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Synthesis of Peptides related to Corticotropin (ACTH). VII.[1] Syntheses and Biological Activity of Leucine^{7}-α^{1-24}-ACTH and N$^{\alpha}$-Methyltryptophan^{9}-α^{1-24}-ACTH[2]

In previous communication,[1] we have reported the syntheses and the biological activites of α^{4-23NH_2}-ACTH, α^{5-23NH_2}-ACTH, α^{6-24}-ACTH and α^{7-23NH_2}-ACTH, and suggested that the sequence -His-Phe-Arg-Trp- in the ACTH molecule could be an important region of this hormone for the steroidogenesis.

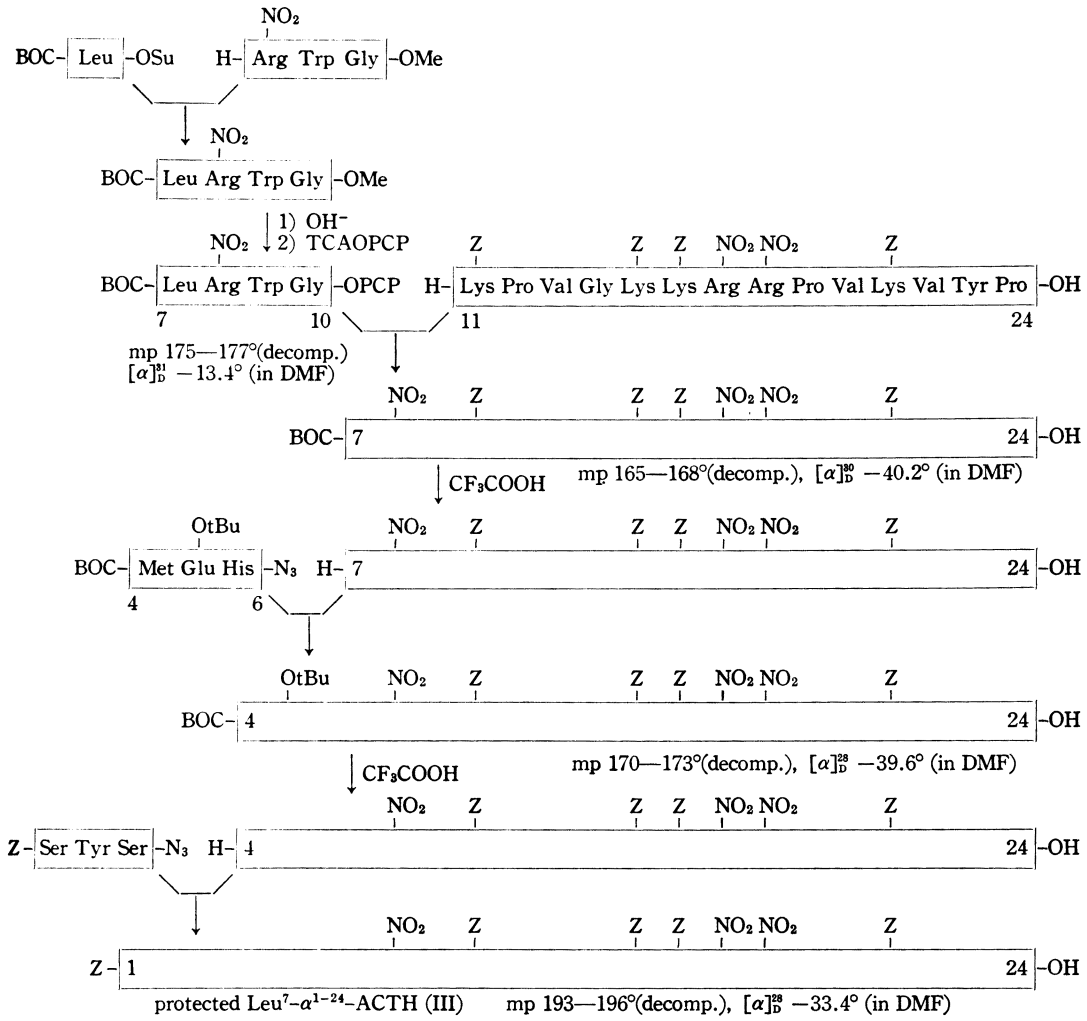

Chart 1. Synthetic Route to Protected Leu^{7}-α^{1-24}-ACTH

1) Part VI: M. Fujino, C. Hatanaka, and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **19**, 1066 (1971).
2) The amino acids, peptides and their derivatives (except glycine) mentioned in this communication are of the L-configuration. Abbreviations used are: Orn, ornithine; Pyr-Ala, β-(pyrazolyl-3)-alanine; Metrp, N$^{\alpha}$-methyltryptophan; Z, benzyloxycarbonyl; BOC, *t*-butyloxycarbonyl; OtBu, *t*-butyl ester; NO_2, nitro; N_3, azide; OPCP, pentachlorophenyl ester; TCAOPCP, pentachlorophenyl trichloroacetate; OSu, N-hydroxysuccinimide ester; HOSu, N-hydroxysuccinimide; DCC, N,N'-dicyclohexylcarbodiimide.

