Syntheses of Peptides Related to the N-Terminal Structure of Corticotropin. I. Synthesis of Ser-Tyr-Ser-Met Sequence

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The hypophyseal polypeptide hormones, the corticotropin and melanocyte-stimulating hormone (MSH), have a common amino acid sequence within their structures[1,2].

By Harris and Lerner[1,3] it has been shown that hog α-MSH is an *N*-acetyltridecapeptide amide and its amino acid sequence, Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val, is identical with that occurring in the first tridecapeptide portion of corticotropin. This evidence affords a chemical basis for the intrinsic MSH activity[4,5] of corticotropin.

Considerably large fragments can be enzymatically or chemically removed from the carboxyl end of the molecule of corticotropin without loss of its biological activity[6]. While it has been reported that periodate oxidation of the *N*-terminal serine of corticotropin[7] or the cleavage of peptide bonds in the *N*-terminal Ser-Tyr-Ser segment of the molecule[6] results in complete depression of its ACTH activity, at the same time significant increase in the melanocyte expanding activity is observed[7].

The further detailed relationship between the two biological activities and the chemical structure must await the synthetic studies of active polypeptides.

Synthesis of *N*-terminal twenty sequence of corticotropin was done by Boissonnas et al. and the product exhibited a positive though limited ACTH activity[8]. Guttmann and Boissonnas[9] have recently synthesized the complete structure of α-MSH and the synthetic hormone has been shown to possess the same properties as those of the natural hog α-MSH. Hofmann et al. have reported that the blocked tridecapeptide amide, Cbz-Ser-Tyr-Ser-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-(ε-Tos)-Lys-Pro-Val-NH_2, has 0.8×10^8 MSH units per gram[10] and the replacement of the carbobenzoxy group with an acetyl group results in some twenty-five fold increase in its activity[11]. The substituted polypeptide sequence of β-MSH has also been synthesized by Schwyzer et al.[12]

Here we wish to write concerning a part of our studies of synthetic peptides corresponding to the amino acid sequence common to corticotropin and α-MSH. The synthesis of Ser-Tyr-Ser-Met sequence will be described.

In the syntheses of serine-containing peptides we used *O*-benzylserine[13,14] as a starting material in place of serine to avoid unexpected side reactions which may be caused by the presence of the free hydroxyl group of the latter.

Carbobenzoxy-*O*-benzylseryltyrosine and -*O*-benzylserylmethionine methyl esters were obtained in good yields as compared with the corresponding serylpeptide derivatives[15,16], though as for the method of coupling, the anhydride procedure was different from the procedures used by the others.

The advantageous effect of *O*-benzylserine is much more remarkable in saponification of the peptide esters with alkali. It has been reported that the saponification of carbobenzoxyseryltyrosine methyl[16] or ethyl[15] ester gave only a 50 per cent yield of the desired product and the yield of carbobenzoxyserylmethionine was also significantly low (67%)[15]. However, we could derive carbobenzoxy-*O*-benzylseryltyrosine and -*O*-benzylserylmethionine and formyl-*O*-benzylserylmethionine in very high yields (near 90%) from the preceding methyl esters. The peptide bonds adjacent to the serine residues in corticotropin are sensitive to any alkali treatment[6] and Guttmann and

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14) K. Okawa, This Bulletin, **29**, 486 (1956).
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16) St. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, **41**, 1852 (1958).

Boissonnas[16] have, moreover, shown the fact that the saponification of serine peptide esters is accompanied by the cleavage of peptide bond at the carboxyl side of serine residue. However, we could not observe such a fact in regard to the *O*-benzylserine peptides.

On removal of carbobenzoxy and *O*-benzyl groups from carbobenzoxy-*O*-benzylserylmethionine methyl ester by hydrobromic acid/acetic acid or catalytic hydrogenation we failed to obtain the desired dipeptide ester, presumably because of the instability of the thioether bond. Sheehan and Yang[17] have shown that a formyl group, which is easily removed by mild acid solvolysis, can be used on peptide syntheses as an amino protecting function in conjugation with the carbodiimide procedure without racemization of the products. Following this procedure, formyl-*O*-benzylserine and methionine methyl ester were coupled with *N,N'*-dicyclohexylcarbodiimide (DCCI) to give formyl-*O*-benzylserylmethionine methyl ester which could be deformylated to *O*-benzylserylmethionine methyl ester without any difficulties.

