Adrenocorticotropin. 51. Synthesis and Properties of Analogues of the Human Hormone with Tyrosine Residues Replaced by 3,5-Diiodotyrosine

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Abstract: Synthesis of α_h-ACTH, [3,5-I_2Tyr^2]-α_h-ACTH, [3,5-I_2Tyr^{23}]-α_h-ACTH, and [3,5-I_2Tyr^{2,23}]-α_h-ACTH was achieved by the solid-phase method. 3,5-Diiodotyrosine was introduced as its N^α-Boc-O-(3-bromobenzyl) derivative. The completed peptides were purified by gel filtration on Sephadex G-10, chromatography on CMC, partition chromatography on Sephadex G-50, and gel filtration on Sephadex G-25. The final products were each characterized and found to be homogeneous by seven different analytical criteria. The steroidogenic activity of α_h-ACTH was much more dependent on the integrity of Tyr^2 than on that of Tyr^{23}.

Iodination of biologically active peptides with either ^{125}I or ^{131}I has been extensively used for radioimmunoassay, binding, and metabolic studies. A major objection to this approach was raised inasmuch as this modification can cause unpredictable and undesirable changes in the physicochemical properties of the peptide under study.[1-3] Moreover, selective labeling is not generally possible unless the peptide contains only a single tyrosine residue, and even in this case a mixture of mono- and diiodinated species can be expected. These difficulties can be obviated by use of 3,5-diiodotyrosine in a strategy of total synthesis. This approach allows a means for investigating the importance of a specific tyrosine residue for biological activity as well as for obtaining radioactive peptide by catalytic reduction with tritium. In this paper, we report the solid-phase synthesis[4] of all three possible 3,5-diiodotyrosine(3,5-I_2Tyr) analogues of α_h-ACTH (Figure 1).[5] The use of [3,5-I_2Tyr^{23}]-α_h-ACTH for preparation of tritium labeled α_h-ACTH will be reported elsewhere.

Results and Discussion

It has been established that benzyl protection of tyrosine is not satisfactory for solid-phase peptide synthesis.[6,7] In addition to being unstable under acidolytic cleavage of the Boc group, a side product, 3-benzyltyrosine, is formed in HF.[7] The 2-bromobenzyloxycarbonyl (2-BrZ) group has been developed to circumvent these two difficulties.[8] However, since the ortho positions to the phenolic hydroxyl group in 3,5-diiodotyrosine are already occupied by iodine atoms, we judged that only a stable protecting group for this function would be required. Therefore, the 3-bromobenzyl protecting group[9] was tested. This protection for 3,5-diiodotyrosine was found to be stable in 50% TFA in CH_2Cl_2 for 24 h and to be completely removed in HF at 0 °C in 1 h without side reaction. The suitability of N^α-Boc-O-(3-bromobenzyl)-3,5-diiodotyrosine for solid-phase peptide synthesis was tested by preparation of the model peptide H-Lys-3,5-I_2-Tyr-Phe-OH. The tripeptide was hydrolyzed to its constituent amino acids by leucine aminopeptidase.

Synthesis of α_h-ACTH, [3,5-I_2Tyr^2]-α_h-ACTH, [3,5-I_2Tyr^{23}]-α_h-ACTH, and [3,5-I_2Tyr^{2,23}]-α_h-ACTH was carried out by the solid-phase method[4] with substantial improvements in methodology over our previous synthesis of α_h-ACTH.[10] These include: (a) 2-BrZ protection of tyrosine;[8] (b) coupling with symmetrical anhydrides preformed with DCC;[11-13] (c) a technique for reducing *tert*-butylation of methionine residues.[14] α_h-ACTH is now obtained in 20–30% yields as compared to our earlier yield of 3%.[10] The synthetic products were homogeneous (see Table I) on thin layer chromatography, paper electrophoresis, isoelectric focusing, and disc electrophoresis. It is noteworthy that modification of a single tyrosine residue in α_h-ACTH resulted in a drastic change in physical characteristics. Modification of both tyrosine residues caused a further change. These changes noticeably affected behavior of the analogues on TLC, chromatography on CMC, and partition chromatography on Sephadex G-50.

