European Colloquium on Hypothalamic Hormones *

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Organized by D. Gupta and W. Voelter

Abstract of the Lectures **

Isolation and Characterization of Hypothalamic Hormones

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The hypothalamic neurohormones were already postulated almost 40 years ago by Hinsey [1] and later on by Green and Harris [2]. The isolation of the porcine thyrotropin releasing hormone (TRH) was reported by Schally [3] *et al.*, the extraction from sheep hypothalami was first done by Burgus and Guillemin [4]. The elucidation of the primary structure of TRH [5, 6] and its synthesis [7, 8] were reported in 1969 to 1970.

The isolation of the luteinizing hormone-releasing hormone (LH-RH) was published in 1971 by Schally *et al.* [9] and Guillemin *et al.* [10]. In 1971 and 1972 LH-RH was synthesized by different groups [11–16].

For the following reasons our group works on the synthesis of peptides with biological activity of

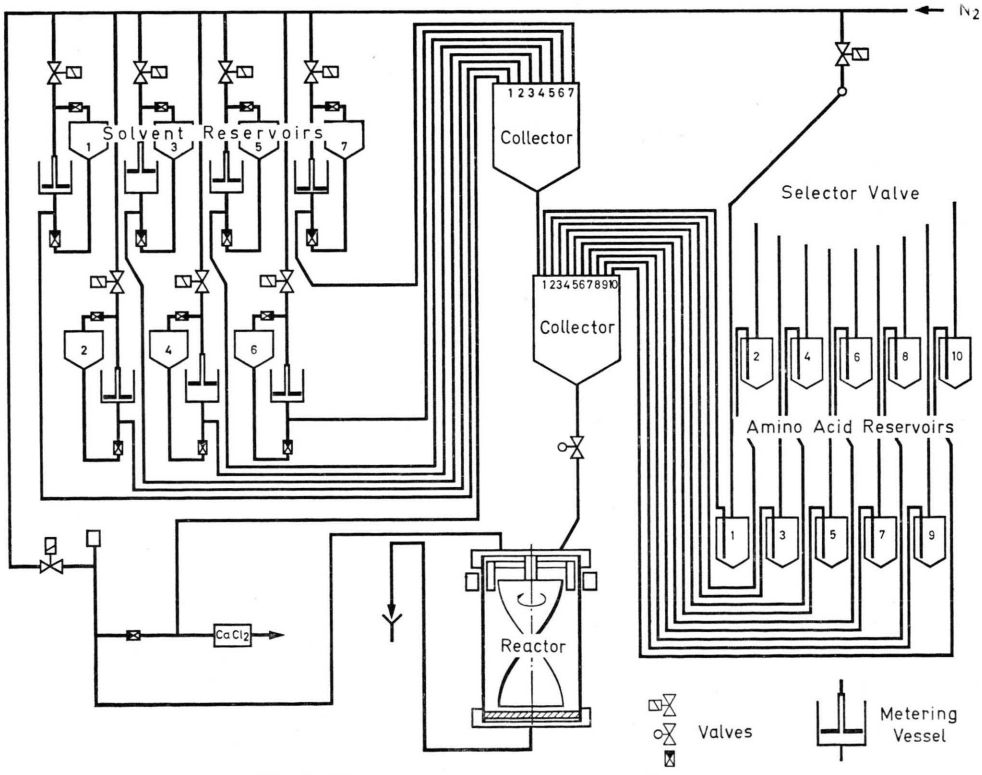

Fig. 1. Diagram of automatic peptide synthesizer.

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** The lectures and discussions will be published in full text in due time.

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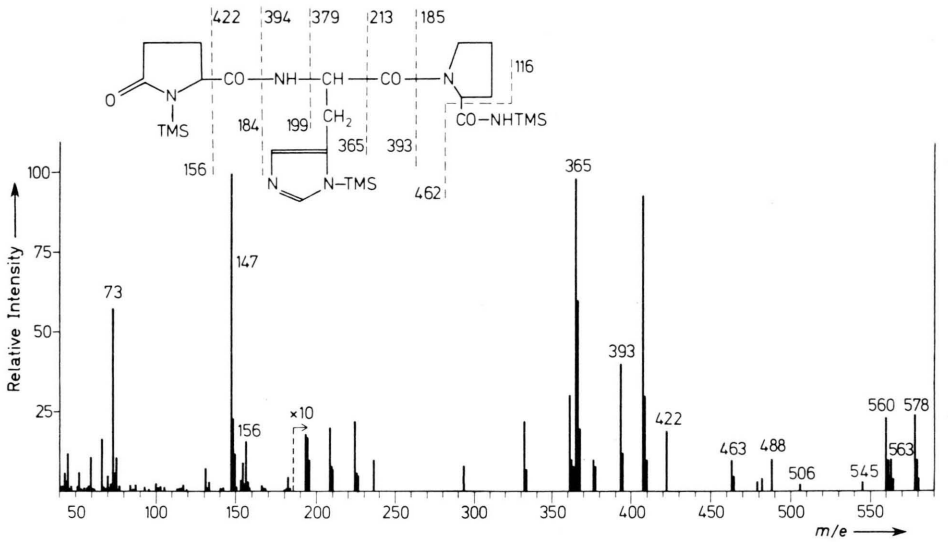


Fig. 2. Mass spectrum of the trimethylsilyl derivative of TRH (LKB 9000, 70 eV, 3.5 kV).

