Synthesis of a Heptacosapeptide Corresponding to the Human Corticotropin 1-27 Sequence

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In previous communications we described the syntheses and biological properties of corticotropin (ACTH)-octadecapeptides including [β-Ala^1]-ACTH(1-18)-NH_2.[1-3] We wish now to report the synthesis of a peptide corresponding to the amino acid sequence 1-27 of human ACTH, *viz.*, H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asp-Ala-Gly-OH, h-ACTH (1-27)-OH (I).[4]

Z-Val-Lys(Boc)-Val-Tyr-Pro-Asp(OBu^t)-Ala-Gly-OBu^t (II) ($[\alpha]_D^{25}$ $-69.2°$ (*c* 1, methanol). Found: C, 60.30; H, 7.84; N, 10.28. Calcd for $C_{60}H_{91}N_9O_{16}$: C, 60.34; H, 7.68; N, 10.55%) was synthesized stepwise starting from the Gly-OBu^t at *C*-terminal, in which Z-Ala, Z-Asp(OBu^t) and Z-Pro were incorporated with DCC and Z-Tyr(Z), Z-Vals and Z-Lys(Boc) by the *p*-nitrophenyl ester method in combination with the hydrogenolytic removal of the N^α-protecting group. Z-Arg(NO_2)-Arg(NO_2)-Pro-OMe (mp 115—120°C decomp, $[\alpha]_D^{27}$ -37.1 (*c* 1, AcOH), $-30.5°$ (*c* 1, DMF)) was treated with HBr/AcOH for removal of the Z-group, followed by acylation with Z-Lys(Boc)-OCOOEt to yield a tetrapeptide ester which was readily saponified to Z-Lys(Boc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (III) ($[\alpha]_D^{28}$ $-36.7°$ (*c* 1, methanol)). Compound III and an octapeptide ester, derived from II by catalytic hydrogenolysis, were coupled by the *N*-hydroxysuccinimide-mediated DCC method[5] to give Z-Lys(Boc)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Asp(OBu^t)-Ala-Gly-OBu^t (IV) ($[\alpha]_D^{22}$ $-78.5°$ (*c* 1, methanol). Found: C, 54.14; H, 7.53; N, 15.88. Calcd for $C_{88}H_{140}N_{22}O_{26}\cdot H_2O$: C, 54.48; H, 7.38; N, 15.88%). Compound IV was submitted to hydrogenolysis and the product was coupled with Z-Lys(Boc)-Pro-Val-Gly-Lys(Boc)-N_3[6] to give a heptadecapeptide, Z-Lys(Boc)-Pro-Val-Gly-Lys(Boc)-Lys(Boc)-Arg-Arg-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Asp(OBu^t)-Ala-Gly-OBu^t, which was converted into the N^α-free peptide (V) ($[\alpha]_D^{23}$ $-74.2°$ (*c* 0.7, 50% AcOH)) by catalytic hydrogenolysis.

A decapeptide Z-Ser-Tyr-Ser-Met-Glu(OBzl)-His-Phe-Arg-Trp-Gly-OH (mp 205—210°C decomp, $[\alpha]_D^{22}$ $-13.0°$ (*c* 1, DMF)), which was synthesized from Z-Ser-Tyr-Ser-N_3[7] and H-Met-Glu-(OBzl)-His-Phe-Arg-Trp-Gly-OH,[8] was esterified with *N*-hydroxysuccinimide by the DCC method[9] and the resulting active ester was allowed to react with V to afford a protected heptacosapeptide, which was treated with hydrogen fluoride[10] to liberate the free peptide (I). The crude product was purified by chromatography on a carboxymethyl cellulose column; $[\alpha]_D^{23}$ $-89.9°$ (*c* 0.5, 0.1N AcOH), $\lambda_{max}^{0.1N\ NaOH}$ $=282$ mμ ($E_{1cm}^{1\%}=25.3$), 288 mμ ($E_{1cm}^{1\%}=26.7$). Amino acid ratios in acid hydrolysate: Asp 1.07, Ser 1.72, Glu 1.02, Pro 3.17, Gly 3.15, Ala 1.03, Val 3.00, Met 0.98, Tyr 2.06, Phe 1.03, Lys 4.11, His 0.91, Arg 2.95. The Tyr/Trp ratio in intact I was 1.8 as determined spectrophotometrically. Peptide I was found to possess an adrenal-stimulating activity of 263 units/mg (3rd USP standard as standard) in the in vivo steroidogenesis.[11]

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4) All amino acid residues are of the L-configuration. The abbreviated designation of amino acids, peptides and their derivatives accords with the proposal of the IUPAC-IUB Commission of Biochemical Nomenclature, which appeared in *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967). Other abbreviations used in this paper are: Et=ethyl, Ac=acetyl, DMF=dimethylformamide, and DCC=*N*,*N*'-dicyclohexylcarbodiimide.

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11) The assay was performed by Dr. Akira Tanaka of this Laboratory according to Lipscomb and Nelson (H. S. Lipscomb and D. H. Nelson, *Endocrinology*, **71**, 13 (1962)) with a minor modification.