Communications to the Editor

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Synthesis of Peptides Related to Corticotropin (ACTH). VI.[1] Syntheses and Biological Activity of the Peptides Corresponding to the Amino Acid Sequences 4—23, 5—23, 6—24 and 7—23 in ACTH[2]

Recently, much work on the syntheses and the structure-activity relationship of adrenocorticotropin (ACTH) fragments has been done to show that the amino-terminal part of ACTH is not essential for the biological activity. Geiger, *et al.*[3] have synthesized glycine[1]-, phenylalanine[2]-, alanine[3]-, and deserine[1]-analogs of α^{1-23NH_2}-ACTH, and Otsuka, *et al.*[4] have synthesized glycine[1]-analogs of α^{1-17NH_2}-ACTH and α^{1-18NH_2}-ACTH. These analogs showed significant biological activities.

Furthermore, Boissonnas, *et al.*[5] and Kappeler, *et al.*[6] synthesized D-serine[1]-analogs, and Geiger, *et al.*[7] and the authors[8] independently synthesized β-alanine[1]-analogs. It is interesting to note that all these analogs possessed an apparently higher potency than the corresponding L-serine[1]-analogs.

On the other hand, Elpiner, *et al.*[9] have reported, from the studies on the action of ultrasonic waves on natural ACTH, that the lack of the N-terminal tetrapeptide (Ser-Tyr-Ser-Met) did little affect the activity, while the lack of the hexapeptide (Ser-Tyr-Ser-Met-Glu-His) eliminated the activity of this hormone.

In order to derive more information on the structure-activity relationship of the N-terminal part of this hormone, we prepared α^{4-23NH_2}-ACTH, α^{5-23NH_2}-ACTH, α^{6-24}-ACTH and α^{7-23NH_2}-ACTH and examined their *in vivo* steroidogenic potencies.

In our previous synthetic works[8,10] of α^{1-23NH_2}-ACTH and α^{1-24}-ACTH, we obtained protected heptadecapeptide amide BOC-Phe-Arg(NO_2)-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-NH_2(I), protected octadecapeptide BOC-Phe-Arg(NO_2)-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-OH (II) and protected eicosapeptide amide BOC-Met-Glu(OtBu)-His-Phe-Arg(NO_2)-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-NH_2 (III).

For preparing protected nonadecapeptide amide Z-Glu(OtBu)-His-Phe-Arg(NO_2)-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-NH_2 (IV) [mp 179—182° (decomp), $[\alpha]_D^{22.5}=-38.0°$ ($c=0.5$ in DMF)], the N^{α}-BOC-group of the protected peptide I was deblocked by treatment with trifluoroacetic acid and the resulting partially protected peptide was then acylated with Z-Glu(OtBu)-His-N_3 which was prepared from the

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2) The amino acids, peptides and their derivatives (except glycine) mentioned in this communication are of the L-configuration. Abbreviations used are: ACTH, adrenocorticotropic hormone; MSH, melanocyte-stimulating hormone; Z, benzyloxycarbonyl; BOC, *t*-butyloxycarbonyl; OtBu, *t*-butyl ester; NO_2, nitro; N_3, azide; $NHNH_2$, hydrazide.
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5) R.A. Boissonnas, S. Guttmann and J. Pless, *Exper.*, **22**, 526 (1966).
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corresponding hydrazide.[11] Protected nonadecapetpide Z-His-Phe-Arg(NO_2)-Trp-Gly-Lys-(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-OH (V) [mp 140.0—150.0° (decomp.), $[\alpha]_D^{23.2}=-39.9°$ (c=1.0 in DMF)] was prepared from the protected peptide II and Z-His-$NHNH_2$[12] by the usual azide method.

These four protected peptides (I, III, IV, and V) were subjected to acidolysis with anhydrous hydrogen fluoride[13] as described in the previous paper,[8] and the resulting products were purified by the routine chromatography on a carboxymethyl cellulose column to give the free peptides.

The purified peptides were homogeneous as judged by the thin-layer chromatography and paper electrophoresis.

The Heptadecapeptide Amide ($\alpha^{7\text{-}23NH_2}$-ACTH)

$[\alpha]_D^{22.8}=-80.6°$ (c=0.1 in 1% AcOH), UV $\lambda_{max}^{0.1N\ NaOH}$ mμ ($E_{1cm}^{1\%}$): 282.5 (24.97), 289.4 (23.99). Amino acid ratios in an acid hydrolysate: Lys 4.00, Arg 3.14, Pro 1.97, Gly 2.00, Val 2.83, Tyr 0.93, Phe 1.00 (average recovery 99.3%).

The Nonadecapeptide ($\alpha^{6\text{-}24}$-ACTH)

$[\alpha]_D^{24.5}=-86.8°$ (c=0.5 in 1% AcOH), UV $\lambda_{max}^{0.1N\ NaOH}$ mμ ($E_{1cm}^{1\%}$): 282.5 (22.04), 289.2 (21.50). Amino acid ratios in an acid hydrolysate: Lys 4.00, His 1.07, Arg 3.07, Pro 2.89, Gly 1.86, Val 3.04, Tyr 0.96, Phe 1.00 (average recovery 94.0%).

