Tritiated Peptides. Part 3.[1] Synthesis of [4-^{3}H-Phe^7]-β-Corticotrophin-(1—24)-tetracosapeptide †

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The synthesis is described of β-corticotrophin-(1—24)-tetracosapeptide ‡ labelled with tritium in the phenylalanine residue at position 7 to a specific radioactivity of 27 Ci mmol^{-1} by reductive deiodination of a protected precursor. Evidence for the integrity of the final product is provided by amino-acid analysis, column chromatography, and bioassay, supported by chemical and enzymic analytical data on the protected precursor and the derived free tetracosapeptide containing iodophenylalanine.

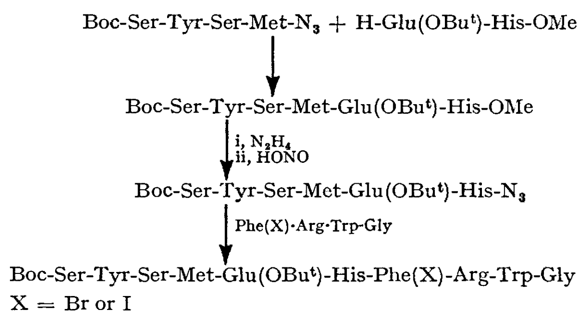
HAVING prepared two labelled species of Synacthen with the labels in tyrosine residues [1,2] adjacent to the terminal amino-acid residues, we turned our attention to other sites for labelling. The phenylalanine residue at position 7 was attractive for two reasons, first that it would allow us to label again by catalytic deiodination and secondly that the residue is in a central position in the molecule and so important metabolic data might be obtained with such a labelled compound.

We decided to prepare as precursors for labelling both the protected [7-(bromophenylalanine)]- and the [7-(iodophenylalanine)]-tetracosapeptides. These were expected to provide fundamental information on the efficiency of replacement of different halogen atoms, and examination of the halogen-containing peptides has allowed an interesting structure–activity correlation to be seen where a steric change is in the active centre.[3]

4-Bromophenylalanine was isolated in optically pure form from the mixture produced by the action of bromine on phenylalanine. We separated the mixture by ion-exchange chromatography and assigned the orientations of the monobrominated products by i.r. spectroscopy and those of the dibrominated products by degradation to the known dibromobenzoic acids with hot alkaline permanganate. Our assignments agree with those of Faulstich and his co-workers [4] made from n.m.r. and i.r. evidence. We found 3-bromophenylalanine in significant amounts in our mixture, but this was not reported by Faulstich.

To economise on the amounts of the halogenated amino-acids used in the syntheses, we adopted a different approach to the *N*-terminal decapeptide unit from that usually employed,[5] in that the last peptide bond was formed at the histidine residue (rather than at methionine) by the azide procedure [6] (see Scheme). The *C*-terminal tetrapeptides containing the phenylalanine analogues were rigorously purified by ion-exchange chromatography before coupling. An approach to the synthesis of a decapeptide containing 2,5-dibromophenylalanine was abandoned when difficulties of solubility were encountered.

The two decapeptides were purified without undue difficulty and converted into the tetracosapeptides in yields of 60% after purification by counter-current distribution.



SCHEME

Trial reduction of the protected bromophenylalanine precursor by the method that had been successfully used for the di-iodotyrosine analogues [1,2] showed that replacement of the bromine atom by hydrogen was unacceptably slow, whereas no similar difficulty was encountered with the replacement of iodine in iodophenylalanine. The poisoning of the catalyst by bromide ion might not be so critical for a peptide not containing sulphur.

The replacement of iodine by tritium and the purification of the radioactive peptide proceeded smoothly with excellent radiochemical efficiency (93%). Replacement of iodine by tritium in tyrosine has not been so efficient in our hands [1,2] and this may be because some radioactivity is exchanged out of the tyrosine ring under the acidic conditions used to deprotect the peptide. No similar mechanism exists for the exchange from the phenylalanine nucleus owing to the absence of a hydroxy-group from the ring. The radioactive peptide was pure as judged by amino-acid analysis following acidic hydrolysis, its complete hydrolysis by enzymes, and its full potency in an isolated adrenal cell bioassay.[7] A small amount (3%) of radioactivity was found in the histidine residue—this was also recorded for the analogues labelled in the tyrosine residues.[1]

The tetracosapeptide containing bromophenylalanine had a potency in the adrenal cell bioassay [7] of 7% in

† β-Corticotrophin-(1—24)-tetracosapeptide: tetracosactrin, Synacthen® (trade name of CIBA-GEIGY Basle), Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro.