Amino acid analyses of acid and enzymic digests are presented in Table II. The results of acid hydrolysis are in good agreement with expected values when it is taken into account that 3,5-diiodotyrosine is converted to tyrosine. The well-known difficulties of obtaining a total enzymic hydrolysate of ACTH were resolved by an initial treatment with acid protease.[15] It should be noted that 3,5-diiodotyrosine in position 2 is readily released, whereas the 3,5-I_2Tyr-Pro moiety in position 23 is not digested as reported previously.[1] Furthermore, the Lys-Pro link appears to be resistant in some instances.

The difference spectra of the synthetic peptides indicated a maximum absorbance at the following wavelengths: [3,5-I_2Tyr^2]-α_h-ACTH, 306 nm; [3,5-I_2Tyr^{23}]-α_h-ACTH, 306 nm; [3,5-I_2Tyr^{2,23}]-α-ACTH, 311 nm. Table I illustrates that these values agree precisely with those obtained by measuring the difference spectra of various model solutions which were made up with the same relative amounts of chromophores as the synthetic peptides. In addition, our values are in agreement with those obtained by Woody et al.[16] for tyrosine and iodinated tyrosine-containing solutions.

The steroidogenic activities of the iodinated analogues in isolated rat adrenal cells are shown in Table III. This activity is almost abolished by iodination of tyrosine in position 2, while tyrosine in position 23 may be iodinated without drastic change. These results are in practically quantitative agreement with a parallel study carried out on corticotrophin-(1–24)-tetracosapeptide and its 3,5-diiodotyrosine analogues.[17]

Experimental Section

Melting points were determined on a Fisher–Johns block and were not corrected. Thin layer chromatography was run on silica gel in the following solvents: chloroform–methanol, 1:1 (CM); chloroform–acetic acid, 15:1 (CA); 1-butanol–acetic acid–water, 4:1:1 (BAW); 1-butanol–pyridine–acetic acid–water, 15:15:3:12 (BPAW). Isoelectric focusing on 5% polyacrylamide gel was carried out between pH 3.5 and 10 for 8 h at 0–5 °C.[18] Amino acid analysis, paper electrophoresis, gel electrophoresis, chromatography on CMC, and partition chromatography on Sephadex G-50 were performed as described previously.[10] For partition chromatography of iodinated analogues, the solvent system was slightly modified in order to counteract the increased hydrophobicity due to iodination (see below). Chloromethylated (0.69 mequiv/g) styrene–1% divinylbenzene (200–400 mesh) was obtained from Bio-Rad Labs. CMC was purchased from

Table I. Some Characterizations of Synthetic α_h-ACTH and Its Diiodotyrosine Analogues

Analysis	Natural α_h-ACTH	Synthetic α_h-ACTH	[3,5-I$_2$Tyr^2]-α_h-ACTH	[3,5-I$_2$Tyr^{23}]-α_h-ACTH	[3,5-I$_2$Tyr^{2,23}]-α_h-ACTH
Paper electrophoresis $(R_f)^a$					
pH 3.7	0.52	0.52	0.52	0.52	0.52
pH 6.9	0.14	0.14	0.14	0.14	0.14
Thin layer chromatography $(R_f)^b$					
Solvent 1	0.56	0.56	0.56	0.56	0.56
Solvent 2	0.26	0.26	0.32	0.32	0.39
Partition chromatography $(R_f)^c$					
System 1	0.25	0.25			
System 2			0.27	0.27	
System 3					0.23
Isoelectric focusing					
pI	8.3	8.3	7.0	7.0	6.4
Difference spectrum, λ_{max}, nm^d		293	306	306	311

