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Synthesis of Peptides related to Corticotropin (ACTH). IV.[1,2] Syntheses of β-Alanine[1]-, γ-Aminobutyric Acid[1]-, Sarcosine[1]-, Proline[1]- and Lysine[1]-α^{1-24}-ACTH[3]

In previous communications,[1,4] we reported the synthesis of α^{1-23NH_2}-ACTH and its β-alanine[1]-analogue by an essentially different route from those reported by several investigators[5] and found that β-alanine[1]-α^{1-23NH_2}-ACTH has a high level of adrenal steroidogenic activity.

The essentiality of terminal amino group in ACTH molecule for biological activity has been demonstrated by Waller, *et al.*[6] on the basis of chemical modification of natural ACTH.

On the other hand, several investigations[7] have disclosed that the N-terminal serine residue was not essential for the activity.

In this communication, we wish to report the syntheses of five analogues of α^{1-24}-ACTH and the relationship of the N-terminal structure and the biological activity of this hormone.

The analogues synthesized involve β-alanine[1]-, γ-aminobutyric acid[1]-, sarcosine[1]-, proline[1]- and lysine[1]-α^{1-24}-ACTH.

The synthesis of the protected tetracosapeptide (XVIIb, XVIIc, XVIId, XVIIe or XVIIf) was achieved by the route illustrated in Chart 1, which was first established in the preparation of α^{1-24}-ACTH (XVIIa).

The C-terminal pentapeptide derivative (VI) was synthesized according to Chart 2, and N-terminal protected tripeptide hydrazides (XVIc, XVId, XVIe and XVIf) were prepared according to Chart 3.

1) Part III: M. Fujino, C. Hatanaka and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **18**, 771 (1970).
2) A part of this work has appeared in Netherlands Patents Report **6**, 27, 1969. During preparation of this communication, a paper on synthesis and physiological activity of β-alanine[1]- and proline[1]-α^{1-23NH_2}-ACTH appeared: R. Geiger, H. Schröder and W. Siedel, *Ann. Chem.*, **726**, 177 (1969).
3) The amino acids, peptides and their derivatives (except glycine, β-alanine, γ-aminobutyric acid and sarcosine) mentioned in this communication are of the L-configuration. Their abbreviated designations are those recommended by IUPAC–IUB Commission on Biochemistry Nomenclature in July, 1965 and July, 1966; *Biochemistry*, **5**, 2485 (1966); **6**, 362 (1967).
4) M. Fujino, C. Hatanaka and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 2186 (1969).
5) For review, see E. Schröder and K. Lübke, "The Peptides," Vol. II, Academic Press, New York, N.Y., **1965**, p. 194.
6) J.P. Waller and H.B.F. Dixon, *Biochem. J.*, **75**, 320 (1960).
7) *a*) R. Geiger, K. Sturm, G. Vogel and W. Siedel, *Z. Naturforsch.*, **196**, 858 (1964); *b*) H. Otsuka, K. Inoye, M. Kanayama and F. Shinozaki, *Bull. Chem. Soc. Japan*, **38**, 679, 1563 (1965); *c*) R.A. Boissonnas, S. Guttmann and J. Pless, *Experientia*, **22**, 526 (1966); *d*) S. Guttmann, J. Pless and R.A. Boissonnas, "Peptides," ed. H.C. Beyerman, A. Van De Linde and W. Maassen Van Den Brink, North–Holland Publ. Co., Amsterdam, 1967, p. 221; *e*) H. Kappeler, B. Riniker, W. Rittel, P.A. Desaulles, R. Maier, B. Schär and M. Staehelin, *ibid.*, p. 214; *f*) W. Rittel, "Pharmacology of Hormonal Polypeptides and Proteins," Plenum Press, New York, 1968, p. 35.