Coupling of carbobenzoxy-*O*-benzylseryltyrosine with *O*-benzylserylmethionine methyl ester by DCCI yielded almost quantitatively the acyltetrapeptide ester, carbobenzoxy-*O*-benzylseryltyrosyl-*O*-benzylserylmethionine methyl ester in crystalline form.

Experimental

All amino acids used are of L-configuration. The melting point was determined in a capillary tube in a sulfuric acid bath and not corrected.

O-Benzylserine Methyl Ester Hydrochloride.—To 20 ml. of anhydrous methanol previously cooled below −10°C, was added dropwise 1.33 ml. (0.0184 mol.) of thionylchloride and then 3 g. (0.0154 mol.) of *O*-benzylserine was introduced[13,14]. The mixture was stirred for 1.5 hr. at room temperature and then allowed to stand overnight. The resulting clear solution was evaporated under reduced pressure. The residue was crystallized from acetone-ether to give 3.44 g. (91%). Recrystallization from methanol-ether yielded needles, m. p. 141.5～142°C with decomposition.

Found: N, 5.86; Cl, 14.41. Calcd. for $C_{11}H_{16}O_3 \cdot NCl$: N, 5.69; Cl, 14.43%.

***N*-Formyl-*O*-benzylserine.**—This was prepared after the usual procedure for optically active formylamino acids of Sheehan and Yang[17]. 1.95 g. of *O*-benzylserine was formylated and the product was recrystallized from water to give 1.77 g., m. p. 137.5～138.5°C, $[\alpha]_D^{26} = +52.9 \pm 1°$ (*c* 2.613 in ethanol).

Found: C, 59.31; H, 6.03; N, 6.39. Calcd. for $C_{11}H_{13}O_4N$: C, 59.3; H, 5.88; N, 6.29%.

***N*-Carbobenzoxy-*O*-benzylseryltyrosine Methyl Ester.**—To a solution of 8.23 g. (0.025 mol.) of *N*-carbobenzoxy-*O*-benzylserine[18] (m. p. 100.5～101°C, $[\alpha]_D^{22} = +12.0 \pm 2°$ (*c* 2.448 in acetic acid)) in 75 ml. of tetrahydrofuran/dioxane (1 : 4) were added 6.56 ml. (0.0275 mol.) of tri-*n*-butylamine and 2.63 ml. (0.0275 mol.) of ethylchloroformate at −5°C. After 15 min. a solution of tyrosine methyl ester (prepared from 6.37 g. (0.0275 mol.) of the hydrochloride[19] and 4.23 ml. (0.030 mol.) of triethylamine) in 8 ml. water and 32 ml. dioxane was introduced. The reaction mixture was stirred at room temperature for 4 hr. and evaporated under reduced pressure. The residue dissolved in 150 ml. of ethyl acetate was successively washed with 1N hydrochloric acid, water, 5% sodium bicarbonate and water and dried over sodium sulfate. The solution was evaporated to a syrupy residue which was crystallized from methanol-ethyl acetate-petroleum ether, 12.3 g. (97.2%), m.p.108～111.5°C. Recrystallization from ethyl acetate-petroleum ether yielded 10.2 g. (80.6%), m. p. 112～114.5°C, $[\alpha]_D^{19} = +14.6 \pm 2°$ (*c* 3.068 in 99% ethanol). (lit.[18] m. p. 111～112°C, $[\alpha]_D^{15} = +15.5°$ (*c* 2.84 in 99% ethanol).

Found: C, 66.32; H, 6.13; N, 5.41. Calcd. for $C_{28}H_{39}O_7N_2$: C, 66.3; H, 5.97; N, 5.53%.