^{a} Relative to Lys. ^{b} Solvent 1: 1-butanol–acetic acid–water (4:3:3). Solvent 2: 1-butanol–pyridine–acetic acid–water (15:15:3:12). ^{c} System 1: 1-butanol–pyridine–0.1% aqueous acetic acid (5:3:11). System 2: 1-butanol–pyridine–0.1 N ammonium acetate (5:3:11). System 3: 1-butanol–pyridine–0.3 N ammonium acetate (5:3:11). ^{d} λ_{max} for difference spectrum of reference solutions of amino acids (molar ratios in parentheses): Trp + Tyr (1:2), 293 nm; Trp + Tyr + 3,5-I$_2$Tyr (1:1:1), 306; Trp + 3,5-I$_2$-Tyr (1:2), 311 nm.

Table II. Amino Acid Analysis of Acid and Enzymic Hydrolysates of Synthetic α_h-ACTH and Analogues

Amino acid	Acid digest				Enzyme digest			
	α_h-ACTH	[3,5-I$_2$Tyr^2]-α_h-ACTH	[3,5-I$_2$Tyr^{23}]-α_h-ACTH	[3,5-I$_2$Tyr^{2,23}]-α_h-ACTH	α_h-ACTH	[3,5-I$_2$Tyr^2]-α_h-ACTH	[3,5-I$_2$Tyr^{23}]-α_h-ACTH	[3,5-I$_2$Tyr^{2,23}]-α_h-ACTH
Trp	0	0	0	0	1.0	1.0	1.0	1.0
Lys	3.8	4.0	4.0	3.9	4.0	3.7	3.3	3.2
His	1.0	1.1	1.0	1.0	1.1	1.0	1.0	1.0
3,5-I$_2$Tyr	0	0	0	0	0	1.0	0	1.0
Arg	2.9	3.0	3.0	3.2	3.3	3.2	3.2	3.1
Asp	2.0	2.0	2.0	2.2	0.9	0.8	0.9	1.0
Ser	3.0	2.9	2.9	3.0	4.1	4.0	3.9	3.9
Asn	0	0	0	0				
Glu	5.0	5.1	5.1	5.2	5.0	5.1	5.0	5.3
Pro	4.0	3.7	4.1	3.9	4.0	3.9	2.5	2.3
Gly	3.0	3.2	3.2	3.3	2.8	2.8	2.5	2.9
Ala	3.1	3.0	3.1	3.0	2.9	3.1	3.1	3.0
Val	2.9	3.1	3.0	3.2	3.1	3.0	2.9	3.0
Met	0.8	1.2	1.0	1.0	0.9	0.8	0.9	0.8
Leu	1.0	1.1	1.0	1.0	1.2	1.3	1.2	1.2
Tyr	1.9	2.0	1.9	1.9	2.2	1.2	1.1	0
Phe	3.1	2.9	3.0	3.1	3.1	3.2	3.0	3.0

H-Ser- Tyr -Ser- Met-Glu- His- Phe -Arg- Trp-Gly- Lys- Pro- Val-
          5   10

Gly- Lys- Lys -Arg-Arg- Pro- Val- Lys- Val- Tyr- Pro- Asn- Gly-
      15   20   25

Ala- Glu- Asp- Glu- Ser- Ala- Glu- Ala- Phe- Pro- Leu- Glu- Phe- OH
           30   35

Figure 1. Structure of α_h-ACTH.

Schleicher and Schuell. 3,5-Diiodotyrosine was a product of Sigma.

The synthetic peptides were also characterized by their difference spectra, which were measured on a Beckman DK-2 recording spectrophotometer at 25 °C as follows. The peptides were dissolved in 0.1 N acetic acid and equivalent aliquots were placed in both the sample and reference cuvettes (matched silica, 1-cm path length). The sample solution was brought to pH 12, at which all tyrosines are ionized, by the addition of a small amount of concentrated NaOH, while the reference solution was kept at pH 3.0. The difference in the absorbance of the ionized peptide vs. the un-ionized form was measured from 360–260 nm.

