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METHOD OF CYCLIZATION OF CARBA ANALOGUES OF OXYTOCIN

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Introduction

Recently carba analogues of oxytocin found use in both veterinary and human medicine. [2-O-Methyltyrosine]deamino-1--carba-oxytocin (CARBETOCIN) has been introduced into veterinary therapy as oxytocicum with protracted activity and [2-p-ethylphenylalanine]deamino-6-carba-oxytocin (NACARTOCIN) is being clinically tested as saluretícum.

The purpose of the present study is to find an easy method of cyclization without significant side reactions which would enable to prepare even large quantities of analogues of neurohypophyseal hormones.

Results

The method is exemplified by the following procedure: The solution of Nps-Tyr(Me)-Ile-Gln-Asn-Cys(C_3H_6COOH)-Pro-Leu-Gly--NH_2 (17.4 g; pure by chromatography) was treated with 50 ml of 2M-HCl in ether. After 15 min standing at room temperature the hydrochloride of the octapeptide amide was precipitated with ether, dried and dissolved in the mixture of DMFA (240 ml) and dioxane (150 ml). The cooled (0°C) solution was

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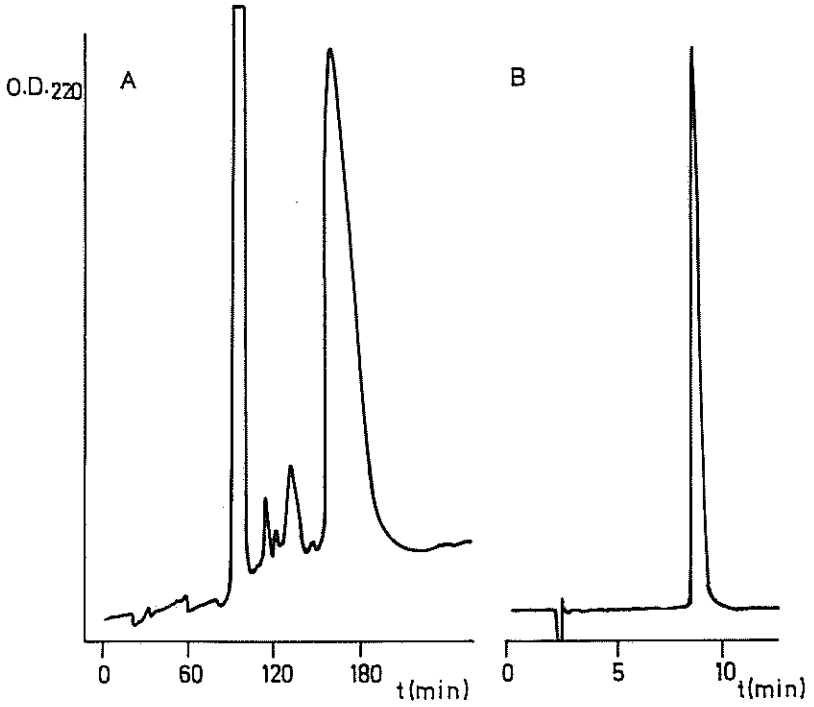
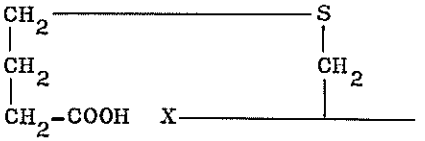
stirred with DCCI (23.1 g) and 1-hydroxybenzotriazole (21 g) for 2 h at 20°C. The solution was filtered and poured drop by drop into the stirred mixture of methanol (4 000 ml) and triethylamine (35 ml). After 3 h at room temperature the reaction mixture was evaporated to dryness; the residue was dissolved in 50% aqueous acetic acid and purified on Sephadex G-15 column (4 000 ml) eluted with the same solvent. The crude product obtained by freeze drying (9.3 g) was further purified on Separon SI-C-18 column (500 g) using mobile phase composed of 55% methanol and 45% of 0.05M ammonium acetate (pH 7.0) by volume, see Fig. 1A. Evaporation and freeze drying yielded 3.38 g (22%) of [2-O-methyltyrosine]deamino-1--carba-oxytocin. The purity of the product was checked by HPLC, see Fig. 1B.



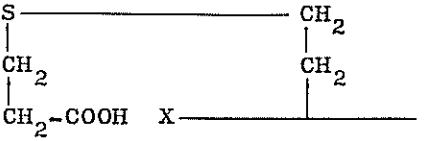
A: Preparative HPLC
B: Analytical HPLC: CGC column (0.3 x 15 cm) with SEPARON SI-C-18, mobile phase : 55% methanol and 45% 0.05M ammonium acetate (pH 7)

In the above way we prepared the following carba analogues: deamino-1-carba-oxytocin, [2-O-methyltyrosine]deamino-1--carba-oxytocin, deamino-6-carba-oxytocin, [2-p-ethylphenylalanine]deamino-6-carba-oxytocin, all in 20-25% yield of HPLC pure products. The procedure is represented by the following scheme:

Carba 1:

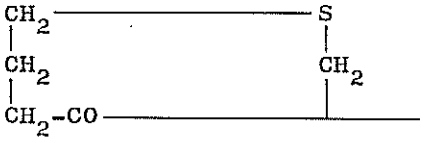


Carba 6:



X = Nps, Boc, Z

1 - splitting off the protecting group
2 - protection by protonation
3 - activation of carboxylic group
4 - cyclization



Discussion

With the various types of peptides different cyclization methods were used for closing the carba bridge (see e.g. 1-3). The most commonly used active ester method with the active ester prepared from properly substituted aryl sulphite suffers from difficulties connected with the preparation of the particular sulphite the stability of which is also limited. The synthesis of the active ester is time consuming. The most serious side reaction accompanying the cyclization with p-nitro- or 2,4,5-trichlorophenylesters in pyridine at eleva-

ted temperature is the one leading to products of higher molecular weight. Another disadvantage of the mentioned methods consists in the necessity to remove the protecting group after the synthesis of the active ester (often a discomforting feature). We found it possible to prepare the N-hydroxybenzotriazole active ester in situ with DCCI from a peptide protected only by protonation. Cyclization proceeds almost instantaneously after liberation of the amino group by pouring the solution of the active ester salt into alkaline medium where the intramolecularly cyclized product is predominantly formed.

The reaction is fast enough even at room temperature. There was found no transesterification in methanol (on comparison by HPLC with an authentic sample of the corresponding linear peptide methyl ester). Trials were done also with other solvents (acetonitrile, tert-butanol, pyridine, isopropanol, DMFA).

Sufficiently high reaction rate enables the reaction to be performed without risk in a small volume compared with other methods of cyclization. The described procedure was tested on cyclization of both small (30 mg) and big (26 g) batches.

References

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