The Nonadecapeptide Amide ($\alpha^{5\text{-}23NH_2}$-ACTH)

$[\alpha]_D^{22.0}=-79.0°$ (c=0.1 in 1% AcOH), UV $\lambda_{max}^{0.1N\ NaOH}$ mμ ($E_{1cm}^{1\%}$): 282.5 (23.90), 289.4 (23.08). Amino acid ratios in an acid hydrolysate: Lys 3.81, His 1.00, Arg 3.00, Glu 1.00, Pro 2.02, Gly 2.08, Val 3.00, Tyr 1.00, Phe 1.04 (average recovery 90.3%).

The Eicosapeptide Amide ($\alpha^{4\text{-}23NH_2}$-ACTH)

$[\alpha]_D^{22.8}=-79.6°$ (c=0.5 in 1% AcOH), UV $\lambda_{max}^{0.1N\ NaOH}$ mμ ($E_{1cm}^{1\%}$): 282.5 (23.78), 289.4 (23.05). Amino acid ratios in an acid hydrolysate: Lys 4.00, His 1.11, Arg 3.05, Glu 1.04, Pro 2.05, Gly 2.02, Val 2.83, Met 0.98, Tyr 0.94, Phe 1.00 (average recovery 94%).

The steroidogenic activities of these four synthetic peptides were assessed by the *in vivo* method[14] and compared with $\alpha^{1\text{-}23NH_2}$-ACTH and $\alpha^{1\text{-}24}$-ACTH.[10]

As shown in Table I, the synthetic $\alpha^{4\text{-}23NH_2}$-ACTH possessed a remarkable steroidogenic potency (*ca.* 15—20 IU/mg), and the $\alpha^{5\text{-}23NH_2}$-ACTH and $\alpha^{6\text{-}24}$-ACTH possessed very low, but definite activity (1 IU/mg and 0.1 IU/mg, respectively), whereas the $\alpha^{7\text{-}23NH_2}$-ACTH was inactive.

Since the tridecapeptide amide $\alpha^{1\text{-}13NH_2}$-ACTH (or deacetylated α-MSH)[15] and $\alpha^{(1\text{-}10)\text{-}(15\text{-}19)}$-ACTH-pentadecapeptide[16] have been reported to have a low, but consistent, steroidogenic activity, it may be assumed that the sequence His-Phe-Arg-Trp-Gly is not only the active site of the MSH action or the lipolytic action of this hormone[17] but also an important region of this hormone for its steroidogenic action.

As has been indicated by Hofmann, *et al.*,[18] methionine residue at position 4 may play an important role as a hydrophobic binding site. This may account for the fact that $\alpha^{4\text{-}23NH_2}$-ACTH possesses a relatively high potency as compared with $\alpha^{5\text{-}23NH_2}$-ACTH.

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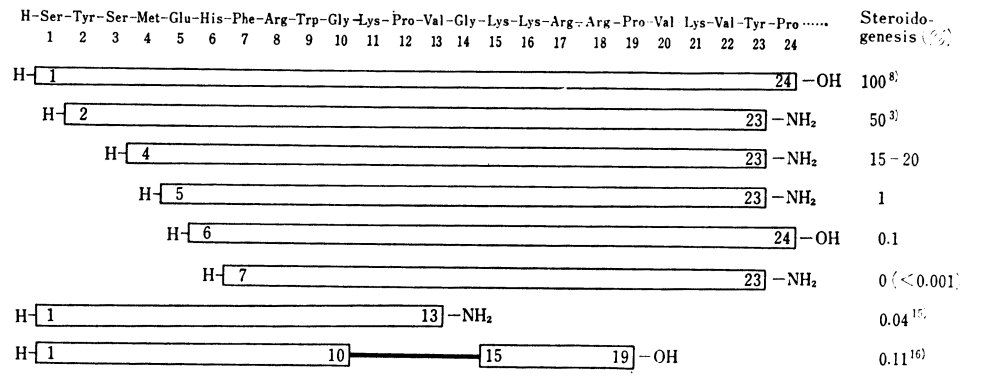
TABLE I. Relationship between Activity and Chain Length of ACTH



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Imine-enamine Tautomerism and Ring Contraction of 2-Methoxy-4,5,6,7-tetrahydro-1H-azepin-4-ones

Recent investigations have established that enamineketone (I) and lactim ether (II) are in imine-enamine tautomeric equilibrium, and that the enamine form predominates in the former case[1] and the imine form does in the latter case.[2] However, there has been no information on imine-enamine tautomerism of β-alkoxy-enamineketone.[3] In the present work, it is shown that 6,6-dimethyl-2-methoxy-4,5,6,7-tetrahydro-1H-azepin-4-one (III), prepared by Curtius-type rearrangement of 3-azido-5,5-dimethyl-2-cyclohexen-1-one, exists as enamine form (IIIA) in polar solvent such as ethanol and dimethyl sulfoxide, as imine form (IIIB) in non-polar solvent such as carbon tetrachloride and as a mixture of both forms in chloroform. The result would show that the enamine and imine forms of III have comparable stabilities in solution. It is also shown that the azepinone (III) undergoes ring contraction by treatment with dilute acid to give methyl 4,4-dimethyl-2-pyrrolidylideneacetate (V). It

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