SYNTHESIS OF A PROTECTED FRAGMENT (24–27) OF THE AMINO ACID SEQUENCE OF CYTOCHROME C

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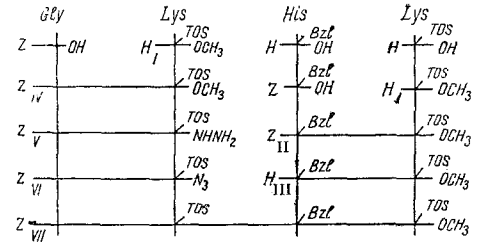
Continuing investigations [1] on the nature of the bond of the apoenzyme with the prosthetic group in the natural hemoproteins and, in particular, cytochrome C, we have synthesized a protected tetrapeptide (VII) corresponding to the sequence 24–27 of the protein chain of cytochrome C of animal origin. The fragment contains the amino acids histidine and lysine, which are capable of forming coordination bonds with the iron of heme [2]. The synthesis of the tetrapeptide (VII) was effected by the following route:



The methyl ester of N^{ε}-tosyl-L-lysine (I) [1] was obtained by the esterification of N^{ε}-tosyl-L-lysine with methanol in the presence of thionyl chloride. Then compound I was condensed with benzyloxycarbonyl-N^{im}-benzyl-L-histidine [4] in dimethylformamide, giving the dipeptide II. Dicyclohexylcarbodiimide was used as the condensing agent. The use of sec-butyl chlorocarbonate in the synthesis of the dipeptide II led to the formation of by-products difficult to separate and lowered the yield of end-product. The treatment of compound II with a 36% solution of hydrogen bromide in glacial acetic acid gave the hydrobromide of the dipeptide III. The hydrazide of benzyloxycarbonylglycyl-N^{ε}-tosyl-L-lysine (V) was used as the second component in the synthesis.

The condensation of benzyloxycarbonylglycine [5] with the methyl ester of N^{ε}-tosyl-L-lysine by the mixed anhydride method gave the dipeptide IV [3]. Although compound IV was isolated in the chromatographically pure state, it was impossible to obtain it in the crystalline form. Then, without further purification, the dipeptide IV was converted into the hydrazide (V) by the action of hydrazine hydrate. The subsequent stages comprised the preparation of the azide (VI) and its condensation with the dipeptide III. This gave the tetrapeptide VII. Its purity was shown by the results of chromatography in a thin layer of silica in various systems, and its structure was confirmed by acid hydrolysis in 6 N hydrochloric acid. Paper chromatography showed the presence in the hydrolysate of all the amino acids present in the original VII.

EXPERIMENTAL

The compounds were chromatographed in a thin layer of silica in the systems 1) chloroform–acetone–methanol (8 : 1 : 1) and 2) ethyl acetate–methanol (19 : 1).

Hydrochloride of the methyl ester of N^{ε}-tosyl-L-lysine (I). The compound was obtained by a published method [3]. Yield 90.8%, mp 136–137° C [from ether–methanol (10 : 1)], $[\alpha]_D^{18}$ +13.6° (c 4.7; DMFA), R_f 0.60 (system 1).

Hydrazide of benzyloxycarbonylglycyl-N^{ε}-tosyl-L-lysine (V). A solution of 0.30 g of benzyloxycarbonylglycine in 15 ml of chloroform was treated at −15° C, with stirring, with 0.4 ml of triethylamine and 0.4 ml of sec-butyl chlorocarbonate. After 10 min, a solution of 0.50 g of the hydrochloride of the methyl ester of N^{ε}-tosyl-L-lysine and 0.2 ml of triethylamine in 5 ml of chloroform was added, and the mixture was stirred for 2 hr and left for 20 hr. Then it was washed successively with water, 3% sodium bicarbonate solution, water, 5% HCl solution, and water again, and was dried with magnesium sulfate. The chloroform was driven off in vacuum, the residue was dissolved in 3 ml of ethanol, and the solution was evaporated in vacuum. This operation was repeated three times. Then the residue was

again dissolved in 2 ml of ethanol, 0.25 ml of hydrazine hydrate was added to the solution, and it was left for 24 hr. The reaction mixture was evaporated to a volume of 0.5 ml and the precipitate was separated off. Yield 0.63 g (87.3%), mp 92–93° C (from methanol), $[\alpha]_D^{22}$ −4.2° (c 1.6; DMFA), R_f 0.54 (system 2).