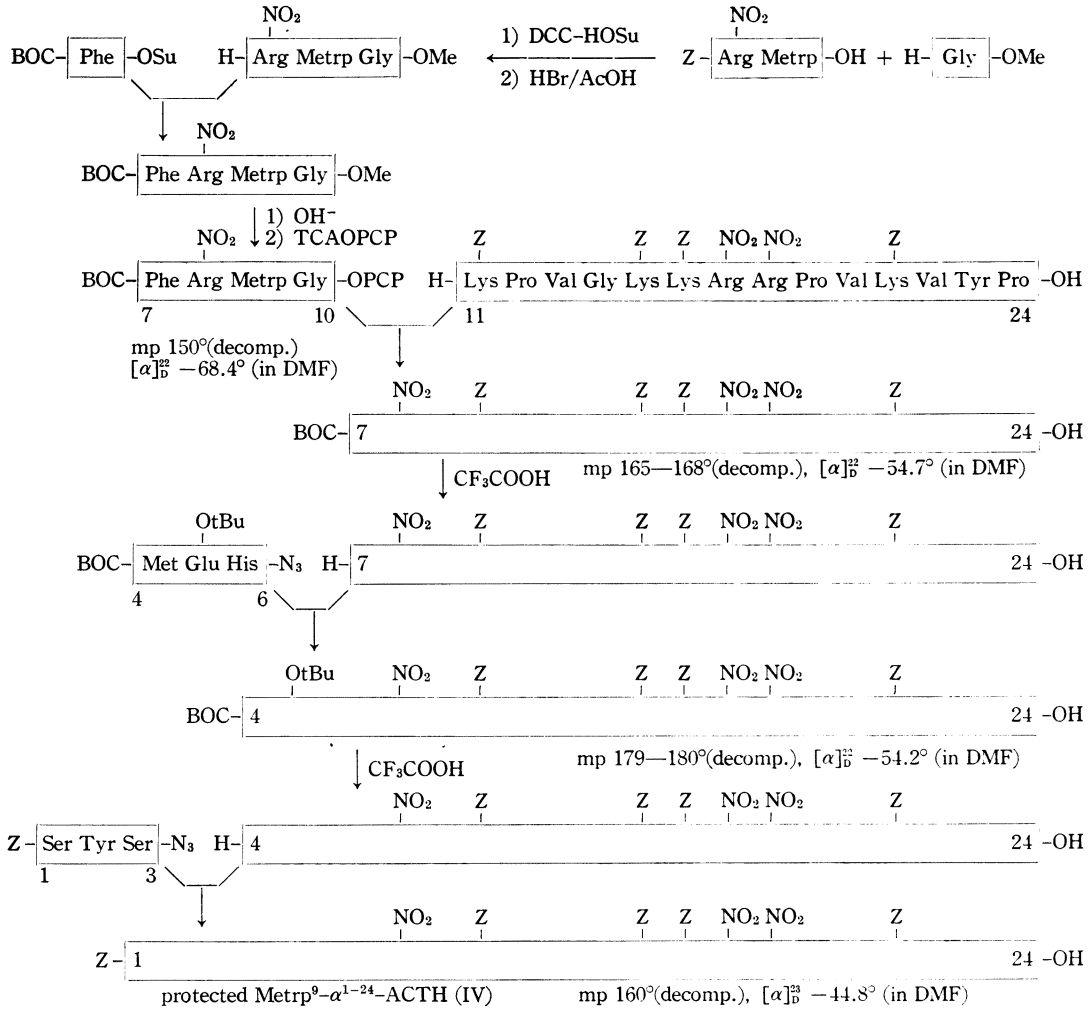

Chart 2. Synthetic Route to Protected Metrp^{9}-α^{1-24}-ACTH

Hofmann, *et al.*,[3] recently, replaced tryptophan^{9} in Gln^{5}-$\alpha^{1-20^{NH_2}}$-ACTH by phenylalanine and found that this replacement decreased the steroidogenic activity from 100 to 1 U/mg, and Li, *et al.*[4] and Tesser, *et al.*[5] have found that their synthetic analogs, Lys^{8}-$\alpha^{1-17^{NH_2}}$-ACTH[4] and Orn^{8}-α^{1-24}-ACTH,[5] exhibited the activities of 1 and 2 U/mg, respectively.