‡ In this paper, all amino-acids are L unless otherwise stated.

[1] Part 2, D. E. Brundish, J. R. Martin, and R. Wade, preceding paper.

[2] D. E. Brundish and R. Wade, *J.C.S. Perkin I*, 1973, 2875.

[3] R. Schwyzer, P. Schiller, S. Seelig, and G. Sayers, *F.E.B.S. Letters*, 1971, **19**, 229.

[4] H. Faulstich, H. O. Smith, and S. Zobeley, *Annalen*, 1973, 765.

[5] R. Schwyzer and H. Kappeller, *Helv. Chim. Acta*, 1961, **44**, 1991.

[6] J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2333.

[7] P. J. Lowry, C. McMartin, and J. Peters, *J. Endocrinol.*, 1973, **59**, 43.

comparison with Synacthen, and the iodophenylalanine was even less active (0.4%). This would be an expected effect of substituting foreign atoms of increasing sizes in a region of the peptide that is thought to be important as the active centre of the hormone.

EXPERIMENTAL

The general remarks given in Part 1 apply. The bromo- and iodo-phenylalanines were stable under conditions of acidic hydrolysis prior to amino-acid analysis.

Bromophenylalanines.—Phenylalanine (25 g) was kept on a watch glass in a desiccator in the presence of a beaker of bromine for 5 days at room temperature.[8] Excess of bromine was removed by evacuation and the residue was partitioned between chloroform and water. The aqueous phase was repeatedly extracted with chloroform until no more colour could be extracted, evaporated to a small volume, and adjusted to pH 7 (NH_4OH), and the precipitate was filtered off. Further evaporation of the filtrate afforded two more crops of solid to give a total of 18 g of mixed bromo-phenylalanines. This was applied in M-acetic acid (1 500 ml) to a column (130 × 2 cm) of Zeo-Karb 226 (H^+ form; 100 mesh), which was eluted at 1 ml min^{-1} with a concave gradient (8 l; 1—5M) of aqueous acetic acid (25 ml fractions) (see Table 1). Fractions were examined by paper electrophoresis at pH 1.8 (acetic acid, 1M–formic acid, 1.67M) and amino-acid analysis (see Table 2). Fractions 59—72 afforded substantially pure 4-bromophenylalanine (2.8 g) and fractions 85—104 gave 2,5-dibromophenylalanine (1.4 g). Rechromatography of material recovered from fractions 1—58 afforded further 4-bromo-derivative (3.85 g).

TABLE 1

Elution pattern of brominated phenylalanines from a column of Zeo-Karb 226 (H^+ form) resin

Phe deriv.	2-Br	3-Br	4-Br	2,5-Br_2	2,3-Br_2	3,4-Br_2
Fractions	1—39	1—62	1—84	73—116	105—131	137—160

TABLE 2

Retention times of halogenated phenylalanines on amino-acid analysis *

Phe	2-Br	3-Br [a]	4-Br	2,5-Br_2 [b]	2,3-Br_2 [a,c]	3,4-Br_2 [b]	4-I
t/min	18.6	21.1	25.4	31.4	43.3	57.8	14.0 [d]

* The short column (6.5 × 0.9 cm) of a Beckman 120C Analyser was packed with 7% cross-linked sulphonated polystyrene resin, particle diam. 7 ± 3 μm, and maintained at 55 °C. Elution was performed with 0.35N-sodium citrate buffer (pH 5.3) at 70 ml h^{-1}.

[a] Minor product (not isolated pure). [b] Substitution assignments verified by degradation to the dibromobenzoic acids with alkaline $KMnO_4$. [c] Orientation assumed by analogy with results of Faulstich *et al.*[4] [d] Column eluted with 0.38N-sodium citrate buffer (pH 4.23).

