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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).
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7) We are grateful to Dr. R. Nakayama and his staff of this division for performing the assays.

From the above finding, it may be pertinent to assume that the decrease of the biological activity by oxidation of ACTH would be due to in part the change of the polar property at the position of methionine-residue.

For the synthesis of I (or II), H–Glu(OtBu)–His–OMe[8] was acylated with BOC–Leu–OPCP (or BOC–Ile–OPCP) to give BOC–Leu (or Ile)–Glu (OtBu)–His–OMe [Leu-analog. mp 131.0—135.0°, $[\alpha]_D^{22}$ −26.0° (c=1.0 in MeOH). *Anal.* Calcd. for $C_{28}H_{47}O_8N_5 \cdot \frac{1}{2}H_2O$: C, 57.03; H, 8.20; N, 11.87. Found: C, 57.07; H, 8.09; N, 12.00, Ile-analog. mp 125.0—127.0°, $[\alpha]_D^{22}$ −28° (c=1.0 in MeOH). *Anal.* Found: C, 57.03; H, 8.21; N, 12.27].

The tripeptide ester was converted to the corresponding hydrazide [Leu-analog. mp 142.0—145.0°, $[\alpha]_D^{22}$ −34.0° (c=1.0 in MeOH). *Anal.* Calcd. for $C_{26}H_{45}O_7N_7 \cdot \frac{1}{2}H_2O$: C, 54.15; H, 8.05; N, 16.99. Found: C, 54.44; H, 8.06; N, 16.79, Ile-analog. mp 168.0—171.0°, $[\alpha]_D^{22}$ −36.4° (c=1.0 in MeOH), *Anal.* Found: C, 54.00; H, 7.96; N, 17.04] and the hydrazide was then coupled with the partially protected octapeptide H–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[1] by the azide method to give the protected heneicosapeptide BOC–Leu(or Ile)–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–Pro–OH [Leu-analog. mp 183.0—188.0° (decomp.), $[\alpha]_D^{23}$ −39.0° (c=1.0 in DMF), Rf^2 0.50 Rf^3 0.73, Rf^4 0.88. *Anal.* Calcd. for $C_{158}H_{224}O_{39}N_{40} \cdot 6H_2O$: C, 55.56; H, 6.96; N, 16.40. Found: C, 55.51; H, 6.86; N, 16.33. Ile-analog. mp 183.0—188.0° (decomp.), $[\alpha]_D^{23}$ −38.8° (c=1.0 in DMF), Rf^2 0.40, Rf^3 0.78, Rf^4 0.88. *Anal.* Found: C, 55.31; H, 6.58; N, 16.33].

The protected heneicosapeptide was treated with cold trifluoroacetic acid under nitrogen gas, and the resulting partially protected heneicosapeptide was coupled with Z–Ser–Tyr–Ser–N_3, which was produced *in situ* from the corresponding hydrazide.[8] The resulting protected tetracosapeptide was isolated by chromatography on silica gel[9] [Leu-analog. mp 187.0—192.0° (decomp.), $[\alpha]_D^{23}$ −33.4° (c=1.0 in DMF), Rf^2 0.33, Rf^3 0.65, Rf^4 0.70. *Anal.* Calcd. for $C_{177}H_{239}O_{47}N_{43} \cdot 7H_2O$: C, 55.40; H, 6.64; N, 15.69. Found: C, 55.03; H, 6.64; N, 16.04. Ileanalog. mp 189.0—193.0° (decomp.), $[\alpha]_D^{23}$ −33.6° (c=1.0 in DMF), Rf^2 0.33, Rf^3 0.65, Rf^4 0.70. *Anal.* Found: C, 55.67; H, 6.60; N, 15.46].

These protected tetracosapeptides were deblocked by exposure to anhydrous hydrogen fluoride[10,11] the resulting hydrogen fluoride salts of the tetracosapeptides were converted to the acetates by the use of Amberlite IRA–400 (acetate), and the products were then purified by chromatography on carboxymethylcellulose.

