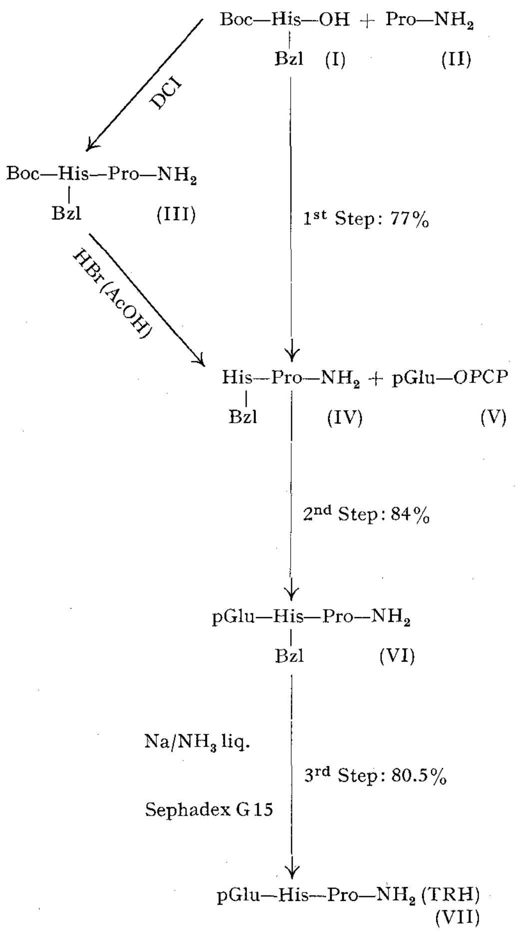
A New Improved Synthesis of Thyrotropin Releasing Hormone

Thyrotropin releasing hormone TRH is a tripeptide of hypothalamic origin which stimulates the synthesis and release of thyrotropin (TSH) by the hypophysis. During the sixties, investigators, such as SCHALLY and GUILLEMIN, carried out intensive studies to extract the thyrotropin releasing factor from swine and ovine hypothalami which led, after various attempts, to the determination of its chemical structure which is as follows: L-pyroglutamyl-L-histidyl-L-prolinamide.

After the discovery of TRH, many syntheses of this tripeptide were proposed by various researchers[1–12]. However, the methods of synthesis described were not suitable for preparation of the hormone on a vast scale and the greater part required the use of protected forms of the amino acids. This involved successive stages of deprotection of the tripeptide which were particularly difficult. On the other hand, when unprotected amino acids were coupled together and with the dipeptides, the yields were very low. When we take into account that at least two theoretical stages of coupling are necessary to obtain the desired product, the final yield, when compared to the original material, was not sufficient for an industrial preparation.

In the overall economic evaluation of the procedure, it must be borne in mind that difficult stages of fractionation and purification are necessary in order to recover the final products from the reaction mixtures which inevitably contain a considerable percentage of collateral products.



Synthesis and purification of TRH.

In this paper, a new and economic industrial procedure for the synthesis of highly purified TRH, giving high yields, is reported.

The design used for the synthesis is shown in the Figure. TRH is obtained by preparing the protected tripeptide, L-Pyroglutamyl-N^{im}Benzyl-L-Histidyl-L-Prolinamide (VI), deprotecting this tripeptide by reduction with metallic sodium in liquid ammonia and purifying the L-Pyroglutamyl-L-Histidyl-L-Prolinamide (VII) thus obtained by gelfiltration on Sephadex G–15.

The protected tripeptide (VI) is prepared by coupling N^{α}Boc-N^{im}Benzyl-L-Histidine (I) with L-Prolinamide hydrochloride (II) in the presence of N,N′-dicyclohexylcarbodiimide (DCI), in order to obtain the dipeptide N^{α}-Boc-N^{im}Benzyl-L-Histidyl-L-Prolinamide (III) which, without being isolated, is deprotected with HBr 2 *N* in glacial acetic acid to give N^{im}Benzyl-L-Histidyl-L-Prolinamide dihydrobromide (IV). The latter is purified by crystallization with methanol-anhydrous ether and reacted with an active ester of pyroglutamic acid (V), pentachlorophenyl-L-pyroglutamate, which results in the protected tripeptide pGlu-(Bzl)His-Pro-NH_2 (VI) which is purified by crystallization with methanol-anhydrous ether. The deprotection is carried out by dissolving the protected tripeptide in liquid ammonia and adding sodium to the solution.