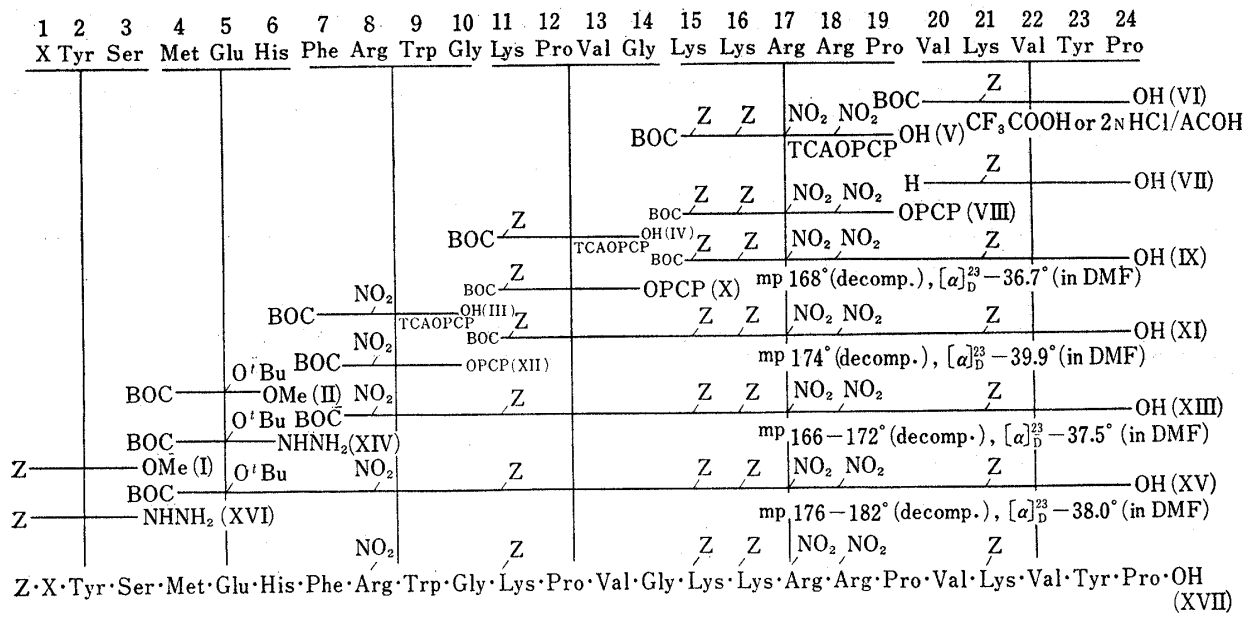


Chart 1. Synthetic Route to N–Protected Tetracosapeptides

TCAOPCP: pentachlorophenyl trichloroacetate -OPCP: pentachlorophenyl ester
XVII a (X=serine): mp 197—203° (decomp., 164—168° sinter), $[\alpha]_D^{23}$ −32.8° (in DMF)
XVII b (X=β-alanine): mp 195° (decomp., 175—183° sinter), $[\alpha]_D^{22}$ −32.5° (in DMF)
XVII c (X=γ-aminobutyric acid): mp 215° (decomp., 168—176° sinter), $[\alpha]_D^{22}$ −32.2° (in DMF)
XVII d (X=sarcosine): mp 190—194° (decomp., 156° sinter), $[\alpha]_D^{26.5}$ −32.4° (in DMF)
XVII e (X=proline): mp 197° (decomp., 160° sinter), $[\alpha]_D^{22}$ −39.0° (in DMF)
XVII f (X=lysine): mp203° (decomp., 145° sinter), $[\alpha]_D^{22.5}$ −33.5° (in DMF)