The purified peptide I [Leu^4-α^{1-24}-ATCH: $[\alpha]_D^{24.5}$ −71.7° (c=0.24 in 1% AcOH), UV $\lambda_{max}^{0.1N\ NaOH}$ mμ ($E_{1cm}^{1\%}$): 282.5 (24.64). 289.5 (25.53), Amino acid ratios in acid hydrolysate:[12] Lys 4.00, His 1.00, Arg 2.90, Ser 2.00, Glu 1.00, Pro 2.94, Gly 1.82, Val 2.83, Leu 0.90, Tyr 1.80, Phe 0.94]behaved as a single component on paper electrophoresis at pH 1.9 and 6.5, and on thin-layer chromatography (Rf^4 0.51).

The purified peptide II [Ile^4-α^{1-24}-ACTH: $[\alpha]_D^{24.5}$ −72.1° (c=0.18 in 1% AcOH), UV $\lambda_{max}^{0.1N\ NaOH}$ mμ ($E_{1cm}^{1\%}$): 282.5 (24.98), 289.5 (25.72). Amino acid ratios in acid hydrolysate:[12] Lys 4.00, His 1.00, Arg 2.90, Ser 2.06, Glu 1.08, Pro 3.08, Gly 1.00, Val 2.93, Ile 0.98, Tyr 1.90, Phe 1.00] behaved as a single component on paper electrophoresis at pH 1.9 and 6.5, and on thin layer chromatography (Rf^4 0.51).

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12) We are grateful to Mr. I. Yoshida of this division for performing the amino acid analysis.

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Formation of New Crystallizable Pigments from D-Glucose or D-Fructose and *p*-Toluidine

It was reported by Kato[1] that melanoidins was formed through D-glucosone and 3-deoxy-D-glucosone derived from D-glucose in the presence of amine or amino acid. But the structure of colored materials, melanoidins, remained unknown as it was. The present paper describes the formation of some new crystallizable pigments in amino–carbonyl reaction of D-glucose or D-fructose and *p*-toluidine.

In our model system of amino–carbonyl reaction in ethanol used D-glucose or D-fructose and *p*-toluidine, a cleavage of C_2—C_3 bond in sugars was occured and by the reaction of C_1—C_2 fragment formed by this cleavage with *p*-toluidine, some pigments colored reddish brown or violet were isolated as pure crystals.

When N-*p*-tolyl-D-glucosylamine (GPT) prepared from D-glucose and *p*-toluidine was allowed to stand in ethanol solution containing *p*-toluidine at room temperature, a free radical assumed to be F_1 formed and then autoxidative cleavage of glucose C-bond was occured.

From the reddish brown solution, three pigments were isolated in addition to the next decomposed products, N,N′-di-*p*-tolylformamidide, N,N′-di-*p*-tolyloxalamide, N,N′-di-*p*-tolylurea, N-*p*-tolyl-D-arabonamide, D-erythronic acid, D-glyceric acid, glycolic acid and non-crystallizable melanodins.[2]

They were 5-methyl-2,3-di-*p*-tolylimino-indoline (I), reddish brown needles, mp 206—207°, 5,5′-dimethylindirubin (II), light violet needles, mp>300° and 5,5′-dimethyl-3-deoxo-3-*p*-tolylimino-indirubin (III), dark violet needles, mp 256—258°.

Under the similar condition, N-*p*-tolyl-D-fructosylamine (FPT) prepared from D-fructose and *p*-toluidine was cleaved to the same decomposition products as GPT and moreover, an another pigment IV was obtained, which was 5,5′-dimethyl-2-deoxo-2-*p*-tolylimino-isoindigo, reddish orange prisms, mp 276—278°.

The structure of these pigments were identified by comparison with authentic specimens derived from 5-methylisatin (V).[3]

II was obtained by reduction[4] of V with $LiAlH_4$ and condensation of II with *p*-toluidine gave III. The reaction of V with diphenyldiazomethane produced 5-methyl-3′,3′-diphenyl-spiro[indoline-3,2′-oxiran]-2-one and hydrolysis[5] of it with hydrochloric acid gave 5,5′-dimethylisoindigo (VI). This sample was identical with the hydrolysate of IV.

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