Tetrahedron Letters No.17, pp. 2037-2039, 1968. Pergamon Press. Printed in Great Britain.

SOLID PHASE SYNTHESIS OF OXYTOCIN

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(Received in Germany 8 January 1968)

Oxytocin has been synthesized by various laboratories, using classical methods of peptide synthesis (1, 2, 3, 4, 5). We wish to report the synthesis of oxytocin by the solid phase method of Merrifield (6). Using this method we have synthesized oxytocin in a matter of days in extremely high yields.

The synthesis was carried out in a manner similar to that described by Bayer et al. (7) for apoferredoxin. Boc-amino acids were prepared according to the pH-Stat method of Schnabel (8). 3.2 mMol t-butyloxycarbonyl glycine were esterified to 10 g of chloromethylated Bio-Beads SX-2 (Bio Rad Laboratories, Richmond, California, Control Number 3553, Capacity 1.8 meq/gm). One coupling cycle consisted of: removal of the Boc-group with 1 N HCl in glacial acetic acid, neutralization of the resulting hydrochloride with triethylamine in N,N'-dimethylformamide, coupling the new amino acid to the free amino group with N,N'-dicyclohexylcarbodiimide (DCCI) as coupling agent in methylene chloride as solvent, except in cases of Asn and Gln which were introduced as p-nitro-phenyl esters of the Boc-amino acids in N,N'-dimethylformamide as solvent. Boc-amino acids and Boc-amino acid p-nitrophenyl esters were used in 4.5 fold excess.

Reaction times used were 2 hours in case of coupling with DCCI and 8 hours in case of the active esters. Excess reagents were removed by washing with methylene chloride, ethanol, glacial acetic acid and N,N-dimethylformamide. By titration of the chloride in triethylamine hydrochloride formed during the neutralization step, the free amino groups available for the next coupling step were determined indirectly. According to these titrations the first four coupling steps occured with 100% yield, the following three with 99% each.

The fully protected nonapeptide esterified to the resin was finally washed with methanol and suspended in 100 ml methanol. The suspension was saturated with ammonia at $-20^{\circ}C$ and allowed to stand at room temperature in a closed vessel for 24 hours. The resin was filtered

off, washed with methanol and N,N'-dimethylformamide. Methanol and ammonia were removed in vacuo and the Boc-O-Benzyl-S-benzyl-peptideamide precipitated with ether. The precipitate was centrifuged off and dried in vacuo.

The protective groups with the exception of the S-Benzyl-groups were removed in trifluoroacetic saturated with HBr. After 90 min. most of the trifluoroacetic acid was removed in vacuo. The S-Benzyl peptide amide was precipitated with ether, dissolved in DMF and reprecipitated with ether. Yield 2.95 g (2.49 mM) corresponding to 78%, calculated on the basis of glycine esterified to the resin. A sample of this product was hydrolyzed in 6 N hydrochloric acid at 110°C for 20 hours in vacuo and analyzed on a Beckman Amino Acid Analyzer (Model Unichrom). The following molar ratios of amino acids and ammonia were obtained, with the value of glycine taken as 1.0: Gly 1.0, Leu 1.1, Pro 1.0, Cys(S-Bzl) 2.0, Asp 1.0, Glu 0.9, Ile 1.0, Tyr 1.0, ammonia 2.9.

600 mg of the S-benzyl protected peptide were dissolved in 250 ml liquid ammonia, redistilled over sodium. Reductive cleavage of the S-benzyl groups was carried out by adding sodium, dissolved in liquid ammonia, dropwise from a cooled dropping funnel. After a stable blue color was obtained for 60 sec. the ammonia was evaporated to a volume of 50 ml. Remaining ammonia was removed by freeze drying. The residue (725 mg) was dissolved in 250 ml 0.2% acetic acid, the pH adjusted to 7.0 and air bubbled through the solution for 8 hours. The solution was allowed to stand in air contact for an additional 8 hours. Complete oxidation was assured by addition of ferricyanate.

After freeze drying the white powder was dissolved in 0.2 N acetic acid and purified on a column of Sephadex G-25 (118 x 2.5 cm). The main Folin-Lowry (9) positive peak was collected and lyophylized. 400 mg of a coarse white powder were obtained. Amino acid analysis revealed the following composition: Gly 0.9, Leu 1.2, Pro 1.1, Cys 2.0, Asp 1.0, Glu 1.0, Ile 1.1, Tyr 1.0, ammonia 3.0, with the value of aspartic acid taken as 1.0.

Oxytocic activity of this product was tested on isolated rat uterus and it was found to be active with about 220 units (10) per mg[+]. 200 mg of the partially purified oxytocin sample was subjected to partition chromatography on Sephadex G-25 in the solvent system 1-butanol-benzene-pyridine-0.1% acetic acid (6:2:1:9) at room temperature (11). An elution pattern similar to that described by Yamashiro (11) was obtained. The Folin-Lowry positive peak with an R_f value of 0.26 was collected and the product isolated by freeze drying. 80 mg of oxytocin with a potency on the isolated rat uterus of about 450 units/mg were obtained.

[+] The authors are indebted to Dr. H. Uehleke, Pharmakologisches Institut der Universität Tübingen, for carrying out the bioassay.

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