N^α-*tert*-**Butyloxycarbonyl-3,5-diiodotyrosine.** 3,5-Diiodo-L-ty-

rosine·2.5H$_2$O (11.6 g, 24 mmol) was converted to the N^α-Boc derivative by standard procedures.[19] The product was crystallized from ethyl acetate–petroleum ether at room temperature: 10.5 g (76%); mp 187–189 °C; TLC (CA) R_f 0.47; $[\alpha]^{24}$D −10.2° (c 2, DMF). Anal. Calcd for C$_{14}$H$_{17}$NO$_5$I$_2$ (532.96): C, 31.55; H, 3.19; N, 2.63. Found: C, 31.74; H, 3.13; N, 2.74.

N^α-*tert*-**Butyloxycarbonyl-** O-**(3-bromobenzyl)-3,5-diiodotyrosine 3-Bromobenzyl Ester.** N^α-*tert*-butyloxycarbonyl-3,5-diiodotyrosine (8 g, 15 mmol) and 3-bromobenzyl bromide (8 g, 32 mmol) were stirred in DMF (32 ml) in the presence of diisopropylethylamine (7.7 ml, 45 mmol) for 2 h at room temperature. The reaction was stopped by addition of 150 ml of ice-cold water and the following workup was performed at 0–4 °C. The pH was slowly increased to 11.0 with 4 N NaOH. The product was then extracted with three 50-ml portions of ether. The extract was filtered in order to break a white emulsion and then 150 ml of ice-cold water was added to the filtrate and the pH lowered to 2.0 with 3 N HCl. The aqueous layer was removed and washed with 75 ml of ether. The combined ether extracts were then washed with three 100-ml portions of water, dried with MgSO$_4$, filtered, and evaporated in vacuo to crystals. Recrystallization from ethyl acetate–petroleum ether gave 10.2 g (78%); mp 114–115 °C; TLC (CA) R_f 0.78; $[\alpha]^{24}$D −13° (c 2, DMF). Anal. Calcd for C$_{28}$H$_{27}$NO$_5$I$_2$Br$_2$ (871.18): C, 38.63; H, 3.13; N, 1.61. Found: C, 38.68; H, 3.14; N, 1.57.

Table III. Steroidogenic Activity of Synthetic Diiodotyrosine Analogues of Human ACTH

Hormone	Dose, ng	Response[a]	%	Relative potency[b] 95% confidence limit	λ
Natural human ACTH	0.1	144.0 ± 4.4	100		
	0.33	165 ± 3.6			
[3,5-I$_2$Tyr^2]-α$_h$-ACTH	5	150.4 ± 1.9	2.4	2.0–2.8	0.13
	15	164.2 ± 3.0			
[3,5-I$_2$Tyr^{23}]-α$_h$-ACTH	0.1	130.6 ± 3.6	64	42–89	0.13
	0.33	156.0 ± 5.1			
[3,5-I$_2$Tyr^{2,23}]-α$_h$-ACTH	5	140.4 ± 37	2.2	1.7–2.5	0.17
	15	158.4 ± 5.0			

[a] Nanograms of corticosterone/10^6 cells/h; values in mean ± S.E. [b] Compared with the potency of the natural human ACTH.

N^α-*tert*-Butyloxycarbonyl-O-(3-bromobenzyl)-3,5-diiodotyrosine. N^α-Boc-O-(3-bromobenzyl)-3,5-diiodotyrosine 3-bromobenzyl ester (10 g, 11.5 mmol) was dissolved in 100 ml of absolute ethanol. After lowering the temperature to 0 °C, 30 ml of 4 N NaOH was slowly added to the solution while stirring. The solution was allowed to warm to room temperature. When TLC (CM) indicated completion of hydrolysis (1 h), 150 ml of ice-cold water was added. Ethanol was removed by evaporation in vacuo and the resulting solution was washed with two 100-ml portions of ether. After lowering the temperature to 0 °C, the pH was brought to 2.5 with 3 N HCl. The solution was then extracted with two 75-ml portions of ethyl acetate. The extract was washed with three 50-ml portions of water, dried with MgSO$_4$, filtered, and evaporated in vacuo to a white powder. Recrystallization from ethyl acetate–petroleum ether gave 6 g (74%): mp 183–185 °C; TLC (CA) R_f 0.52; [α]^{24}D −10° (c 2, DMF). Anal. Calcd for C$_{21}$H$_{22}$NO$_5$I$_2$Br (701.95): C, 35.93; H, 3.13; N, 1.99. Found: C, 35.77; H, 3.03; N, 2.02.