Optical Purity of Brominated Amino-acids.—Samples (2.5 mg) of amino-acids were incubated overnight at 37 °C with L-amino-acid oxidase from *C. adamanteus* venom (2.5 mg) in pH 7.2 Tris buffer (0.5 ml) in an atmosphere of oxygen. After incubation the solution was examined on the amino-acid analyser, after removal of protein by addition of sulphosalicylic acid. The acids were totally oxidised.

N-*t-Butoxycarbonyl-4-bromophenylalanine* (I).—This was prepared in 65% yield from 4-bromophenylalanine in the usual manner [9] at pH 10.3 and 20 °C during 16 h and crystallised from ethyl acetate–petroleum (b.p. 60—80°); the *product* had m.p. 117—118°, $[\alpha]_D^{20}$ 9.6 ± 0.2° (*c* 2.0 in EtOAc) (Found: C, 48.7; H, 5.3; Br, 23.5; N, 4.1. $C_{14}H_{18}BrNO_4$ requires C, 48.8; H, 5.25; Br, 23.3; N, 4.05%).

N-*t-Butoxycarbonyl-2,5-dibromophenylalanine* (II).—This was prepared as described for (I) (reaction time 48 h) in 100% yield; the *product* had m.p. 156—157°, $[\alpha]_D^{20}$ +4.1 ± 0.5° (*c* 1.5 in EtOAc) (Found: C, 39.9; H, 4.0; Br, 37.75; N, 3.45. $C_{14}H_{17}Br_2NO_4$ requires C, 39.7; H, 4.0; Br, 37.8; N, 3.3%).

N-*t-Butoxycarbonyl-4-bromophenylalanylarginyltryptophylglycine Methyl Ester Hydrochloride* (III).—Triethylamine (2.13 ml) and (I) (5.25 g) were dissolved in alcohol-free chloroform (25 ml) and dioxan (25 ml) and cooled to −10 °C. Isobutyl chloroformate (2.00 ml) was added and the mixture was stirred with exclusion of moisture for 30 min at −10 °C. A solution of Arg-Trp-Gly-OMe,2HCl (7.7 g) and triethylamine (2.13 ml) in dimethylformamide (40 ml) at −10 °C was added and the mixture was allowed to come to room temperature over 3 h with stirring, and was then stirred for a further 18 h. The mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in butan-1-ol (250 ml) and ethyl acetate (125 ml) and the organic phase was extracted at 0 °C with saturated aqueous citric acid (3 × 100 ml), water (100 ml), and saturated aqueous $NaHCO_3$ (2 × 100 ml), dried (Na_2SO_4), and evaporated. The residue was dissolved in methanol (50 ml) and added dropwise to vigorously stirred ether (1 l). The precipitate was filtered off and dried *in vacuo* to give amorphous *product* (9.20 g, 77%), $[\alpha]_D^{20}$ −11.6 ± 1.2° (*c* 4.0 in MeOH) (Found: C, 51.2; H, 5.95: Br, 10.0; Cl, 4.3; N, 13.9. $C_{34}H_{46}BrClN_8O_7$ requires C, 51.4; H, 5.8; Br, 10.1; Cl, 4.45; N, 14.1%).

4-Bromophenylalanylarginyltryptophylglycine Acetate (IV).—Compound (III) (6.5 g) was dissolved in methanol (130 ml), N-NaOH (13.0 ml) was added, and the mixture was stirred for 45 min at room temperature. N-HCl (13.0 ml) was added with vigorous stirring and the solution was evaporated to dryness below 30 °C. The residue was treated with cold saturated citric acid solution (50 ml) and extracted twice with a mixture of butan-1-ol (100 ml) and ethyl acetate (100 ml). The combined extracts were washed with cold brine (5 × 100 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in glacial acetic acid (150 ml), N-HCl in glacial acetic acid was added (420 ml), and the mixture was kept for 45 min at room temperature, then evaporated to dryness. The residue was stored for 16 h *in vacuo* (KOH), then dissolved in M-acetic acid (250 ml) and passed through a column (250 ml) of Dowex 1 (AcO^- form) resin. The eluate was evaporated to dryness and the residue redissolved in water (250 ml) and applied to a column (35 × 2 cm) of carboxymethyl-cellulose (CMC) (H^+ form) which was eluted with a linear gradient (4 l; 0—0.5M) of acetic acid (25 ml fractions). Fractions were examined by t.l.c. in solvent No. 7 of von Arx and Neher,[10] and fractions 12—48 were combined and freeze-dried to give 816 mg of product. A further 481 mg was obtained by rechromatography of material recovered from fractions 1—11 and 49—65. The *product* (1.30 g, 22%) had $[\alpha]_D^{28}$ +6.7 ± 0.7° (*c* 0.4 in acetic acid) (Found: C, 50.9; H, 5.4; Br, 11.2; N, 15.75. $C_{28}H_{35}BrN_8O_5,CH_3CO_2H$ requires C, 51.2; H, 5.55; Br, 11.4; N, 15.95%).