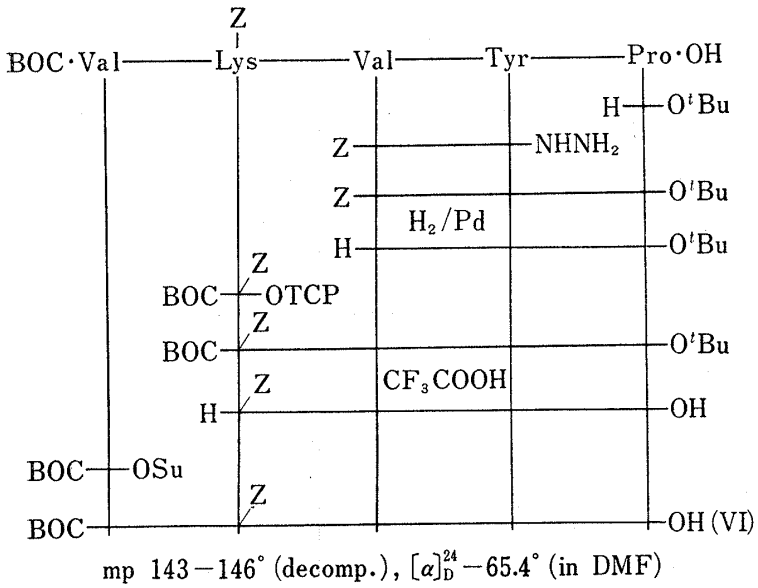
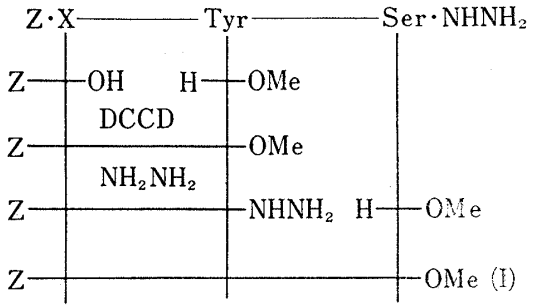
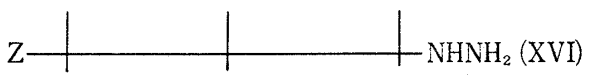


Chart 2. Synthesis of N–Protected C–Terminal Pentapeptide (VI)

-OTCP: 2,4,5-trichlorophenyl ester
-OSu: N-hydroxysuccinimide ester



c) mp 152−154°, $[\alpha]_D^{22}$ −1.8° (in DMF)
d) mp 159°, $[\alpha]_D^{22}$ +9.4° (in DMF)
e) mp 153−155°, $[\alpha]_D^{23}$ −53.0° (in MeOH)
f) mp 166−168°, $[\alpha]_D^{22}$ −23.0° (in MeOH)



c) mp 218−220° (decomp.), $[\alpha]_D^{23}$ −1.5° (in 1N HCl)
d) mp 215° (decomp.), $[\alpha]_D^{23}$ +4.4° (in DMF)
e) mp 205° (decomp.), $[\alpha]_D^{22}$ −45.0° (in DMF)
f) mp 218° (decomp.), $[\alpha]_D^{22}$ −29.0° (in DMF)

Chart 3. Syntheses of N–Terminal Tripeptide Subunits (XVIc—f)

c: x=γ-aminobutyric acid, d: x=sarcosine,
e: x=proline, f: x=lysine
DCCD: N,N'-dicyclohexylcarbodiimide

The syntheses of other peptide subunits (Ia, Ib, II, III, IV, V, XIV, XVIa and XVIb) and active esters (VIII, X and XII) were already described in detail in the previous papers.[1,8]

Removal of the protecting groups from N-protected tetracosapeptides was carried out with anhydrous hydrogen fluoride[9,10] to give the corresponding deprotected peptide hydrofluorides which were converted to the acetates by the treatment with Amberlite IRA-400 (acetate) and the products were purified by column chromatography on carboxymethylcellulose (ammonium acetate buffer). Their physicochemical constants and steroidogenic activity *in vivo*[11] are listed in Table I.