In order to ascertain the suitability of this compound for solid-phase peptide synthesis, a sample (20 mg) was treated with 50% TFA in CH$_2$Cl$_2$ (1 ml) at room temperature for 24 h. The 3-bromobenzyl protection was found to be completely stable as indicated by TLC (BAW and BPAW). Another 20-mg sample was treated with 10 ml of liquid HF for 1 h at 0 °C in the presence of anisole.[20,21] After removal of HF and anisole, TLC of the product gave a single spot, comigrating with 3,5-diiodotyrosine (BAW, R_f 0.60; BPAW, R_f 0.63).

Model Peptide. In order to ensure that N^α-Boc-(3-BrBzl)-3,5-I$_2$Tyr-OH would react well during the coupling step in solid-phase synthesis and that the resulting coupled amino acid would be optically pure, the model peptide H-Lys-3,5-I$_2$Tyr-Phe-OH was synthesized. Esterification of N^α-Boc-Phe to the chloromethylated resin was carried out as described[10] following some modifications of the Loffet procedure.[22] A sample (200 mg) of the resulting N^α-Boc-Phe resin (0.37 mmol of Phe/g) was submitted to the modified techniques[13] of solid-phase synthesis for the attachment of the two other residues. N^α-Boc-O-(3-BrBzl)-3,5-I$_2$Tyr-OH was found to couple well, since no trace of free amino group could be detected by the picric acid test[23] after its coupling to Phe resin. The finished protected peptide resin was submitted to 1 h of treatment with liquid HF in the presence of anisole. After removal of HF and anisole, the product (41 mg) migrated as a single spot on TLC (BAW, R_f 0.36; BPAW, R_f 0.50) and paper electrophoresis at pH 3.7 (R_f 0.60, relative to Lys). Amino acid analysis of an acid hydrolysate gave: Lys$_{1.0}$, Tyr$_{0.93}$, and Phe$_{1.0}$ (3,5-I$_2$Tyr was converted to Tyr during hydrolysis in HCl). After digestion with leucineaminopeptidase, amino acid analysis gave: Lys$_{0.99}$, 3,5-I$_2$Tyr$_{1.0}$, and Phe$_{1.0}$. 3,5-I$_2$-Tyr was eluted on the short column of the amino acid analyzer at 10 min after the peak for ammonia. The UV spectrum of the model peptide was identical with that of 3,5-I$_2$Tyr in 1 N acetic acid between 260 and 310 nm.

[3,5-I$_2$Tyr^{23}]-α$_h$-ACTH. Starting with 1 g of N^α-Boc-Phe resin (0.37 mmol), synthesis of [3,5-I$_2$Tyr^{23}]-α$_h$-ACTH was achieved following the same procedures as those described for the synthesis of β_{C2}-MSH.[24] Coupling reactions were carried out by the use of preformed symmetrical anhydride[12] of N^α-Boc amino acids (ca. 1.1 mmol of anhydride from 2.22 mmol of N^α-Boc amino acid and 1.1 mmol of DCC). N^α-Boc protection was used throughout with the following side-chain protecting groups: Lys[10] and Tyr[8], 2-BrZ; 3,5-I$_2$Tyr, 3-BrBzl; His, Boc[25]; Arg, Tosyl[25]; Asp, Ser and Glu, Bzl. The asparagine
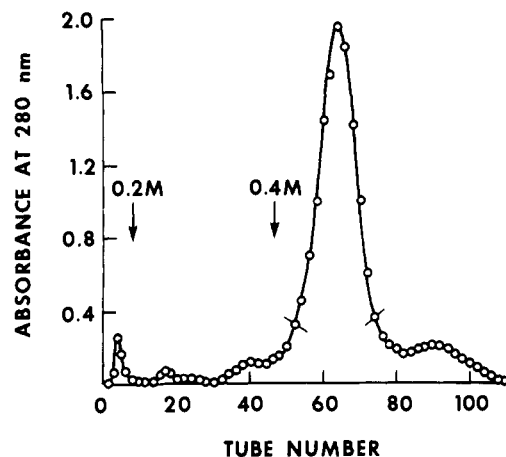

