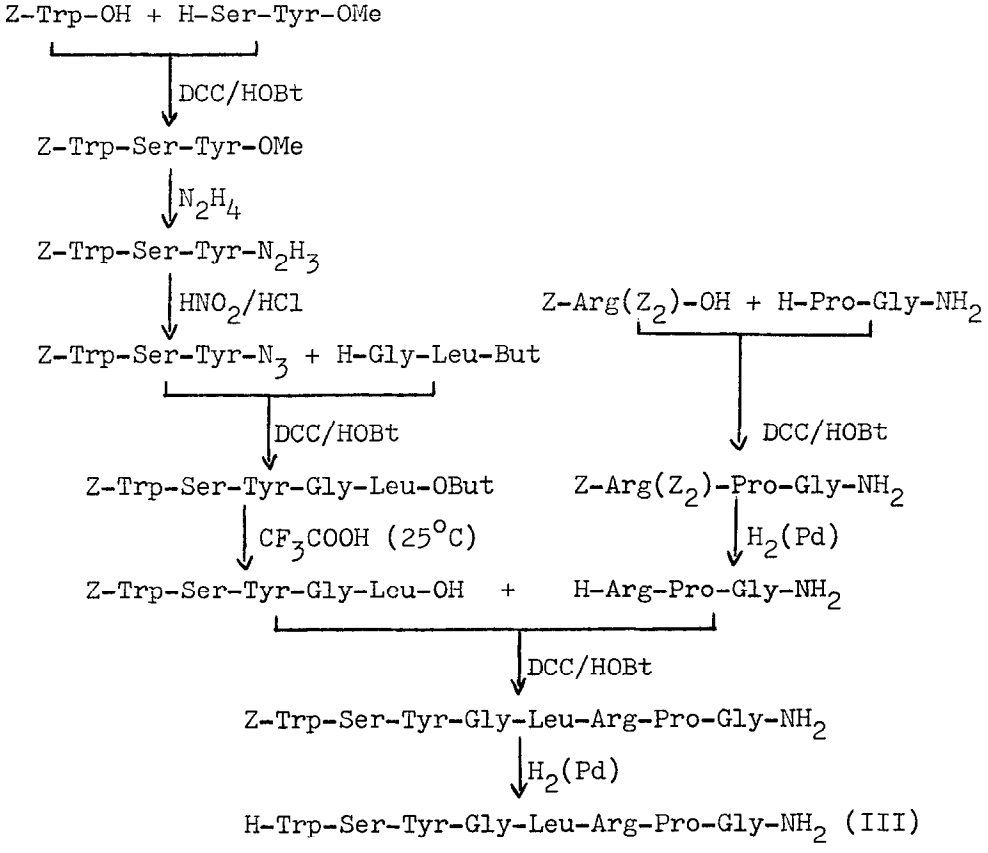
Scheme 1. SYNTHESIS OF DECAPEPTIDE I



Scheme 2. SYNTHESIS OF DIPEPTIDE II



Scheme 3. SYNTHESIS OF OCTAPEPTIDE III



His-OH quantitatively yielded Pyr-His-OH, when it was treated with trifluoroacetic acid/anisole over a period of 30-60 min. at 70°C.