***N*-Carbobenzoxy-*O*-benzylseryltyrosine.**—2.533 g. (0.005 mol.) of *N*-carbobenzoxy-*O*-benzylseryltyrosine methyl ester was saponified in 12.5 ml. of methanol with 5.0 ml. (0.01 mol.) of 2N Sodium hydroxide at room temperature for one hour, yielding 2.34 g. (95.0%). Recrystallization from ethyl acetate-petroleum ether gave 2.20 g. (89.5%), m. p. 158.5～159.5°C, $[\alpha]_D^{23} = +39.4 \pm 1°$ (*c* 2.086 in ethanol).

Found: C, 65.38; H, 5.88; N, 5.72. Calcd. for $C_{27}H_{28}O_7N_2$: C, 65.6; H, 5.74; N, 5.70%.

***N*-Carbobenzoxy-*O*-benzylserylmethionine Methyl Ester.**—4.12 g. (0.0125 mol.) of *N*-carbobenzoxy-*O*-benzylserine and methionine methyl ester (prepared from 2.80 g. (0.014 mol.) of the hydrochloride) were coupled in a tetrahydrofuran/dioxane solution almost exactly as in the case of *N*-carbobenzoxy-*O*-benzylseryltyrosine methyl ester. Yield 5.1 g. (86%), m. p. 69.0～70.5°C. Recrystallization for analysis from ethyl acetate-petroleum ether gave a sample with m. p. 69.5～71°C.

Found: C, 60.56; H, 6.48; N, 5.76. Calcd. for $C_{24}H_{30}O_6N_2S$: C, 60.8; H, 6.38; N, 5.91%.

***N*-Carbobenzoxy-*O*-benzylserylmethionine.**—3.56 g. (0.0075 mol.) of *N*-carbobenzoxy-*O*-benzylserylmethionine methyl ester was saponified in 18 ml. of methanol with 4.2 ml. of 2N sodium hydroxide at room temperature for 30 min. The product was recrystallized from ethyl acetate-ether to yield 3.17 g. (92%), m. p. 152～152.5°C.

Found: N, 5.95; S, 7.00. Calcd. for $C_{23}H_{28}O_6 \cdot N_2S$: N, 6.09; S, 6.97%.

***N*-Formyl-*O*-benzylserylmethionine Methyl Ester.**—To a suspension of 6.59 g. (0.033 mol.) of methionine methyl ester hydrochloride in 50 ml. of ether was added 20 ml. of ice-cold 50% potassium carbonate. The mixture was shaken at 0°C until the solid disappeared. The ether layer which separated was dried over sodium sulfate at 0°C and concentrated

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18) K. Okawa, This Bulletin, **29**, 488 (1956).

19) R. A. Boissonnas, St. Guttmann, P. -A. Jaquenoud and J. -P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

in vacuo to an oil. This oily residue was dissolved in 50 ml. of methylene chloride and the solution was mixed with a suspension of 6.69 g. (0.03 mol.) of *N*-formyl-*O*-benzylserine in 50 ml. of methylene chloride. To the resulting clear solution was added at 0°C 6.20 g. (0.03 mol.) of dicyclohexylcarbodiimide dissolved in 15 ml. of methylene chloride. The reaction mixture was stirred at room temperature for 5 hr. and then refrigerated. The separated dicyclohexylurea was collected by filtration, yielding 6.45 g. (95.8%). The filtrate was cooled in ice, washed with ice-cold 1 N hydrochloric acid, ice-water, 5% sodium bicarbonate and finally with water and dried over sodium sulfate. The concentration of the solution gave a syrupy residue which was crystallized from tetrahydrofuran-ether to yield colored needles, 7.55 g., m. p. 73~76°C. An additional 1.0 g. of crystal was obtained from the mother liquor. Total yield, 8.55 g. (77.4%). These crops, reddish orange in color, were combined and dissolved in ethyl acetate and chromatographed on 125 g. of aluminum oxide. Elution with the same solvent gave colorless fractions which were combined and concentrated in vacuo. Crystallization of the resulting syrup from ethyl acetate ether yielded colorless and odorless needles, 7.025 g. (63.5%), m. p. 78.5~79.5°C, $[\alpha]_D^{25} = -23.2 \pm 1°$ (*c* 2.08 in methanol).