Figure 2. Chromatography of crude [3,5-I$_2$Tyr^{23}]-α$_h$-ACTH (333 mg) on CMC (1 × 60 cm column) at 24 °C. Solutions of 0.2 and 0.4 M ammonium acetate, pH 4.5, were introduced as indicated through a 500-ml mixing chamber containing the starting buffer, 0.01 M ammonium acetate, pH 4.5. Fractions of 12.5 ml were collected; flow rate, ca. 200 ml/h.

residue was introduced as its p-nitrophenyl ester.[26] After removal of the last N^α-Boc protecting group with 50% TFA in CH$_2$Cl$_2$ to avoid butylation of methionine in HF,[14] the finished peptide resin was dried in vacuo over P$_2$O$_5$ overnight to yield 3.26 g.

A sample (0.7 g) of the protected peptide resin was treated with 10 ml of liquid HF in the presence of 2.1 ml of anisole at 0 °C for 1 h. Extraction of the peptide from the resin was performed as described.[24] The resulting material was submitted to gel filtration on a 2.2 × 25 cm Sephadex G-10 column in 0.5 N acetic acid. One peak (280-nm detection) was eluted, yielding 333 mg of material after lyophilization. Chromatography of this material on CMC (Figure 2) gave essentially one peak (280 nm). This latter material (220 mg) was submitted to partition chromatography on Sephadex G-50. Since the hydrophobicity of [3,5-I$_2$Tyr^{23}]-α$_h$-ACTH was much greater than that of α$_h$-ACTH, it eluted with an undesirably high R_f in the solvent system already described for α$_h$-ACTH.[10] Use of 0.1 N ammonium acetate in the aqueous portion of this solvent system gave for [3,5-I$_2$Tyr^{23}]-α$_h$-ACTH the elution pattern shown in Figure 3A [Folin–Lowry detection].[27] Rechromatography of the major peak (125 mg) on the same column gave a single peak with an R_f value of 0.27 (Figure 3B). This material was collected, evaporated to a small volume (3–5 ml), and submitted to gel filtration on a 2.5 × 133 cm column of Sephadex G-25 in 0.5 N acetic acid. A sharp symmetrical peak (280 nm) was eluted (Ve 350 ml), yielding 95 mg of synthetic [3,5-I$_2$Tyr^{23}]-α$_h$-ACTH (ca. 25% yield based on starting N^α-Boc-Phe resin).

