Synthesis of D-Ser[1]-Nle[4]-(Val-NH$_2$)[25]-β-Corticotropin(1-25), a Highly Potent Analogue of ACTH

In the hope of obtaining an ACTH-analogue more resistant to deactivation by air oxidation and amino- or carboxypeptidase attack than the natural hormone[1] and its active synthetic analogues prepared so far[2–7], we have synthesized a new analogue, which contains an aminopeptidase resistant D-serine residue at its amino end, a carboxypeptidase resistant L-valinamide residue at its carboxyl end and, instead of the readily oxidizable methionine residue in position 4, an isologous norleucine residue.

This analogue represents a pentacosapeptide with the following sequence: D-seryl-L-tyrosyl-L-seryl-L-norleucyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophanyl-glycyl-L-lysyl-L-prolyl-L-valyl-glycyl-L-lysyl-L-lysyl-L-arginyl-L-arginyl-L-prolyl-L-valyl-L-lysyl-L-valyl-L-tyrosyl-L-prolyl-L-valinamide.

This pentacosapeptide was synthesized by methods known to avoid racemization. Intermediary peptides were built up by recurrent addition of Z-amino acids[8] and condensed together by the azide method or at the level of glycine or proline residues.

By using the mixed anhydride method Z-Val-Gly-Lys(Boc)-Lys(Boc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (m.p. 151° dec.; $[\alpha]_D^{20} = -38°$ in methanol; $\log \varepsilon = 4.48$ at $\lambda_{max} = 268.5$ nm) was condensed with H-Val-Lys(Boc)-Val-Tyr-Pro-Val-NH_2 (m.p. 145° dec.; $[\alpha]_D^{20} = -68°$ in methanol; $\log \varepsilon = 3.15$ at $\lambda_{max} = 277$ and 283 nm) into Z-Val-Gly-Lys(Boc)-Lys(Boc)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Val-NH_2 (m.p. 190° dec.; $[\alpha]_D^{20} = -36°$ in dimethylformamide; $\log \varepsilon = 4.47$ at $\lambda_{max} = 269$ nm).

After elimination of the Z- and NO_2-groups by catalytical hydrogenation H-Val-Gly-Lys(Boc)-Lys(Boc)-Arg-Arg-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Val-NH_2 (m.p. 191° dec.; $[\alpha]_D^{20} = -56°$ in acetic acid/water (95:5); $\log \varepsilon = 3.15$ at $\lambda_{max} = 284.5$ nm) was obtained, which, after conversion into the corresponding tritosylate, was coupled by the DCCI method with Trt-Glu(OBu^t)-His(Trt)-Phe-Arg-Trp-Gly-Lys(Boc)-Pro-OH (m.p. 209° dec.; $[\alpha]_D^{20} = -14°$ in dimethylformamide; $\log \varepsilon = 3.63$ at $\lambda_{max} = 280.5$ nm) into Trt-Glu(OBu^t)-His(Trt)-Phe-Arg-Trp-Gly-Lys(Boc)-Pro-Val-Gly-Lys(Boc)-Lys(Boc)-Arg-Arg-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Val-$NH_2 \cdot 3$Tos-OH (m.p. 184° dec.; $[\alpha]_D^{20} = -53°$ in methanol; $\log \varepsilon = 3.82$ at $\lambda_{max} = 280.5$ nm). After selective elimination of the α-Trt-group, the resulting enecosapeptide H-Glu(OBu^t)-His(Trt)-Phe-Arg-Trp-Gly-Lys(Boc)-Pro-Val-Gly-Lys(Boc)-Lys(Boc)-Arg-Arg-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Val-$NH_2 \cdot 3$ Tos-OH (m.p. 170° dec.; $[\alpha]_D^{20} = -50°$ in methanol; $\log \varepsilon = 3.88$ at $\lambda_{max} = 281$ nm) was condensed with Boc-D-Ser-Tyr-Ser-Nle-N_3 (prepared from the corresponding hydrazide of m.p. 211° and $[\alpha]_D^{20} = +8°$ in dimethylformamide) into Boc-D-Ser-Tyr-Ser-Nle-Glu(OBu^t)-His(Trt)-Phe-Arg-Trp-Gly-Lys(Boc)-Pro-Val-Gly-Lys(Boc)-Lys(Boc)-Arg-Arg-Pro-Val-Lys(Boc)-Val-Tyr-Pro-Val-$NH_2 \cdot 3$Tos-OH and purified by chromatography on CM-Cellulose (m.p. 198° dec.; $[\alpha]_D^{20} = -36°$ in acetic acid/water (95:5); $\log \varepsilon = 3.93$ at $\lambda_{max} = 279$ nm). After cleavage of all protecting groups by trifluoracetic acid and treatment with IRA-410 in the acetate form, the free pentacosapeptide was obtained as dodecaacetate decahydrate in analytically pure state (m.p. 172° dec.; $[\alpha]_D^{20} = -74°$ in 1 n acetic acid; $\log \varepsilon = 3.85$ at $\lambda_{max} = 275.5$ nm in 1 n acetic acid; calculated for $C_{142}H_{222}N_{42}O_{31} \cdot 12CH_3CO_2H \cdot 10H_2O$: C 50.9; H 7.5; N 15.0; O 26.6; CH_3CO_2H 18.4%. Found: C 50.8; H 7.6; N 14.7; O 26.1; CH_3CO_2H 18.8%).