Table I. Properties and Biological Activities of α^{1-24}-ACTH-Analogues

Compound	$[\alpha]_D^{23}$ ($c=0.5$, 1% AcOH)	Paper electrophoresis pH 1.9 (AcOH–HCOOH) 500V, 60 min	Amino acid *Anal.* (5.7N HCl, 110°, 30 hr)	Biological activity[11] (steroidogenic, *in vivo*) u/mg
α^{1-24}-ACTH	−80[12]	− 9.5 cm (=synacthen®)[13]	$Lys_{4.0}$ $His_{0.9}$ $Arg_{3.0}$ $Ser_{2.1}$ $Glu_{1.0}$ $Pro_{2.9}$ $Gly_{2.0}$ $Val_{3.0}$ $Met_{0.8}$ $Tyr_{2.0}$ $Phe_{1.0}$	90
β-Alanine[1]-analog.	−78	− 9.6 cm	$Lys_{4.0}$ $His_{1.0}$ $Arg_{3.0}$ $Ser_{1.1}$ $Glu_{1.0}$ $Pro_{3.0}$ $Gly_{2.0}$ $Val_{3.1}$ $Met_{1.0}$ $Tyr_{2.0}$ $Phe_{1.0}$ β-$Ala_{1.0}$	100—160[a]
γ-Aminobutyric acid[1]-analog.	−75	− 9.6 cm	$Lys_{4.0}$ $His_{0.9}$ $Arg_{3.0}$ $Ser_{1.2}$ $Glu_{1.2}$ $Pro_{3.1}$ $Gly_{2.0}$ $Val_{3.1}$ $Met_{0.9}$ $Tyr_{2.0}$ $Phe_{1.0}$ γ-$Abu_{1.0}$	100—110[a]
Sarcosine[1]-analog.	−78	− 9.3 cm	$Lys_{4.0}$ $His_{1.0}$ $Arg_{3.2}$ $Ser_{1.1}$ $Glu_{1.0}$ $Pro_{3.0}$ $Gly_{2.0}$ $Val_{3.0}$ $Met_{1.0}$ $Tyr_{2.1}$ $Phe_{1.0}$ $Sar_{1.0}$	100—160[a]
Proline[1]-analog.	−75	− 9.4 cm	$Lys_{4.1}$ $His_{1.0}$ $Arg_{3.0}$ $Ser_{1.1}$ $Glu_{1.1}$ $Pro_{4.2}$ $Gly_{2.0}$ $Val_{3.0}$ $Met_{0.8}$ $Tyr_{2.0}$ $Phe_{1.0}$	50—65
Lysine[1]-analog.	−81	−11.0 cm	$Lys_{5.1}$ $His_{1.1}$ $Arg_{3.1}$ $Ser_{0.9}$ $Glu_{1.0}$ $Pro_{3.0}$ $Gly_{2.0}$ $Val_{2.9}$ $Met_{0.8}$ $Tyr_{2.0}$ $Phe_{1.0}$	30—50

a) These data were just based on the comparison of blood steroid level at 15 min after injection. When assays were done at 25—40 min after administrations, β-alanine[1]-, γ-aminobutyric acid[1]-and sarcosine[1]-analogues indicated very high level of activities and the order of their potencies were as follows:
sarcosine[1]-analog. $\geq$ β-alanine[1]-analog. $>$ γ-aminobutyric acid[1]-analog. $>$ α^{1-24}-ACTH

From the results as shown in Table I, it appears that the N-terminal α-amino group is not essential for the biological activity of this hormone, and that the replacement with an amino-peptidase-resistant amino acid at the N-terminal position of α^{1-24}-ACTH enhances the biological activity, as described by other investigators[7c-f].

Moreover, it is interesting to note that sarcosine[1]-α^{1-24}-ACTH possesses a particularly high level of biological activity, whereas proline[1]-α^{1-24}-ACTH possesses a relatively low activity in the *in vivo* testings.

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8) M. Fujino, O. Nishimura and C. Hatanaka, *Chem. Pharm. Bull.* (Tokyo), **17**, 2135 (1969).
9) 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).
10) Anisole and Methionine were used as a scavenger to protect tyrosine, tryptophan and methionine residues from benzylation, and also thioglycolic acid and tryptophan were used to protect tryptophan and methionine residues from oxidation.
11) Steroidogenic potencies in the dexamethasone-blocked rat (after 15 min of administration) of these analogues were determined by Dr. R. Nakayama, *et al.* in this Division. The detailed biological properties of these compounds will be separately reported elsewhere by the staff of Biological Research Laboratories of this Division.
12) R. Schwyzer and H. Kappeler gave $[\alpha]_D^{25}=-88.6\pm2°$ ($c=0.511$ in 1% AcOH); *Helv. Chim. Acta*, **46**, 1550 (1963).
13) Trade name, CIBA Limited.