On paper electrophoresis, the synthetic product gave one spot (ninhydrin detection) at both pH 3.7 and 6.9 with respective R_f values (relative to Lys) of 0.52 and 0.14 (Table I). Thin layer chromatography of synthetic [3,5-I$_2$Tyr^{23}]-α$_h$-ACTH also gave one spot in two different systems (Table I). One band was detected (precipitation with 12% TCA) on isoelectric focusing with an approximate pI of 7.0 (Table I). Gel electrophoresis of the product at pH 4.5 for 1 h also gave
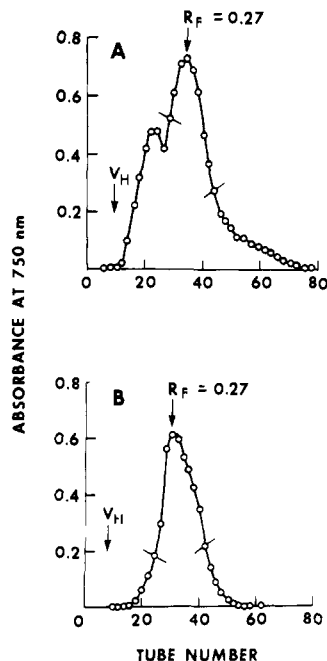
Figure 3. (A) Partition chromatography on Sephadex G-50 of purified [3,5-I$_2$-Tyr^{23}]-α_h-ACTH (220 mg) obtained from CMC. It was run in a 2.5 × 42 cm column at 24 °C in the solvent system: 1-butanol–pyridine–0.1 M ammonium acetate, 5:3:11. Fraction size, 5.9 ml; holdup volume, V_H; flow rate, ca. 10 ml/h. (B) Re-chromatography on Sephadex G-50 partition column of highly purified [3,5-I$_2$Tyr^{23}]-α_h-ACTH (125 mg) obtained from A. Fraction size, 6.6 ml; holdup volume, V_{II}; flow rate, ca. 10 ml/h. Detection by Folin–Lowry method.

a single band. Amino acid analyses of acid and enzymic hydrolysates are shown in Table II. For complete enzyme digestion, a sample (0.5 mg) was first treated with acid protease (50 μg) in 0.2 ml of 0.01 N HCl at 37 °C for 24 h.[15] The lyophilizate was then submitted to tryptic (5 μg) and chymotryptic (5 μg) digestions in 0.2 ml of Tris-HCl buffer (pH 8.5) containing 0.01 M MgCl$_2$ at 37 °C for 5 h, followed by a 24-h treatment with 12 μg of leucineaminopeptidase.

α_h-**ACTH, [3,5-I$_2$Tyr^2]-α_h-ACTH, and [3,5-I$_2$Tyr^{2,23}]-α_h-ACTH.** Synthetic α_h-ACTH, [3,5-I$_2$Tyr^2]-α_h-ACTH, and [3,5-I$_2$Tyr^{2,23}]-α_h-ACTH were prepared by the same procedures as those described for [3,5-I$_2$Tyr^{23}]-α_h-ACTH with the exception that the solvent systems for partition chromatography of α_h-ACTH and [3,5-I$_2$Tyr^{2,23}]-α_h-ACTH were respectively as follows: 1-butanol–pyridine–0.1% aqueous acetic acid, 5:3:11; and 1-butanol–pyridine–0.3 N ammonium acetate, 5:3:11. Methods for characterization of the purified synthetic products were the same as those described for [3,5-I$_2$Tyr^{23}]-α_h-ACTH and the results of these analyses are presented in Tables I and II.

Biological Assay. Steroidogenic activity (Table III) was measured in isolated rat adrenal cells as described.[28] Adrenal cells were isolated by digestion of decapsulated adrenal glands of adult male Sprague-Dawley rats with collagenase. Peptides were dissolved in 0.001 N HCl and the concentrations determined by UV absorbance at 280 nm.

Dilutions for the assay were made in plastic tubes in Krebs-Ringer bicarbonate buffer containing 0.5% bovine serum albumin. Peptides were incubated with 10^5 adrenal cells in 0.5 ml of Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.2% BSA and 0.01% lima bean trypsin inhibitor for 1 h under atmosphere of 95% O$_2$:5% CO$_2$ at 37 °C. Corticosterone was estimated by specific radioimmunoassay.[29]

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(5) Symbols and abbreviations are in accord with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature, Collected Tentative Rules and Recommendations (1973). Other abbreviations are: ACTH, adrenocorticotropin; α_h-ACTH, human adrenocorticotropin: TFA, trifluoroacetic acid; TLC, thin layer chromatography; CMC, carboxymethylcellulose; DMF, N,N-dimethylformamide; DCC, dicyclohexylcarbodiimide; β_{c2}-MSH, glutamic acid form of camel β-melanotropin.
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