The pentacosapeptide gives a single spot on thin layer chromatography (n-butanol/water/pyridine/acetic acid 30:24:20:6; silica gel) and paper electrophoresis (mobility: 1.25 Glu at pH 1.9 and 0.95 His at pH 5.8), ninhydrine, chlorine, bromophenol blue, Folin's, Pauli's, Ehrlich's and Sakaguchi's reagents, being used for staining. On counter current distribution in the system sec-butanol/water/trifluoroacetic acid (120:160:1) it gives a single peak of K = 0.5 having the theoretical pattern. On total acid hydrolysis the following amino acid composition is obtained: $Ser_{2.0}$; $Tyr_{2.1}$; $Nle_{1.0}$; $Glu_{1.0}$; $His_{1.0}$; $Phe_{1.0}$; $Arg_{3.1}$; $Gly_{2.0}$; $Lys_{4.1}$; $Pro_{3.0}$; $Val_{3.9}$. After incubation for 24 h with either leucine aminopeptidase or carboxypeptidase no amino acids are released and the pentacosapeptide is recovered intact.

Modifications at the amino terminal end of the ACTH sequence[7,9] or replacement of the methionine residue by an α-aminobutyric residue[10] are known to cause a decrease of the biological activity. Therefore, the high and hitherto unsurpassed level of corticotropic activity exhibited by this pentacosapeptide (ca. 625 I.U./mg free base), both in the rat[11] and in the human[11,12], is remarkable.

Résumé. Le nouveau pentacosapeptide D-séryl-L-tyrosyl-L-séryl-L-norleucyl-L-glutamyl-L-histidyl-L-phénylalanyl-L-arginyl-L-tryptophanyl-glycyl-L-lysyl-L-prolyl-L-valyl-glycyl-L-lysyl-L-lysyl-L-lysyl-L-arginyl-L-arginyl-L-prolyl-L-valyl-L-lysyl-L-valyl-L-tyrosyl-L-prolyl-L-valinamide a été synthétisé par des méthodes évitant la racémisation. Ce pentacosapeptide possède une activité corticotrope très élevée (env. 625 U.I./mg base libre; cf. les deux communications suivantes), ne contient pas le reste méthionine facilement oxydable de l'ACTH naturelle, et est résistant à l'action des amino- et carboxypeptidases.

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[8] The following abbreviations are used: Z = benzyloxycarbonyl; Boc = tert. butyloxycarbonyl; Trt = triphenylmethyl; NO_2 = nitro; OBu^t = oxy-tert. butyl; Tos = toluenesulphonyl; DCCI = dicyclohexylcarbodiimid. UV-spectra are taken in methanol/water/triethylamine 57:14:1. All products were pure on paper electrophoresis and thin layer chromatography and gave correct elementary analyses. For the sake of brevity, we have included here analysis results for the final product only.
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