[8] L. Birkofer and K. Hempel, *Chem. Ber.*, 1963, **96**, 1373.
[9] E. Schnabel, *Annalen*, 1967, **702**, 188.
[10] E. von Arx and R. Neher, *J. Chromatog.*, 1963, **12**, 329.

N-*t-Butoxycarbonyl-4-iodophenylalanine* (V).—This was prepared in 86% yield from 4-iodophenylalanine [11] in the usual manner [9] at pH 10.3 and 20 °C. The *product* was crystallised twice from ether–cyclohexane and had m.p. 120—21°, $[\alpha]_D^{22}$ +21.3 ± 0.02° (*c* 1.2 in EtOAc) (Found: C, 43.05; H, 4.75; I, 32.6; N, 3.65. $C_{14}H_{18}INO_4$ requires C, 42.95; H, 4.6; I, 32.5; N, 3.6%).

4-Iodophenylalanylarginyltryptophylglycine Actetate (VI).—This was prepared from (V) by the series of reactions described above for the bromo-analogue with the difference that the CMC column was eluted with a gradient of acetic acid in aqueous 10% DMF and the compound was also loaded onto the column in aqueous 10% DMF. The *product* was obtained in an overall yield of 16% from Boc-iodophenylalanine and had $[\alpha]_D^{20}$ +9.8 ± 0.2° (*c* 0.6 in AcOH) (Found: C, 47.75; H, 5.15; I, 17.1; N, 14.7. $C_{28}H_{35}IN_8O_5,CH_3CO_2H$ requires C, 48.0; H, 5.2; I, 16.95; N, 14.9%).

N-*t-Butoxycarbonylseryltyrosylserylmethionyl-γ-t-butylglutamylhistidine Methyl Ester* (VII).—Boc-Ser-Tyr-Ser-Met-N_2H_3 (5.0 g) was dissolved in DMF (35 ml) and cooled to −7 °C, and 5N-HCl (2.35 ml) was added with the temperature kept below −5 °C. Isopentyl nitrite (1.17 ml) was added and the mixture was stirred at −7 °C for 7 min.[6] Triethylamine (1.64 ml) was added and a cooled solution of Glu(OBu^t)-His-OMe,AcOH [from the *N*-benzyloxycarbonyl derivative [5] (5 g)] and triethylamine (1.42 ml) in DMF (31 ml) was added. The mixture was stirred at −10 °C for 2½ h and then at 4 °C for 60 h. The mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in butan-1-ol (100 ml) and ethyl acetate (75 ml) and extracted at 0 °C with saturated citric acid solution (3 × 150 ml), brine (150 ml), and saturated aqueous $NaHCO_3$ (2 × 150 ml), dried (Na_2SO_4), and evaporated to dryness. The ester (5 g, 65%) was adequate for conversion into the hydrazide without further purification. A *sample* crystallised twice from aqueous methanol had m.p. 174—175°, $[\alpha]_D^{25}$ 27.4 ± 1.3° (*c* 1.0 in MeOH) (Found: C, 53.2; H, 6.75; N, 12.0; S, 3.65. $C_{41}H_{62}N_8O_{14}S$ requires C, 53.4; H, 6.7; N, 12.15; S, 3.45%).

