SYNTHESIS OF β-ALANYL-ARGINYL-GLYCYL-PHENYLALANYL-PHENYL-ALANYL-TYROSINE AMIDE LABELLED WITH TRITIUM OR CARBON-14

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SUMMARY

The synthesis of β-Ala-Arg-Gly-Phe-Phe-Tyr-NH_2 labelled with tritium on the tyrosine residue or with carbon-14 on the arginine residue is described. The tritium labelled compound VII was prepared in 49% radiochemical yield from L-[3,5-^{3}H]tyrosine and benzyloxycarbonyl-β-alanyl-nitro-arginyl-glycyl-phenylalanyl-phenylalanine. The carbon-14 labelled compound XVI was synthesized in 17% radiochemical yield from L-[U-^{14}C]arginine and glycyl-phenylalanyl-phenylalanyl-tyrosine amide.

Key Words: Syntheses, [^{3}H]DP-432, [^{14}C]DP-432

Twenty three years ago, Sanger[1] suggested that the sequence Phe-Phe-Tyr (B 24-26) is an interesting part of insulin and this sequence could play a role in the biological activity. From the studies of the mechanism of insulin action[2,3] and the relationship between structure and activity of chemically or enzymatically modified insulins[4,5], amino acid sequence of peptide which possesses an insulin-like effect has been shown. On the basis of these results, many peptides modified the sequence have been synthesized, and their insulin-like effects have also been measured. Among these synthetic peptides, β-Ala-Arg-Gly-Phe-Phe-Tyr-NH_2 (DP-432), a new compound possessing an insulin-like

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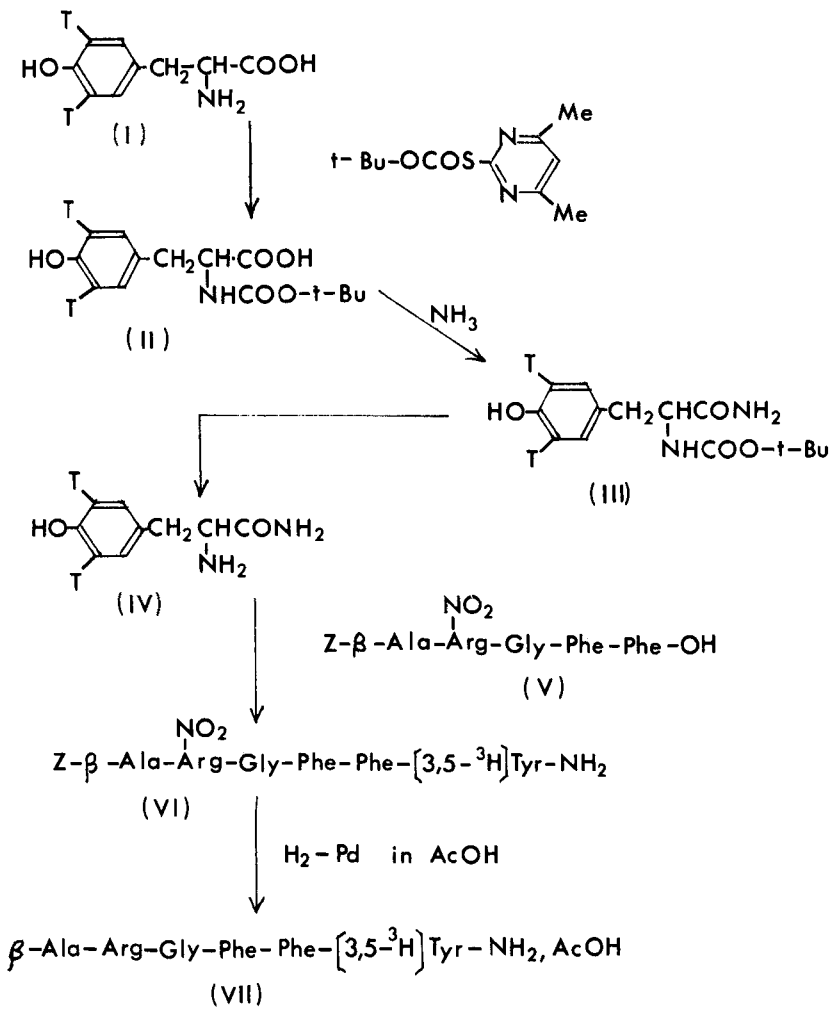
activity has been found[6]. This paper deals with the syntheses of β-Ala-Arg-Gly-Phe-Phe-[3,5-^{3}H]Tyr-NH_2 (VII) and β-Ala-[U-^{14}C]-Arg-Gly-Phe-Phe-Tyr-NH_2 (XVI) for the study of metabolic fate in animals.

