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SYNTHESIS OF THYROLIBERIN

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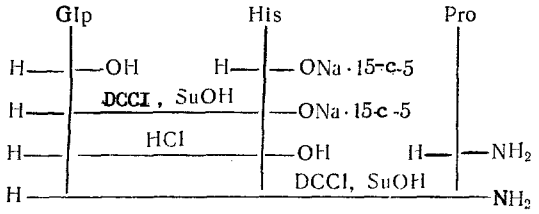
A two-stage method for the synthesis of thyroliberin is proposed. A complex of the sodium salt of histidine with 15-crown-5 is used for the protection of the carboxylic fraction of histidine.

Since the time of the isolation of thyroliberin (TRH) – a hormone of the hypothalamus – in 1969 [1], and the discovery of its antidepressant properties [2], several multistage methods for its preparation from protected amino acids have been published [3].

We have performed a two-stage synthesis of TRH with the minimum use of protective groups by the following scheme*



The condensation was performed by the carbodiimide method in dimethylformamide in the presence of N-hydroxysuccinimide. The use of a 1.1-molar excess of N-hydroxysuccinimide substantially suppressed racemization [4]. The carboxylic function of histidine was protected by salt-formation. An aqueous solution of sodium salt of histidine was mixed with an equivalent amount of 15-crown-5 [5]† and dimethylformamide, and then the water and 2-3 ml of the dimethylformamide were driven off in vacuum and the residue was treated with a mixture of pyroglutamic acid, N-hydroxysuccinimide, and N,N'-dicyclohecylcarbodiimide in dimethylformamide. The sodium salt of pyroglutamylhistidine was decomposed with an equivalent amount of HCl solution.

The pyroglutamylhistidine and the thyroliberin were purified by column chromatography on silica gel. The physicochemical characteristics of the thyroliberin obtained corresponded to those given in the literature [1].

EXPERIMENTAL

The purity of the compounds obtained was monitored by the TLC method on Silufol plates (Kavalier, Czechoslovakia). Reanal (Hungary) L-amino acids were used. Amino acid analysis was performed on a Hitachi amino acid analyzer. The amino acid and elementary analyses corresponded to the compositions of the peptides aimed at.

* Abbreviations: DDCI) N,N'-dicyclohexylcarbodiimide; SuOH) N-hydroxysuccinimide; Glp) pyroglutamic acid; His) histidine; Pro) proline.
† The nomenclature of the crown ethers has been taken from [5].

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Glp-His. A solution of 465 mg (3 mmole) of histidine in 3 ml of 1 N NaOH was treated with 660 mg (3 mmole) of 15-crown-5 and 15 ml of dimethylformamide. The water and 2-3 ml of the dimethylformamide were evaporated off in vacuum. To the residual solution was added a mixture of 387 mg (3 mmole) of pyroglutamic acid, 378 mg (3.3 mmole) of N-hydroxysuccinimide, and 660 mg (3.3 mmole) of dicyclohexylcarbodiimide. The reaction mixture was stirred at room temperature for 24 h and filtered, and the filtrate was evaporated in vacuum at 40-50°C. The residue was neutralized with 3 ml of 1 N HCl solution and, after the water had been evaporated off in vacuum, it was chromatographed on silica gel L 100/250 (0.8 × 75 cm column). Chloroform–methanol (1:1) first eluted 15-crown-5, N-hydroxysuccinimide, and pyroglutamic acid, and then methanol eluted 717 mg (90%) of pyroglutamylhistidine, mp 215-217°C, $[\alpha]_D^{20}$ – 3.26° (c 0.46; CF_3CH_2OH). R_f 0.34 (ethyl acetate–pyridine–acetic acid–water (5:5:1:3) – system 1).

Glp-His-Pro-NH_2. A mixture of 532 mg (2 mmole) of pyroglutamylhistidine, 228 mg (2 mmole) of prolinamide, 253 mg (2.2 mmole) of N-hydroxysuccinimide, and 453 mg (2.2 mmole) of dicyclohexylcarbodiimide in 5 ml of dimethylformamide was stirred for 20 h and was filtered, the precipitate was washed with 2 ml of dimethylformamide, the filtrate was evaporated, and the residue was chromatographed with chloroform–method (1:1). The fraction containing thethyroliberin was treated with 1 ml of acetic acid, the solution was evaporated in vacuum, and the residue was triturated with ethyl acetate. This gave 440 mg (60%) of thyroliberin. Hygroscopic substance, $[\alpha]_D^{20}$ – 58° (c 1; H_2O), R_f 0.74 (system 1).

SUMMARY

1. A two-stage method for the synthesis of thyroliberin has been proposed.

2. The possibility of using complexes of the sodium salts of amino acids with crown ethers for the temporary protection of a carboxylic function has been shown.

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PREPARATION OF ESTERS OF AMINO ACIDS AND OF PEPTIDES UNDER MILD CONDITIONS

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A new method for obtaining esters of N-protected amino acids and peptides from complexes of their sodium salts with 15-crown-5 that are soluble in organic solvents is proposed.

Esters of N-protected amino acids and of peptides are frequently used in peptide synthesis [1]. The most convenient among known methods for their synthesis [2, 3] is their preparation from the cesium salts of the N-protected amino acids or peptides [3]. However, the performance of esterification in a heterogeneous system according to the latest method increases the reaction time and, in a number of cases, lowers the yield.

We have proposed a method for synthesizing esters of N-protected amino acids and peptides (II) in dimethylformamide solution from their sodium salts in the presence of equimolar amounts of 15-crown-5 [1]*

* The nomenclature of the crown ethers is given in accordance with [4].

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