The reaction time is calculated from the moment in which the solution becomes a persistent blue. After a very short period, which varies from 30 to 60 sec, the reaction is interrupted by adding glacial acetic acid. This reaction time is an important factor in the procedure. Fewer than 30 sec may be insufficient to get a substantially complete deprotection of the tripeptide, while more than 30 sec may cause the deterioration of the product.

It is well known that reduction by sodium in liquid ammonia is a somewhat drastic method. As such, it has never been proposed as a means for deprotecting synthetic TRH in its protected form. Another characteristic of our method is the facility with which the deprotected tripeptide can be purified by gel filtration, and its high yield. The chemico-physical properties of the intermediates and of the final product are shown in the Table.

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Chemico-physical properties of the intermediates and of TRH

Compound (formula)	PF (°C)	$[\alpha]_D^{20}$	TLC[a]			Analysis (calc./found)		
			Rf¹	Rf²	Rf³	C	H	N
His–Pro–NH$_2$ · 2HBr (IV) | Bzl ($C_{18}H_{25}O_2N_5Br_2$)	196°–198°	−28.2° (c 1.0 in MeOH)	0.41	0.50	0.61	42.96 43.04	5.01 4.98	13.92 13.72
pGlu–His–Pro–NH$_2$ (VI) | Bzl ($C_{23}H_{28}O_4N_6$)	120°–121° (decomp.)	−24.5° (c 1.0 in MeOH)	0.48	0.54	0.76	61.05 61.35	6.24 6.12	18.57 18.50
pGlu–His–Pro–NH$_2$[b] (TRH) ($C_{16}H_{22}O_4N_6$–0.5 H_2O)	amorph.	−40° (c 1.0 in MeOH)	0.24	0.60	0.39	51.74 51.54	6.24 6.18	22.63 22.81

[a] The Rf¹, Rf² and Rf³ values (Silica Gel G according to Stahl activated to 110 °C for 1 h) refer to the eluent systems '*n*-butanol-glacial acetic acid-ethyl acetate-H_2O' (1:1:1:1); 'chloroform-methanol-NH_3 concentrated' (60:45:20); 'chloroform-methanol-acetic acid 30%' (60:45:20).
[b] The amino-acid analysis gave the following results: Glu 1.00; His 0.98; Pro 0.99; NH_3 0.96. The peptide hydrolysis was carried out in a closed ampoule in HCl 6 *N* at 110 °C for 48 h.

The homogeneity and the high grade of purity of the TRH obtained by the method reported in this paper were shown by thin layer chromatography and analysis of the amino acids and confirmed by its high biological activity. The valuation of the biodynamic activity was carried out on isolated rat pituitaries according to the method of SCHALLY et al.[13] which determines by radioimmunoassay the amount of TSH released by the pituitary after stimulation with TRH.

Summary. This synthesis of thyrotropin releasing hormone (TRH) is particularly suitable for large scale production of highly purified TRH, thanks to its high yields of the coupling and deprotection reactions together with the very simple final purification step.

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Systemic Fungicidal Activity of *N*-[2,2,2-Trichloro-1-(3-pyridylamino)ethyl]-formamide

Triforine (I), *N,N'*-[piperazine-1,4-diyl-*bis*(2,2,2-trichloroethylidene)]-*bis*formamide[1–4], has attracted considerable interest as a systemic fungicide in plants particularly against powdery mildew and rust diseases. Several related *bis*formamides have been patented as fungicides[5]. We have recently been interested[3,6–9] in determining the salient chemical features necessary for systemic activity in compounds of this type. From these studies it emerged that compounds of structure (II) where R is O-alkyl, NH-alkyl or S-alkyl[7,8] were systemically active following root application against powdery mildews. The best compounds were fully inhibitory at 100 ppm. In contrast compounds (II) where R is O-aryl, NH-aryl or S-aryl were much less effective[7–9]. It has also been found that the 3 chlorine groups in (II; R=O-alkyl) can be replaced by bromine without significant loss of systemic activity although the hexabromo analogue of triforine was inactive[9].

We have now studied several compounds of type (II) were R is a pyridylamino or related heterocyclic moiety. One of these compounds, *N*-[2,2,2-trichloro-1-(3-pyridylamino)-ethyl]formamide (III) has been found to be outstanding as a systemic fungicide against *Erysiphe graminis* following root application to sand-grown wheat seedlings.

The compounds (Table I) were prepared by the following typical method (cf.[3]). To 1-(2,2,2-trichloro-1-formamidoethyl)pyridinium chloride[10] (0.005 mol.) suspended in

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