Found: C, 55.84; H, 6.84; N, 7.42; S, 8.69. Calcd. for $C_{17}H_{24}O_5N_2S$: C, 55.5; H, 6.57; N, 7.61; S, 8.70%.

***N*-Formyl-*O*-benzylserylmethionine.** — 1.54 g. (0.0042 mol.) of *N*-formyl-*O*-benzylserylmethionine methyl ester was saponified in 11 ml. of dioxane with an equivalent amount of 1 N sodium hydroxide at room temperature for one hour. The resulting solution was diluted with ice-water to 30 ml. and neutralized with ice-cold 1 N hydrochloric acid to a separate crystalline precipitate, 1.30 g. (87.8%), m. p. 161~162°C. Recrystallization from ethanol-water gave 1.19 g. of needles, m. p. 163°C, $[\alpha]_D^{16} = -8.8 \pm 2°$ (*c* 2.095 in methanol).

Found: C, 54.49; H, 6.41; N, 7.96; S, 9.13. Calcd. for $C_{16}H_{22}O_5N_2S$: C, 54.20; H, 6.24; N, 7.88; S, 9.03%.

***O*-Benzylserylmethionine Methyl Ester.**—4.42 g. (0.012 mol.) of formyl-*O*-benzylserylmethionine methyl ester was dissolved in 40 ml. of *N*-methanolic hydrochloric acid and stored at room temperature overnight. After most of the methanol had been removed in vacuo the resulting colored syrup was dissolved in 40 ml. of water and washed several times with ethyl acetate. The colorless aqueous solution was concentrated in vacuo to a small volume and mixed with 15 ml. of 50% potassium carbonate and 40 ml. of ether at 0°C. The ether layer was separated and the alkaline phase was twice extracted with ether. The combined ether solution was dried over sodium sulfate at 0°C and concentrated to give a colorless and clear oil, 3.95 g. (97%). A sample was chromatographed on paper in the system of *n*-butanol-acetic acid-water (2 : 1 : 2) and only one spot positive to both ninhydrin and PtI_6^{2-} reagent[20] was detected.

***N*-Carbobenzoxy-*O*-benzylseryltyrosyl-*O*-benzylserylmethionine Methyl Ester.**—4.925 g. (0.01 mol.) of *N*-carbobenzoxy-*O*-benzylseryltyrosine and 3.95 g. of *O*-benzylserylmethionine methyl ester were dissolved in 80 ml. of methylene chloride. To this birefringent solution was added 2.063 g. (0.01 mol.) of dicyclohexylcarbodiimide in 20 ml. of methylene chloride at 0°C and the reaction mixture was stirred at room temperature. The birefringence of flow almost completely disappeared in about 10 min. as the crystalline dicyclohexylurea separated. After the stirring had been continued for 3 hr. the mixture was stored at room temperature overnight to turn into a semi-solid state. It was returned to a solution by gentle warming at the boiling point and the insoluble urea was filtered off. The filtrate was concentrated to dryness in vacuo and the resulting solid was recrystallized from ethyl acetate-ether to yield 8.03 g. (98.7%), m. p. 163~167°C. Repeated recrystallization from methylene chloride-ether afforded 7.00 g. (86%), m. p. 166~169°C. A sample for analyses was further recrystallized from methanol to needles, m. p. 171~173°C, $[\alpha]_D^{20} = -12.9 \pm 0.1°$ (*c* 1.986 in methanol), $[\alpha]_D^{26} = -16.1 \pm 1°$ (*c* 2.008 in dimethylformamide).

Found: C, 63.54; H, 6.34; N, 6.95; S. 3.87. Calcd. for $C_{43}H_{50}O_{10}N_4S$: C, 63.5; H, 6.19; N, 6.88; S, 3.84%.

Summary

1. Carbobenzoxy-*O*-benzylseryltyrosyl-*O*-benzylserylmethionine methyl ester, which has the *N*-terminal tetrapeptide sequence of corticotropin and α-MSH, has been synthesized.

2. It has been shown that the *O*-benzylseryl peptide bond is very stable to an alkali treatment.

3. Syntheses of the relating serine peptide derivatives have been described.

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