Found, %: C 54.81; H 6.32; N 13.88; S 6.33. Calculated for $C_{23}H_{31}O_6N_5S$, %: C 54.64; H 6.18; N 13.86; S 6.34.

Methyl ester of benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-N^{ε}-tosyl-L-lysine (II). At 0° C, 0.47 g of the hydrochloride of the methyl ester of N^{ε}-tosyl-L-lysine, 0.185 ml of triethylamine in 5 ml of dimethylformamide, and then 0.28 g of dicyclohexylcarbodiimide, were added to a solution of 0.51 g of benzyloxycarbonyl-N^{im}-benzyl-L-histidine in 35 ml of dimethylformamide, and the mixture was stirred at 0° C for 0.5 hr and was left for 18 hr. The reaction mixture was evaporated to a volume of 8 ml and the dicyclohexylurea was separated off; the mother liquor was diluted with chloroform to a volume of 30 ml and was washed successively with water, 10% sodium bicarbonate solution, water, 5% HCl, and water again, and was dried with magnesium sulfate. The solvent was driven off in vacuum and the residue was crystallized from ether with cooling. It was recrystallized twice from ethyl acetate to eliminate traces of dicyclohexylurea and was chromatographed on a column of silica. The fraction eluted by a mixture of ethyl acetate and methanol (9 : 1) was evaporated in vacuum. Yield 0.56 g (61.4%), mp 138.5–139.5° C (from ethyl acetate), $[\alpha]_D^{22}$ −5.1° (c 1.2; DMFA), R_f 0.63 (system 2).

Found, %: C 62.31; H 6.27; N 10.10; S 4.55. Calculated for $C_{35}H_{41}O_7N_5S$, %: C 62.20; H 6.12; N 10.36; S 4.74.

Methyl ester of benzyloxycarbonylglycine-N^{ε}-tosyl-L-lysine-N^{im}-benzyl-L-histidyl-N^{ε}-tosyl-L-lysine (VII). To 0.29 g of the dipeptide II was added 0.43 ml of a 36% solution of hydrogen bromide in glacial acetic acid. After 20 min, 50 ml of ether was added and the precipitate was separated off and washed with ether. The weight of the hydrobromide of the dipeptide III was 0.26 g (98.1%). The hydrazide of IV (0.35 g) was dissolved in a mixture of 2 ml of water, 0.8 ml of 6 N HCl, and 0.8 ml of acetic acid, and the solution was cooled to −2° C and treated with 0.08 g of sodium nitrite. After 2 min, the mixture was extracted with 15 ml of chloroform and the azide formed was washed successively with 3% sodium bicarbonate solution and water and was dried with magnesium sulfate at 0° C. The solution of the azide was cooled to −6° C and, with stirring, a cooled solution of 0.26 g of the hydrobromide of the dipeptide III and 0.08 ml of triethylamine in 5 ml of chloroform was added. Then the mixture was stirred at −5° C for 3 hr and was left for 20 hr. The solution was washed with water, 5% HCl, water, and 3% sodium bicarbonate, and was dried with magnesium sulfate. The solvent was driven off in vacuum and the residue was crystallized from ether. Yield 0.19 g (39.2%). After recrystallization from aqueous ethanol and then from ethanol, mp 153–155° C, $[\alpha]_D^{22}$ −16.5° (c 1; DMFA), R_f 0.60 (system 2).

Found, %: C 59.42; H 6.36; N 10.95; S 6.76. Calculated for $C_{50}H_{62}N_8O_{11}S_2$, %: C 59.19; H 6.16; N 11.04; S 6.32.

CONCLUSIONS

The synthesis of the methyl ester of benzyloxycarbonylglycyl-N^{ε}-tosyl-L-lysyl-N^{im}-benzyl-L-histidyl-N^{ε}-tosyl-L-lysine has been effected.

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