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Synthesis of Peptides related to Corticotropin (ACTH). V.[1] Syntheses of Leucine[4]- and Isoleucine[4]-α^{1-24}-ACTH[2]

Hofmann, *et al.*[3] demonstrated the non-essential nature the methionine[4]-residue at position 4 for the ACTH activity by synthesizing α-aminobutyric acid[4]-α^{1-20NH_2}-ACTH which exhibited 30—40% the activity of natural ACTH. Similarly, Boissonnas, *et al.*[4] have recently synthesized norleucine[4]- and norvaline[4]-analogues of α^{1-25NH_2}-ACTH with no marked change in biological activities.

On the other hand, an earlier work[5] on the chemical modification of natural ACTH has suggested the essentiality of the methionine-residue for the biological activity of the molecule.

However, it is indeed a fact that ACTH activity is reversibly abolished by the oxidation of ACTH with hydrogen peroxide or other oxidizing agents. Although the reason for this phenomenon is still unknown, the activity loss may be caused by some steric effect or polar effect due to the conversion of the methionine-residue to the corresponding sulfoxide.[6]

In this paper, we wish to report the syntheses and biological activities of leucine[4]-α^{1-24}-ACTH (I) and isoleucine[4]-α^{1-24}-ACTH (II).

The replacement of the methionine-residue by leucine or isoleucine does not appear to change significantly the polar and functional properties of the peptide, but it appears to change markedly steric conformation at position 4 in the molecule.

Steroidogenic activities of the peptide I and II were determined by the *in vivo* method[7] and compared to that of the synthetic α^{1-24}-ACTH[1] and the 3rd U.S.P. Standard.

The both preparations (I and II) exhibited 55—85% the biological activity of α^{1-24}-ACTH.

1) Part IV: M. Fujino, C. Hatanaka and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **18**, 1288 (1970).
2) The peptides and peptide derivatives mentioned have the L-configuration. For a simpler description the following abbreviations are used: Z=benzyloxycarbonyl, BOC=*t*-butyloxycarbonyl, OMe=methyl ester, OtBu=*t*-butyl ester, OPCP=pentachlorophenyl ester, NO_2=nitro. The following solvent systems are used for thin-layer (Kiesel gel G, Merck) chromatography: *Rf*[1], $CHCl_3$–MeOH–AcOH (9:1:0.5 v/v); *Rf*[2], AcOEt–pyridine–AcOH–H_2O (60:20:6:11 v/v); *Rf*[3], *n*-BuOH–AcOH–H_2O (4:1:1 v/v); *Rf*[4], *n*-BuOH–pyridine–AcOH–H_2O (30:20:6:24 v/v). During preparation of this manuscript, a paper on synthesis and physiological activity of Leucine[4]-α^{1-23NH_2} ACTH appeared; R. Geiger, H. Schröder and W. Siedel, *Ann. Chem.*, **726**, 177 (1969).
3) K. Hofmann, R.D. Wells, H. Yajima and J. Rosenthaler, *J. Am. Chem. Soc.*, **85**, 1546 (1963).
4) R.A. Boissonnas, St. Guttmann and J. Pless, Proceedings of the 7th European Peptide Symposium, Budapest, Sept. 1964. *Acta Chim. Hung.*, **44**, 141 (1965).
5) H.B.F. Dixon, *Biochim. Biophys. Acta*, **18**, 599 (1955); *idem*, *Biochem. J.*, **62**, 25 (1956); J.D. Waller and H.B.F. Dixon, *Biochem. J.*, **75**, 320 (1960).
6) M.L. Dedman, T.H. Farmer and C.J.O.R. Morris, *Biochem. J.*, **78**, 348 (1961); K. Hofmann and H. Yajima, "Recent Progress in Hormone Research," Vol. 18, ed., G. Pincus, Academic Press Inc., New York, N.Y., 1962, p. 41.
7) We are grateful to Dr. R. Nakayama and his staff of this division for performing the assays.