Scheme 1

Synthesis of [Tyr-3,5-^{3}H] DP-432

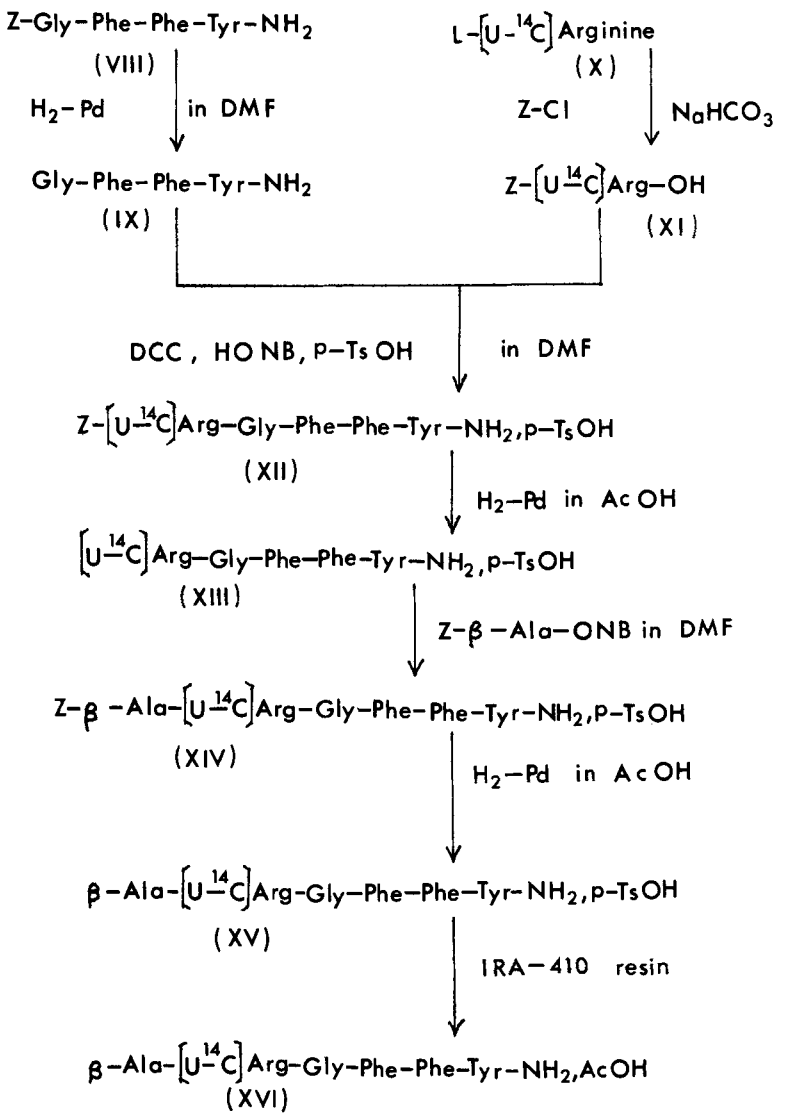


Reaction of L-[3,5-^{3}H]tyrosine (I) with tert-butyl 4,6-dimethyl-pyrimidyl-2-thiol carbonate by a modification of known procedure[7] gave tert-butyloxycarbonyl-L-[3,5-^{3}H]tyrosine (II) in 91% yield. The activated ester which was prepared by mixing II, N-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[8] and dicyclohexylcarbodiimide (DCC), was converted to tert-butyloxycarbonyl-L-[3,5-^{3}H]-tyrosine amide (III) in an aqueous ammonia. Removal of the tert-butyloxycarbonyl group in III by treatment with trifluoroacetic acid led to L-[3,5-^{3}H]tyrosine amide (IV) as shown in Scheme 1. The reaction of IV with benzyloxycarbonyl-β-alanyl-nitroarginyl-glycyl-phenylalanyl-phenylalanine (V) in the presence of HONB and DCC yielded benzyloxycarbonyl-β-alanyl-nitroarginyl-glycyl-phenyl-alanyl-phenylalanyl-[3,5-^{3}H]tyrosine amide (VI) in quantitative yield. Deblocking reactions by the catalytic reduction of VI with hydrogen provided VII. After purification, the product VII with a specific activity of 40.8 mCi/mmole was obtained in 49% radio-chemical yield based on I. The synthetic route of XVI was presented in Scheme 2. L-[U-^{14}C]Arginine (X) was acylated with benzyloxy-carbonyl chloride by the ordinary acylation method[9] to give benzyloxycarbonyl-[U-^{14}C]arginine (Z-Arg-^{14}C, XI). In the presence of DCC, HONB and p-toluene sulfonic acid in dimethyl formamide, coupling XI with Gly-Phe-Phe-Tyr-NH_2 (IX) freshly prepared from benzyloxycarbonyl-Gly-Phe-Phe-Tyr-NH_2 (VIII) gave Z-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XII) in 44% yield based on X.
After removal of the blocking group in XII by the catalytic reduction, the resulting compound [U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XIII) was condensed with 5-norbornene-2,3-dicarboximide ester of benzyloxycarbonyl-β-alanine (Z-β-Ala-ONB) in dimethyl formamide to afford Z-β-Ala-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XIV) in 22% yield based on X. Deblocking by the reduction of XIV with hydrogen in the presence of a palladium-black catalyst provided β-Ala-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XV), and XV was converted to β-Ala-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, AcOH (XVI) with