On the other hand, Hofmann, *et al.*[3,6] have reported that the replacement of histidine^{6} by β-(3-pyrazolyl)alanine did not eliminate the biological activity. In fact, the analog Gln^{5}, Pyr-ala^{6}-$\alpha^{1-20^{NH_2}}$-ACTH exhibits the activity of approximately 50 U/mg.

These findings clearly indicate that the sequence Arg^{8}-Trp^{9} plays an important role in steroidogenesis of the hormone.

In order to derive further information concerning the structure–function relationship in the "important region," we have prepared Leu^{7}-α^{1-24}-ACTH (I) and Metrp^{9}-α^{1-24}-ACTH (II) and evaluated their *in vivo* steroidogenic potencies.

3) K. Hofmann, R. Andreatta, H. Bohn, and L. Moroder, *J. Med. Chem.*, **13**, 339 (1970).
4) D. Chung and C.H. Li, *J. Am. Chem. Soc.*, **89**, 4208 (1967).
5) G.I. Tesser and W. Rittel, *Rec. Trav. Chim. Pays-Bas.*, **88**, 553 (1969).
6) K. Hofmann, H. Bohn, and R. Andreatta, *J. Am. Chem. Soc.*, **89**, 7126 (1967).

For syntheses of I and II, the protected tetracosapeptides III and IV were prepared according to the routes shown in Chart 1 and 2, respectively, which were essentially the same as described in previous papers.[7]

The protecting groups of III and IV were removed by means of the hydrogen fluoride method,[8] and the resulting hydrogen fluoride salts of tetracosapeptides were converted to the corresponding acetates by the use of Amberlite IRA-400 (acetate), and each of the products was purified twice by chromatography on carboxymethylcellulose.

Leu^{7} α^{1-24} ACTH (I): $[\alpha]_D^{25}$ $-78.6°$ (*c*, 0.5 in 1% acetic acid), Amino acid ratios in acid hydrolysate: Lys 3.90, His 1.10, Arg 3.10, Ser 1.95, Glu 1.00, Pro 2.88, Gly 1.94, Val 2.83, Met 0.78, Leu 1.04, Tyr 2.04, Trp 0.89.[9]

Metrp^{9}-α^{1-24}-ACTH (II): $[\alpha]_D^{28}$ $-95.0°$ (*c*, 0.5 in 1% acetic acid), amino acid ratios in acid hydrolysate: Lys 4.02, His 1.05, Arg 3.10, Ser 1.95, Glu 1.00, Pro 3.00, Gly 2.00, Val 2.90, Met 0.80, Tyr 2.00, Phe 0.95, Metrp 0.94.[9]

The steroidogenic activities of these synthetic analogs were examined by the in vivo method[10] and compared with that of α^{1-24}-ACTH.[7b]

The compound I exhibits approximately 20% the activity (15—25 U/mg) when compared with that of the parent peptide.

This indicates that the aromatic ring of phenylalanine at position 7 is not essential for the biological activity.

Replacement of the tryptophan in α^{1-24}-ACTH by N$^{\alpha}$-methyltryptophan decreases dramatically, but not totally, the *in vivo* steroidogenic activity (the compound II exhibiting only 0.05—1 U/mg).

The above mentioned results strongly suggest that none of the so-called "essential/functional amino acid" exists in the molecule of ACTH, although the region of Arg^{8}-Trp^{9} in the molecule is perhaps the most important part for steroidoigenesis.

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Chemical Research Laboratories,
Research and Developmemt Division,
Takeda Chemical Industries, Ltd.
Juso, Higashiyodogawa-ku, Osaka

Masahiko Fujino
Chitoshi Hatanaka
Osamu Nishimura
Susumu Shinagawa

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7) *a*) M. Fujino, C. Hatanaka, and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 2186 (1969); **18**, 711 (1970); *b*) M. Fujino, C. Hatanaka, and O. Nishimura, *ibid.*, **18**, 1288 (1970); *c*) M. Fujino, O. Nishimura, and C. Hatanaka, *ibid.*, **17**, 2135 (1969); **18**, 1291 (1970).
8) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965); S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *ibid.*, **40**, 2164 (1967).
9) Calculated from the UV absorptions.
10) The authors wish to express their appreciation to Dr. R. Nakayama for measurement of the steroidogenic activity (rat, *i.v.*).