N-*t-Butoxycarbonylseryltyrosylserylmethionyl-γ-t-butylglutamylhistidine Hydrazide* (VIII).—Non-crystalline (VII) (4.2 g) was dissolved in methanol (50 ml), hydrazine hydrate (2.25 ml) was added, and the mixture was stirred overnight under N_2. Water (50 ml) was added over 30 min and the mixture was kept at 4 °C for 6 h. The gel was collected by filtration, washed with cold water and ether, and dried *in vacuo* (H_2SO_4). It was recrystallised from aqueous methanol to give the *product* (2.65 g, 63%), m.p. 195—197°, $[\alpha]_D^{25}$ +10.7 ± 1.1° (*c* 1.0 in AcOH) (Found: C, 52.0; H, 6.75; N, 15.1; S, 3.65. $C_{40}H_{62}N_{10}O_{13}S$ requires C, 52.1; H, 6.75; N, 15.2; S, 3.45%).

N-*t-Butoxycarbonylseryltyrosylserylmethionyl-γ-t-butylglutamylhistidyl-4-bromophenylalanylarginyltryptophylglycine* (IX).—Compound (VIII) (1.91 g) dissolved in DMF (19 ml) was cooled to −10 °C and 5N-HCl (1.25 ml) was added with stirring, with the temperature kept below −5 °C. Isopentyl nitrite (0.277 ml) was added and the mixture was stirred at −10 °C for 10 min.[6] Triethylamine (0.89 ml) was added and the mixture was added to a solution of (IV) (1.03 g) in DMF (19 ml) containing triethylamine (0.17 ml) cooled to −10 °C. The mixture was stirred at −10 °C for 30 min and at 4 °C for 16 h, then filtered, and the filtrate was evaporated to dryness below 35 °C. The residue was dissolved in DMF (10 ml) and added dropwise to vigorously stirred ethyl acetate (250 ml) to give a white solid (2.73 g). This was dissolved in hot methanol (35 ml); the solution was filtered and warm water (35 ml) added. After 16 h at 4° the gel formed was collected by filtration, washed with ice-cold 50% aqueous methanol then ether, and dried *in vacuo* (H_2SO_4) to give material (1.36 g, 43%) which (by analysis) was substantially pure. This was dissolved in the upper phase (50 ml) of butan-1-ol–aqueous 10% (v/v) acetic acid (1 : 1),[12] loaded into tubes 3—7 of a 123-tube counter-current distribution machine (10 ml each phase), and subjected to 113 upper-phase transfers. Samples of the upper phases of the fractions were examined by t.l.c. in the solvent system No. 7. The *product* was recovered from tubes 85—101 and was obtained as a white powder (0.52 g, 16% overall) by addition of a solution in DMF (5 ml) to vigorously stirred ether (45 ml); amino-acid analysis: Arg, 0.98; Glu, 1.00; Gly, 1.00; His, 1.02; Met, 0.99; Phe (Br), 1.03; Ser, 1.84; Tyr, 1,00; Trp, 0.89 (Found: C, 53.05; H, 6.05; Br, 5.05; N, 14.5; S, 2.15. $C_{68}H_{93}BrN_{16}O_{18}S$ requires C, 53.2; H, 6.05; Br, 5.2; N, 14.6; S, 2.1%).

N-*t-Butoxycarbonylseryltyrosylserylmethionyl-γ-t-butylglutamylhistidyl-4-iodophenylalanylarginyltryptophylglycine* (X).—This was prepared by the azide method [6] as described above for the 4-bromo-analogue starting from (VI) (1.0 g). After removal of DMF, solid (2.7 g) was recovered by trituration with ice–water. This was recrystallised twice from MeOH–H_2O (9 : 1 v/v) to yield the *product* (0.84 g, 40%); amino-acid analysis: Arg, 0.98; Glu, 1.00; Gly, 0.99; His, 0.97; Met, 1.03; Phe(I), 1.02; Ser, 1.67; Tyr, 1.01; Trp, 0.78. (Found: C, 51.8; H, 6.05; I, 7.85; N, 13.95; S, 2.1. $C_{68}H_{93}IN_{16}O_{18}S$ requires C, 51.65; H, 5.9, I, 8.05; N, 14.2; S. 2.03%).