an ion-exchange resin of Amberlite IRA-410. Purification was chromatographically accomplished with a carboxymethyl cellulose column by the linear gradient method. Thus, the final product XVI with a specific activity of 9.3 mCi/mmole was obtained in 17% radiochemical yield based on X. The purity of both VII and XVI were shown to be greater than 99% based on both radiochromatographic and reverse isotope dilution methods.

Scheme 2

Synthesis of [Arg-^{14}C(U)] DP-432



EXPERIMENTAL

Materials

L-[3,5-^{3}H]Tyrosine and L-[U-^{14}C]arginine were purchased from The Radiochemical Centre, Amersham, England.

tert-Butyloxycarbonyl-L-[3,5-^{3}H]tyrosine (II)

A solution of 82 mCi (specific activity, 54 Ci/mmole) of L-[3,5-^{3}H]tyrosine was added to 362 mg (2 mmole) of inactive L-tyrosine and the solution was concentrated to dryness in vacuo. To a mixture of 1.1 ml of water, 0.42 ml of triethylamine and 362 mg of the residue (diluted L-[3,5-^{3}H]tyrosine) was added 528 mg of tert-butyl 4,6-dimethylpyrimidyl-2-thiolcarbonate in 1.1 ml of dimethyl formamide. After allowing to stand over-night at room temperature with stirring, 4 ml of water was added to the reaction mixture. The resulting solution was washed with ethyl acetate, and the aqueous layer was neutralized to pH 2 with 6N HCl and extracted with ethyl acetate. The extract was washed with cooled N HCl and sat-NaCl. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with petroleum benzine twice and dried in vacuo to give 515 mg of II in 91% yield.

tert-Butyloxycarbonyl-L-[3,5-^{3}H]tyrosine amide (III)

To a solution of 515 mg of II in 3.7 ml of tetrahydrofuran was added 361 mg of HONB and 416 mg of DCC upon cooling at 0°, and the mixture was stirred for 4 h in an ice bath. To the mixture was added 0.18 ml of conc. ammonia and allowing to stand over-night with stirring. After the crystalline dicyclohexylurea was removed by filtration, the filtrate was concentrated to dryness and the residue was dissolved in 15 ml of ethyl acetate. The ester solution was washed with sat-NaCl, sat-$NaHCO_3$ followed by 10% aq-citric acid and sat-NaCl, and then dried over anhydrous Na_2SO_4. The dried ester solution was evaporated to give 494 mg of crude III in 95% yield.

Benzyloxycarbonyl-β-Ala-Arg(NO_2)-Gly-Phe-Phe-[3,5-^{3}H]Tyr-NH_2 (VI)

A mixture of 494 mg of III and 5.3 ml of trifluoroacetic acid was stirred vigorously at room temperature for 10 min and added to 0.32 ml of 6N HCl. Evaporation of the mixture in vacuo left a residue which was washed with ether twice and dried for 2 days in a vacuum desiccator placed NaOH. The dried residue was dissolved in 8.8 ml of dimethyl formamide, and 2.26 ml of dimethyl formamide containing 10% N-ethyl morpholine was added to the solution. To the mixture were added 1.369 g of benzyloxycarbonyl-β-Ala-Arg(NO_2)-Gly-Phe-Phe-OH (V) and 632 mg of HONB upon cooling at -10° in an ice-salt bath, then further added 727 mg of DCC and allowing to stand over-night with stirring. Dicyclohexylurea precipitated was filtered off, and the filtrate was concentrated in vacuo. The residue was solidified by the addition of water. After drying the solid, it was dissolved in dimethyl formamide and an insoluble material was removed. Evaporation of the filtrate in vacuo left a residue which was purified by dissolving in methanol and addition of water. The crystalline powder was dried in vacuo to give 1.71 g of VI.