Table 1. PROPERTIES OF INTERMEDIATES OF THE SYNTHESIS

compound	elementary analysis	$[\alpha]_D^{28}$
Z-Pro-Gly-NH_2 m.p. 132-6°C	$C_{15}H_{19}N_3O_4$ (305.3) calcd C 59.02 H 6.29 N 13.77 found C 59.2 H 6.4 N 13.8	-38.9° c=1, MeOH
Z-Arg(Z_2)-Pro-Gly-NH_2 m.p. 117-22°C	$C_{37}H_{43}N_7O_9$ (729.8) calcd C 60.88 H 5.94 N 13.43 found C 60.8 H 6.0 N 13.45	-29.6° c=1, MeOH
Z-Gly-Leu-OBut	$C_{20}H_{30}N_2O_5$ (378.5) calcd C 63.45 H 7.94 N 7.40 found C 63.7 H 8.1 N 7.4	-30.1° c=1, MeOH
Z-Trp-Ser-Tyr-N_2H_3	$C_{31}H_{34}N_6O_7$ (602.6) calcd C 61.82 H 5.69 N 13.94 found C 61.8 H 5.8 N 13.7	-14.3° c=1, DMF
Z-Trp-Ser-Tyr-Gly-Leu-OBut	$C_{43}H_{54}N_6O_{10}$(814.9) calcd C 63.36 H 6.67 N 10.32 found C 63.4 H 6.7 N 10.2	-25.1° c=1, MeOH
Z-Trp-Ser-Tyr-Gly-Leu-OH, 1.5 H_2O	$C_{39}H_{46}N_6O_{10}$, 1.5 H_2O (785.9) calcd C 59.66 H 6.28 N 10.69 found C 59.8 H 6.2 N 10.5	
Z-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2,TosOH, 1.5 H_2O	$C_{52}H_{69}N_{13}O_{12}$, $C_7H_8O_3S$, 1,5 H_2O (1267.4) calcd C 55.93 H 6.36 N 14.36 found C 55.9 H 6.5 N 14.5	-30.6° c=0.5 in 90% CH_3COOH

After triturating the decapeptide I with N-sodium hydrogencarbonate it was purified by partition chromatography on Sephadex LH-20 in n-butanol-acetic acid-water (8:4:40).

Table 1 shows the properties and purity criteria of the most important intermediates of the synthesis, Table 2 records physical constants and chemical properties of I.

Table 2. CHEMICAL AND PHYSICAL PROPERTIES OF I

Elementary analysis:	$C_{55}H_{75}N_{17}O_{13}$, 2 CH_3COOH, 4 H_2O (1373)	
	calcd C 51,58 H 6.68 N 17.35	
	found C 51.4 H 6.5 N 17.3	
Amino acid analysis:	Ser 0.84 (1)	Leu 1.04 (1)
	Glu 0.98 (1)	Tyr 0.95 (1)
	Pro 0.96 (1)	His 1.00 (1)
	Gly 2.02 (1)	Arg 0.96 (1)

$[\alpha]_D^{28} = 50.5^o \pm 2^o$ (c=1 in 1% acetic acid)

Tyr:Trp[a] = 1.01

a) see ref. (8)

The synthetic decapeptide was also characterized by TLC in 4 diversified solvent systems on cellulose and silicalgel at 4°C. Samples in the range of 3-5 mcg were applied. As standard for this comparison we used our porcine LH-RH preparations and TRH. The R_f-values for the compounds are based upon the Pauly positive spots. The data in Table 3 show that the R_f-values of the synthetic decapeptide and those of one of our two preparations exhibiting LH-RH activity are indistinguishable in all of the systems.

Table 3. R_f-VALUES OF SYNTHETIC DECAPEPTIDE I, LH-RH AND TRH

No.	solvent systems (volume ratio)	adsorbents	R_f x 100 I	LH-RH	TRH
1.	$CHCl_3$ - CH_3OH - CH_3COOH (32%) (60:45:20)	A [a]	54	54	40
2.	$CHCl_3$ - CH_3OH - CH_3COOH (32%) (60:45:20)	B	64	66	48
3.	$CHCl_3$ - CH_3OH - NH_3 (32%) (60:45:20)	A	47	47	64
4.	$CHCl_3$ - CH_3OH - NH_3 (32%) (60:45:20)	B	81	80	71
5.	n-C_4H_9OH - CH_3COOH - H_2O (40:10:50)	A	16	15	10
6.	n-C_4H_9OH - CH_3COOH - H_2O (40:10:50)	B	44	45	31
7.	n-C_4H_9OH - CH_3COOH - C_6H_5N - H_2O (40:10:10:20)	A	41	40	26

a) A = Silica Gel G; B = Cellulose F; precoated plates from E Merck

The decapeptide I has been examined for the characteristic hormonal activities in comparison with our natural porcine LH-RH preparations. By radioimmunoassay we found that 1 nanogram of I was effective in elevating the blood levels of LH significantly in mature, ovariectomized rats, pretreated with estrogen and progesterone. It was active at a dose level of 100 ng in the bioassay. The biological data on our synthetic decapeptide will be reported in detail in companion papers (6,7).

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