N-*t-Butoxycarbonylseryltyrosylserylmethionyl-γ-t-butylglutamylhistidyl-4-bromophenylalanylarginyltryptophylglycyl-*N^ε*-t-butoxycarbonyl-lysylprolylvalylglycyl-*N^ε*-t-butoxycarbonyl-lysyl-*N^ε*-t-butoxycarbonyl-lysylarginylarginylprolylvalyl-*N^ε*-t-butoxycarbonyl-lysylvalyltyrosylproline t-Butyl Ester Sesquisulphate* (XI).—Compound (IX) (411 mg) was dissolved in DMF (3 ml) and cooled to −10 °C, and N-H_2SO_4 (0.27 ml) was added with stirring. Tetradecapeptide monosulphate [13] (0.66 g) and *N*-hydroxybenzotriazole hydrate (HOBt) (46 mg) were added in DMF (3 ml), the mixture was heated to 50 °C, dicyclohexylcarbodi-imide (95 mg) in DMF (1 ml) was added, and the mixture was stirred under N_2 for 16 h at 50 °C. After 6 h at 4 °C, dicyclohexylurea (DCU) was filtered off and the filtrate evaporated to dryness below 35 °C. The residue was dissolved in the lower phase (19 ml) of methanol–ammonium acetate buffer [ammonium acetate (309 g), acetic acid (456 ml), and water to 16 l]–chloroform–carbon tetrachloride (8 : 4 : 5 : 2 v/v), loaded into tubes 3 and 4 of a 123-tube counter-current distribution machine (10 ml each phase), and subjected to 685 upper phase transfers.[13] Samples of the lower phases were examined by t.l.c. in solvent system No. 7. Pure material was collected from tubes 81—95. The contents of tubes 55—80 were retained, all other tubes were refilled with fresh solvents, and the system was given a further 1 000 transfers in the recycling mode. Further pure material was obtained from tubes 38—74. The recovered *product*

[11] E. Abderhalden and E. A. Brossa, *Ber.*, 1909, **42**, 3411.

[12] K. Hofmann, J. Rosenthaler, R. D. Wells, and H. Yajima, *J. Amer. Chem. Soc.*, 1964, **86**, 4991.

[13] R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, 1963, **46**, 1550.

was obtained by evaporation of the solution and trituration with petroleum (b.p. 60—80°) as a white powder (601 mg, 59%); amino-acid analysis: Arg, 3.04; Glu, 1.00; Gly, 2.02; His, 0.99; Lys, 4.00; Met, 0.99; Phe (Br), 1.01; Pro, 3.01; Ser, 1.73; Trp, 0.73; Tyr, 1.97; Val, 3.03 (Found: C, 53.75; H, 7.25; Br, 2.1; N, 14.6; S. 1.95. $C_{169}H_{265}BrN_{40}O_{41}S,1.5H_2SO_4$ requires C, 53.85; H, 7.15; Br, 2.1; N, 14.85; S, 2.1%).

N-*t-Butoxycarbonylseryltyrosylserylmethionyl-γ-t-butylglutamylhistidyl*-4-*iodophenylalanylarginyltryptophylglycyl*-N^ε-*t-butoxycarbonyl-lysylprolylvalylglycyl*-N^ε-*t-butoxycarbonyl-lysyl*-N^ε-*t-butoxycarbonyl-lysylarginylarginylprolylvalyl*-N^ε-*t-butoxycarbonyl-lysylvalyltyrosylproline t-Butyl Ester Trihydrochloride* (XII).—This was prepared as described above for the corresponding bromo-analogue sesquisulphate starting from the protected iodo-decapeptide (X) (560 mg) and the tetradecapeptide dihydrochloride (0.85 g). Purification was carried out by counter-current distribution as described except that tube contents were examined after 750 transfers. Pure material was collected from tubes 95—114. The contents of tubes 71—94 were retained and a further 750 transfers were performed in the recycling mode. Further pure material was collected from tubes 46—74. The product (841 mg, 62%) was recovered as a white powder; amino-acid analysis: Arg, 3.02; Glu, 1,00; Gly, 2.01; His, 0.99; Lys, 4.01; Met, 0.98; Phe(I) 1.05; Pro, 2.98; Ser, 1.77; Trp, 0.76; Tyr, 2,01; Val, 3.02 (Found: C, 53.45; H, 7.25; I, 3.7; N, 14.35; S, 0.6. $C_{169}H_{265}IN_{40}O_{41}S,3HCl$ requires C, 53.7; H, 7.1; I, 3.35; N, 14.8; S, 0.85%).