β-Ala-Arg-Gly-Phe-Phe-[3,5-^{3}H]Tyr-NH_2, AcOH (VII)

A mixture of 1.71 g of VI and 1.36 g of palladium-black in 50 ml of acetic acid was treated with hydrogen until the calculated amount was absorbed. The catalyst was removed by filtration and washed with acetic acid. The combined solution was concentrated in vacuo and 50 ml of water was added to the residue. The aqueous solution was freeze-dried and the resulting residue was chromatographed over 70 ml of carboxymethyl cellulose (ammonium acetate form) using 400 ml of 0.005M NH_4OAc and 400 ml of 0.2M NH_4OAc as an eluent by the linear gradient method. The fraction of VII was freeze-dried to give crystalline powder. The purification of crude VII was achieved by using chromatography as same as the technique described above. The product VII (820 mg) with a specific activity

of 40.8 mCi/mmole was obtained in 49% radiochemical yield based on I.

Z-[U-^{14}C]Arg-OH (XI)

Z-[U-^{14}C]Arg-OH was prepared by the acylation of L-[U-^{14}C]-arginine (10 mCi, 1 mmole) with benzyloxycarbonyl chloride according to an adaptation of the method of Boissonnas[9], and obtained in 54% yield from X.

Z-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XII)

A solution of 360 mg of Z-Gly-Phe-Phe-Tyr-NH_2 in 9 ml of dimethyl formamide was hydrogenated with hydrogen in the presence of a palladium-black catalyst. After the catalyst was removed by filtration, 168 mg of XI, 109 mg of HONB and 106 mg of p-toluene sulfonic acid were added to the filtrate with stirring and cooling in an ice-salt bath. To the resulting solution was added 123 mg of DCC and the mixture was stirred over-night, and then the precipitated dicyclohexylurea was removed by filtration and washed with a small amount of dimethyl formamide. The combined solution was concentrated in vacuo below 35°, and the residue was extracted with a mixture of 50 ml of n-butanol and 15 ml of water. The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with ethyl acetate and dried to give 446 mg of crude XII in 44% yield based on X. The Rf value of XII on silica gel t.l.c. (developing solvent; AcOEt, pyridine, AcOH, water, 60 : 20 : 6 : 11, v/v) was identical with that of an authentic sample.

[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XIII)

A mixture of 446 mg of XII in 20 ml of acetic acid and 470 mg of palladium-black was treated with hydrogen until the calculated amount was absorbed. After the catalyst was removed and washed with acetic acid, evaporation of the combined solution in vacuo below 40° left 427 mg of XIII which appeared as a syrup.

Z-β-Ala-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XIV)

A solution of 427 mg of XIII and 196 mg of Z-β-Ala-ONB in 20 ml of anhydrous dimethyl formamide was stirred at room temperature over-night. The by-product precipitated was filtered off and washed with 10 ml of dimethyl formamide. The combined solution was evaporated to dryness, and the residue was washed with ethyl acetate. The product was purified by dissolving in hot methanol and addition of water. The crystalline powder was filtered off, washed with water and dried in vacuo to afford 244 mg of XIV in 22% yield based on X.

β-Ala-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, p-TsOH (XV)

A mixture of 244 mg of XIV, 14 ml of acetic acid and 250 mg of palladium-black was hydrogenated until absorption ceased. The catalyst was removed by filtration and washed with 20 ml of acetic acid. The combined solution was evaporated in vacuo and 20 ml of water was added to the residue. The aqueous solution was freeze-dried to give 259 mg of XV.

β-Ala-[U-^{14}C]Arg-Gly-Phe-Phe-Tyr-NH_2, AcOH (XVI)

Into 15 ml of water was dissolved 259 mg of XV, and the insoluble solid was filtered off. The filtrate was passed over a column of Amberlite IRA-410 (acetate form) and eluted with water. The eluted fraction was freeze-dried in vacuo to give 178 mg of crude XVI. The crude XVI was dissolved in 5 ml of an aqueous solution of 0.005M NH_4OAc, and chromatographed over carboxymethyl cellulose (ammonium acetate form) column with 400 ml of 0.005M NH_4OAc and 400 ml of 0.2M NH_4OAc as an eluent by the linear gradient method. The fraction of XVI was collected and freeze-dried. In order to remove excess acetic acid, the residue was dissolved in 50 ml of water and the solution was freeze-dried again in vacuo to give 135 mg of XVI with a specific activity of 9.3 mCi/mmole in 17% yield based on X.

Thin-layer chromatography

The t.l.c. plates used were 20 cm glass plates coated with silica gel F_{254} (Art. 5715, Merck) and developed with the following solvent systems using the ninhydrin reagent for visualization; (a) n-BuOH, AcOH, H_2O (4 : 1 : 1, v/v). (b) n-BuOH, pyridine, AcOH, H_2O (30 : 20 : 6 : 24, v/v). (c) AcOEt, n-BuOH, AcOH, H_2O (1 : 1 : 1 : 1, v/v). With these developing solvents (a, b and c), the Rf-values of DP-432 were found at 0.15, 0.54 and 0.58 respectively.

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