The Synthesis of a Heptadecapeptide Amide Corresponding to a Modified Sequence in the Corticotropin Structure

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In order to examine whether or not the level of hormonal potency depends simply on the degree of basicity at positions 15—18 of the corticotropin (ACTH) molecule, we have synthesized a heptadecapeptide amide, H-Gly-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-Gly-Lys-Arg - Arg - NH_2 ($\alpha^{1-14,16-18}$ - Gly^1-ACTH-18-NH_2) (I);*1 The peptide I has bene found to possess 1.26 and 2.72 USP units per mg. of steroidogenic activity, as estimated in vitro[1] and in vivo[2] respectively.*2 On the other hand, the peptide I has been found to elicit a high lipolytic activity at minimal effective doses of 0.0037 μg. in rat adipose tissue and of 0.00042 μg. in rabbit.[3],*2

Cbz-Arg(NO_2)-OH (II) was converted, via

*1 All amino acid residues are of the L-configuration with the exception of glycine. In this communication, the following abbreviations will be used: Cbz, carbobenzoxy; BOC, *t*-butyloxycarbonyl; Ac, acetyl; But, *t*-butyl; CMC, carboxymethyl cellulose.

1) M. Saffran and A. V. Schally, *Endocrinol.*, **56**, 523 (1955).

2) By a slight modification of Lipscomb and Nelson (H. S. Lipscomb and D. H. Nelson, ibid., **71**, 13 (1962)).

*2 The authors are much indebted to Dr. Akira Tanaka for these biological assays.

3) A. Tanaka, B. T. Pickering and C. H. Li, *Arch. Biochem. Biophys.*, **99**, 294 (1962).

a mixed anhydride, into Cbz-Arg(NO_2)-NH_2 (m. p. 220—221°C, $[\alpha]_D^{24}$ +4.9° (*c* 1.95, dimethylformamide); lit.[4] m. p. 219—220°C); this was then treated with HBr/AcOH to obtain H-Arg(NO_2)-NH_2·HBr (III) (m. p. 237—239°C, $[\alpha]_D^{26}$ +13.7° (*c* 2.6, water)). The coupling of II with III by the mixed anhydride procedure gave Cbz-Arg(NO_2)-Arg(NO_2)-NH_2 (IV), (m. p. 115—115.5°C, $[\alpha]_D^{25}$ −6.2° (*c* 2.1, 50% AcOH)). Cbz-Lys(BOC)-Pro-Val-Gly-Lys(BOC)-N_3, which was derived from the hydrazide,[5] was allowed to react with H-Arg(NO_2)-Arg(NO_2)-NH_2·HBr (m. p. 153—156°C decomp., $[\alpha]_D^{23.5}$ +14.1° (*c* 2.0, water)), which had been obtained from IV by the HBr/AcOH treatment, to give Cbz-Lys(BOC)-Pro-Val-Gly-Lys(BOC)-Arg(NO_2)-Arg(NO_2)-NH_2 (V) ($[\alpha]_D^{25.5}$ −40.4° (*c* 1.8, methanol)). Compound V was then hydrogenolyzed to obtain H-Lys(BOC)-Pro-Val-Gly-Lys(BOC)-Arg-Arg-NH_2 (VI) ($[\alpha]_D^{24}$ −44.8° (*c* 1.5, 50% AcOH).

The activated decapeptide ester, BOC-Gly-Tyr-Ser-Met-Glu(γ-Bu^t)-His-Phe-Arg-Try-Gly-ON(CO-CH_2)$_2$[6], was allowed to react with VI to afford BOC-Gly-Tyr-Ser-Met-Glu(γ-Bu^t)-His-Phe-Arg-Try-Gly-Lys(BOC)-Pro-Val-Gly-Lys(BOC)-Arg-Arg-NH_2 (VII). A crude sample of VII was, after having been treated with thioglycollic acid, purified by CMC column chromatography ($[\alpha]_D^{23.5}$ −35.8° (*c* 0.44, 50% AcOH). The purified VII was treated with 90% trifluoroacetic acid in order to liberate the heptadecapeptide I. $\lambda_{max}^{0.1N\ NaOH}$ = 281.5 mμ (ε 7070), 288.5 mμ (ε 6850). $[\alpha]_D^{23.5}$ −57.4° (*c* 0.5, 0.1 N AcOH). Amino acid ratios in acid ratios in acid hydrolysate:[7] Gly 2.81, Tyr 0.99, Ser 0.86, Met 1.00, Glu 1.00, His 1.01, Phe 0.91, Arg 2.74, Lys 2.12, Pro 1.04, Val 1.02, Try 0.57*3, NH_3 1.30. Tyr/Try ratio in the intact I:[8] 1.00 : 1.14.

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4) H. van Orden and E. L. Smith, *J. Biol. Chem.*, **208**, 751 (1954).
5) H. Otsuka, K. Inouye and Y. Jono, This Bulletin, **37**, 1471 (1964).
6) H. Otsuka, K. Inouye, M. Kanayama and F. Shinozaki, ibid., **38**, 679 (1965).
7) D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1191 (1958).
*3 Decomposed partially with acid.
8) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946); G. H. Beavan and E. R. Holliday, *Advances in Protein Chemistry*, **7**, 319 (1952).