[7-(4-*Bromophenylalanine*)]-β-*Corticotrophin*-(1—24)-*tetracosapeptide*.—A sample (36.8 mg) of protected tetracosapeptide (XI) was deprotected and purified by ion-exchange chromatography as described for the di-iodotyrosine analogue.[2] The column was eluted with 0.6M-trimethylammonium acetate buffer (pH 5.5). After evaporation of the bulk of the salt, the last traces were removed from the peptide by gel filtration [column of Biogel P-2 (18 × 0.9 cm)]. The recovered *peptide* (18.4 mg, 63%) had amino-acid analysis after acidic hydrolysis: Arg, 3.07; Glu, 1.00; Gly, 2.00; His, 1.01; Lys, 4.03; Met, 1.02; Phe(Br), 0.97: Pro, 3.03; Ser, 1.83; Trp, 0.73; Tyr, 2.03; Val, 3.03; and after enzymic hydrolysis:[14] Arg, 2.92; Glu, 0.97; Gly, 1.96; His, 1.01; Lys, 4.03; Met, 0.98; Phe (Br), 1.00; Pro, 3.00; Ser, 1.94; Trp, 0.90; Tyr, 2.02; Val, 2.98.

[7-(4-*Iodophenylalanine*)]-β-*Corticotrophin*-(1—24)-*tetracosapeptide*.—This was prepared as described above for the bromo-analogue from the derivative (XII) (5.2 mg). The recovered *peptide* (1.1 mg, 27%) had amino-acid analysis after acidic hydrolysis: Arg, 3.02; Glu, 1.00; Gly, 2.08; His, 1.00; Lys, 4.12; Met, 1.00; Phe(I), 1.04; Pro, 3.11; Ser, 1.78; Trp, 0.67; Tyr, 2.02; Val, 3.07; and after enzymic hydrolysis: Arg, 2.93; Glu, 1.01; Gly, 1.99; His, 0.97; Lys, 3.75; Met, 1.00; Phe(I) 1.02; Pro, 2.82; Ser, 1.97; Trp, 0.91; Tyr, 2,01; Val, 2.77.

Trial Reductions of the Protected Tetracosapeptides.—A sample (8.4 mg) of the bromotetracosapeptide derivative (XI) was catalytically hydrogenated for 90 min as described for the di-iodo analogue.[1] After removal of catalyst and solvent, a portion was hydrolysed with acid and analysed for amino-acids: Arg, 3.00; Phe, 0.59; Phe(Br), 0.36. Reduction was incomplete although uptake of hydrogen had ceased.

A sample (10.0 mg) of the iodo-analogue (XII) was reduced for 50 min and analysed; amino-acid analysis: Arg, 3.04; Phe, 0.96; Phe(I), 0.

{7-([4-^{3}H]*Phenylalanine*)}-β-*Corticotrophin*-(1—24)-*tetracosapeptide*.—A sample (13.6 mg) of (XII) was catalytically tritiated, deprotected, and purified as described for the tyrosine analogue.[2] The recovered peptide (0.645 μmol, 18%) had amino-acid analysis after acidic hydrolysis: Arg, 2.98; Glu, 0.99; Gly, 2.03; His, 1.03; Lys, 4.00; Met, 0.95; Phe, 1.02; Pro, 3.00; Ser, 1.71; Trp, 0.59; Tyr, 1.99; Val, 3.03; and after enzymic hydrolysis: Arg, 2.46; Glu, 1.00; Gly, 1.89; His, 0.99; Lys, 3.82; Met, 0.94; Phe, 0.99; Pro, 2.89; Ser, 1.81; Trp, 0.70; Tyr, 1.94; Val, 3.01. Specific activity was 27.2 Ci mmol^{-1}.

A sample of enzymic hydrolysate was diluted with inactive carrier amino-acid mixture and the mixture was separated with the amino-acid analyser.[1] The column eluate was collected in fractions (0.6 ml) which were assessed for their content of radioactivity. The percentage content was distributed as follows: Phe, 96.7; His, 3.3%.

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