LH-RH resp. TRH: 1. General improvement of synthetic steps in peptide chemistry; *e. g.* this reason initiated us to build a new automatic peptide synthesizer (see Fig. 1) [17]. 2. General improvement of the separation techniques of peptide mixtures *e. g.* by high pressure liquid chromatography [18]. 3. General improvement of the methods of structure elucidation in peptide chemistry. It was reported recently that the trimethylsilyl derivatives of peptide amides

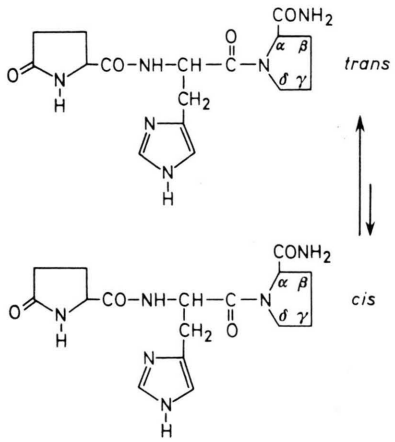
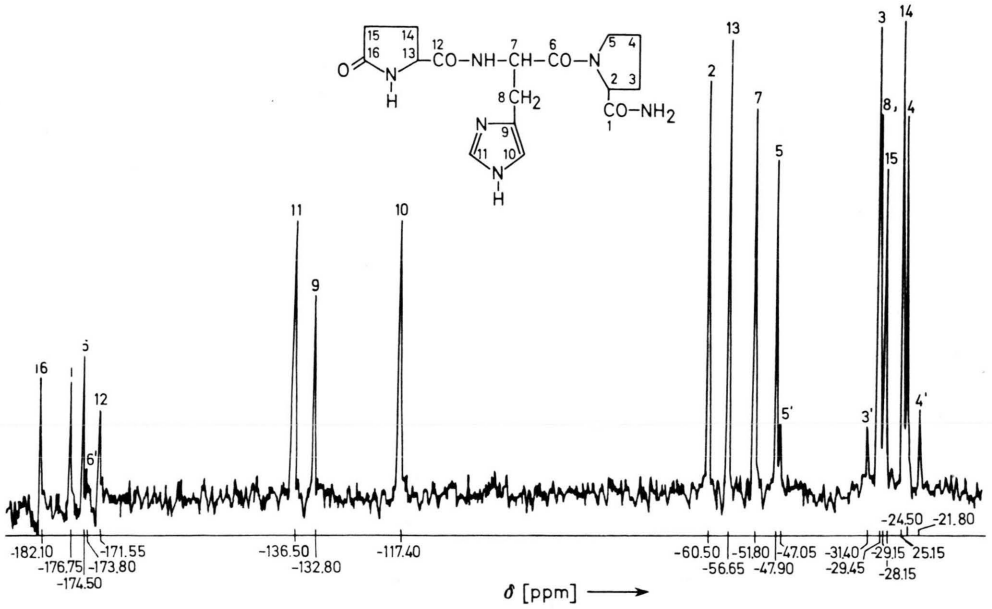


Fig. 3. *cis/trans* isomerism of TRH. →

Fig. 4. Pulse Fourier transform ^{13}C-NMR spectrum of TRH (D_2O, 200 mg, 16 384 accumulations; Bruker-HFX-90 NMR spectrometer). ↓



like TRH are best suited for the structure elucidation by mass spectrometry (see Fig. 2) [19]. Applying ^{13}C-NMR spectroscopy the real structure of TRH in water solutions could be elucidated: The hormone exists to about 15% as *cis* and 85% as *trans* isomer (see Figs 3 and 4) [20, 21]. 4. Correlate all these structural properties of releasing hormones resp. their derivatives with their biological activities [22].

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Synthesis of Gonadotropin-Releasing Hormones

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Out of the peptide hormones produced in the hypothalamus two with a stimulating effect (TRH and LH-RH) and two with an inhibiting effect (MIF and GIF) have so far been elucidated in their structure, with subsequent confirmation. A great many data of the two releasing hormones TRH and LH-RH is available. Without detailing their analogues the syntheses of these two substances are discussed below.

The final product or an intermediate product of the 16 classic and 5 Merrifield TRH syntheses known to us have mostly to be purified by column chromatography or countercurrent distribution. As such purifications encounter difficulties during the production of larger quantities, easier procedures for purification of the peptide are preferred. Since TRH itself cannot be crystallized, intermediate products with good tendency for cristallization should be obtained. Column purification could be dispensed with in various TRH syntheses, introducing the Mbh residue (4,4′-dimethoxybenzhydryl residue) into the amide function. This was possible by the good crystallization properties of the intermediate products and the smooth splitting of the protective groups.

This procedure is less suited for LH-RH with its complicated sequence, because tryptophan and tyrosine, which are sensitive to cations, would be damaged during acid splitting of the protective groups. Even in a synthesis with minimum protection aiming at unprotected LH-RH many problems arise. For example, dioxopiperazine is formed from H-Pro-Gly-NH_2 while ammonia is split off. The cheap Z-arginine or Z-Leu-Arg(HCl)-OH cannot be coupled with H-Pro-Gly-NH_2, because the secondary amine in the pyrrolidine ring shows such a strong alkaline reaction that it deprives the guanidino group of protone protection. Intramolecular lactame formation can be observed. The catalytic hydrogenation of peptides containing tryptophan seems to be dangerous.

In addition to the side reactions described, the missing crystallizability and the reactivity of the unprotected third functions of arginine, serine, tyrosine, tryptophan, and histidine lead to a strongly contaminated final product which has to be purified by means of partition chromatography.

In order to investigate the synthesis of a properly protected LH-RH-chain, splitting conditions at the intact LH-RH are simulated. The by-products appearing during this procedures, are recorded.