The Synthesis and Biological Activities of a Hexadecapeptide Corresponding to a Modified Sequence in the Corticotropin Structure

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The syntheses of adrenocorticotropically active peptides, which have chain lengths of less than a half of the 39-amino acid chain in the corticotropin (ACTH) structure, have been reported by several investigators,[1-7] We wish to describe herein the synthesis of a hexadecapeptide with a modified sequence of the first octadecapeptide portion of the ACTH molecule, in which the *N*-terminal serine is replaced by a glycine and the lysyl-lysyl sequence at positions 15—16 is removed; this substance is, namely, glycyl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycyl-lysyl-prolyl-valyl-glycyl-arginyl-arginine (I),*1 which may be designated as $\alpha^{1-14,17-18}$-Gly^1-ACTH. This synthetic peptide has been shown to have a very low but a consistently reproducible adrenal steroidogenic activity of 0.134 U.S.P. unit per mg., as estimated by the in vitro method;[8] it has also brought about a plasma corticosterone elevation in a hypophysectomized rat.*2 The results may partially validate the finding of Lebovitz and Engel that the hydroxymethyl group of the *N*-terminal serine is not necessary for the ACTH activity.[9] The peptide has been also found to exhibit a high lipolytic potency[10] of the minimal effective doses, 0.00093 μg. in rabbit adipose tissue and 0.031 μg. in the rat,*2 values which were of the same order of magnitude as those reported for the heptadecapeptide, α^{1-17}-ACTH.[3,10]

N^{α}-Cbz-N^{ε}-BOC-lysyl-prolyl-valyl-glycyl-nitroarginyl-nitroarginine benzyl ester (II) (m.p. 114—115°C, $[\alpha]_D^{25}$ −49.4° (*c* 2.0, methanol). Found: C, 52.54; H, 6.49; N, 18.52. Calcd.: C, 52.48; H, 6.78; N, 18.36%) was obtained by the DCCI-mediated condensation of N^{α}-Cbz-N^{ε}-BOC-lysyl-prolyl-valyl-glycine[11] with nitroarginyl-nitroarginine benzyl ester, which had been derived by trifluoroacetic acid treatment from the BOC-nitroarginyl-nitroarginine benzyl ester ($[\alpha]_D^{25}$ −18.1° (*c* 2.5, methanol). Found: C, 47.68; H, 6.78; N, 21.42. Calcd.: C, 47.21; H, 6.27; N, 22.94%). Compound II was hydrogenolyzed to give N^{ε}-BOC-lysyl-prolyl-valyl-glycyl-arginyl-arginine diacetate (III) ($[\alpha]_D^{26.5}$ −50.1° (*c* 1.6, 50% acetic acid). Found: N, 19.51; CH_3CO, 9.68. Calcd.: N, 19.54; CH_3CO, 9.24%), which was then converted into the trihydrochloride (IV) (Found: Cl, 12.05.

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*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; DCCI, *N*,*N*′-dicyclohexylcarbodiimide; CMC, carboxymethyl cellulose.
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Calcd.: Cl, 11.54%).

BOC-glycine was coupled with tyrosine methyl ester (m. p. 134—135°C, $[\alpha]_D^{23.5}$ +26.9° (*c* 2.4, methanol); lit.[12] m. p. 135°C) by DCCI to give the acyldipeptide ester, which was then converted into the BOC-glycyl-tyrosine hydrazide (V) (m. p. 189—190°C, $[\alpha]_D^{26.5}$ +0.5° (*c* 2.0, dimethylformamide). Found: C, 54.56; H, 6.79; N, 15.93. Calcd.: C, 54.53; H, 6.87; N, 15.90%). The BOC-seryl-methionine methyl ester (VI) (m. p. 66—67.5°C, $[\alpha]_D^{25.5}$ −29.5° (*c* 2.0, methanol). Found: C, 48.06; H, 7.61; N, 8.20; S, 9.34. Calcd.: C, 47.98; H, 7.48; N, 7.99; S, 9.15%) was prepared by the DCCI-induced coupling of the methionine methyl ester with the crystalline BOC-serine monohydrate (m. p. 45—47°C, $[\alpha]_D^{25}$ −7.6° (*c* 2.6, water), $[\alpha]_D^{26}$ −3.0° (*c* 2.3, acetic acid). Found: C, 43.25; H, 7.88; N, 6.52; H_2O, 7.19. Calcd.: C, 43.04; H, 7.68; N, 6.28; H_2O, 8.07%). The azide prepared from V was allowed to react with the dipeptide ester, which had beed derived from VI by HCl/ethyl acetate treatment, to obtain the protected tetrapeptide BOC-glycyl-tyrosyl-seryl-methionine methyl ester (VII) (m. p. 183—185°C, $[\alpha]_D^{24}$ −16.5° (*c* 1.15, methanol). Found: C, 52.44; H, 7.01; N, 9.98; S, 5.70. Calcd.: C, 52.62; H, 6.71; N, 9.82; S, 5.62%). Compound VII was treated with hydrazine to give the corresponding hydrazide hemihydrate (VIII) (m. p. 201—203°C decomp., $[\alpha]_D^{27}$ −19.4° (*c* 1.2, 50% acetic acid). Found: C, 50.07; H, 6.93; N, 14.24; S, 5.48. Calcd.: C, 49.73; H, 6.78; N, 14.50; S, 5.53%).

γ-*t*-Butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine[13] and the azide, which had been derived from VIII, were coupled together to give the decapeptide derivative BOC-glycyl-tyrosyl-seryl-methionyl-γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine dihydrate (IX) (m. p. 212—218°C (decomp.), $[\alpha]_D^{26.5}$ −15.5° (*c* 1.7, dimethylformamide). Found: C, 55.17; H, 6.84; N, 15.03; S, 2.14. Calcd.: C, 55.05; H, 6.62; N, 15.33; S, 2.19%). The hydrochloride of IX was then converted into the *N*-hydroxysuccinimide ester,[14] and this active ester was allowed to react with IV in an aqueous pyridine solution to give the hexadecapeptide derivative BOC-glycyl-tyrosyl-seryl-methionyl-γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycyl-N^{ϵ}-BOC-lysyl-prolyl-valyl-glycyl-arginyl-arginine (X), which was then partially purified on a CMC column. Compound X was treated with 90% trifluoroacetic acid to yield the free hexadecapeptide I, which was then purified by CMC column chromatography. The purified material was found to be homogeneous in paper electrophoresis and in paper chromatography. $\lambda_{max}^{0.1N\ HCl}$ = 278 mμ (ε = 5990), $\lambda_{shoulder}^{0.1N\ HCl}$ = 288 mμ (ε = 4490); $\lambda_{max}^{0.1N\ NaOH}$ = 281 mμ (ε = 6950), $\lambda_{max}^{0.1N\ NaOH}$ = 288 mμ (ε = 6840). $[\alpha]_D^{52}$ −54.3° (*c* 0.5, 0.1N acetic acid). Amino acid ratios in the acid hydrolysate:[15] Gly 2.84, Tyr 1.01, Ser 1.05, Met 1.05, Glu 1.00, His 1.12, Phe 1.00, Arg 2.89, Lys 0.97, Pro 1.04, Val 0.94 (average recovery 92.5%).*3 The intact hexadecapeptide was determined spectrophotometrically to contain tyrosine and tryptophan in a molar ratio of 1.00 : 0.95.[16]

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