PENTACHLOROPHENYL AND 2,4,5-TRICHLOROPHENYL ESTERS OF gly-lys(N^{ε}-tos)-lys(N^{ε}-tos) AND THEIR POLYMERIZATION

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As was mentioned in [1, 2], polypeptides of regular structure, containing lysine residues, are of interest for modeling histones. Lysine-containing polypeptides were obtained previously by the polycondensation of the p-nitrophenyl and 2,4,6-trichlorophenyl esters of peptides with the amino acid sequences: gly-lys-lys-, gly-gly-lys, gly-gly-lys-lys-, and gly-pro-lys- [2, 3], and containing the N^{ε}-tosyl group in the ε-position of lysine. In a search for other activated esters in order to synthesize lysine-containing polypeptides we settled on the pentachlorophenyl and 2,4,5-trichlorophenyl esters as having the most promise [4-9]. The high activity of these esters can be seen from the half-life periods of the reaction for the ammonolysis of the pentachlorophenyl, 2,4,5-trichlorophenyl and p-nitrophenyl esters of carbobenzoxy-L-phenylalanine with benzylamine in dioxane, which are respectively 1.34, 4.9, and 23.2 min [10]. In the present paper is reported the preparation of the pentachlorophenyl and 2,4,5-trichlorophenyl esters of a tripeptide, which have the amino acid sequence gly-lys-lys, and their polymerization. We used the carbodiimide method in the synthesis of these peptides, since in our case it is characterized by convenience of use and in a number of cases is accompanied by slight racemization [6]. The ε-amino group of lysine was protected by tosyl protection (tos), while the α-amino group of lysine and glycine was protected by carbobenzoxy protection. The use of tosyl protection is not optimum in the case of preparing the free polypeptides, since its subsequent removal is carried out under fairly drastic conditions, for example, with sodium in liquid ammonia. Here a very undesirable partial destruction of the polypeptide chains occurs. However, when conducting a study of the polymerization possibilities of various activated esters of peptides, or of activating agents, it is sufficiently satisfactory as protection. The combining of these two methods of protection is convenient only in the sense that the tosyl grouping is stable under the conditions employed to remove the carbobenzoxy protection. The reaction of carbobenzoxy-(N^{ε}-tosyl)lysine with either pentachlorophenol or 2,4,5-trichlorophenol and dicyclohexylcarbodiimide (DCDI) gives either the pentachlorophenyl or 2,4,5-trichlorophenyl ester of carbobenzoxy-(N^{ε}-tosyl)lysine, from which the carbobenzoxy protection was removed by hydrobrominolysis (37% HBr/CH_3COOH). The obtained N^{ε}-tosyllysine ester hydrobromide was reacted with carbobenzoxy-(N^{ε}-tosyl)lysine in the presence of dicyclohexylcarbodiimide. The carbobenzoxy group was also removed by hydrobrominolysis from the obtained activated N-acyldipeptide ester. The dipeptide ester hydrobromide was reacted with carbobenzoxyglycine in the presence of DCDI. Then the carbobenzoxy group was also removed from the activated tripeptide esters by hydrobrominolysis. The hydrobromides of the pentachlorophenyl and 2,4,5-trichlorophenyl esters of gly-lys-(N^{ε}-tos)-lys-(N^{ε}-tos) obtained in this manner were polymerized in dimethyl sulfoxide (DMSO) in the presence of the calculated amount of triethylamine at 20°C for 5 days. In preparing the polytripeptides using the 2,4,5-trichlorophenyl esters we found that, depending on the properties of the starting monomers, polypeptides with a variable solubility in methanol are obtained. We associate this circumstance with the variable degree of polymerization of the polytripeptides. The average molecular weight of the polypeptides was determined by the van Slyke method. It was equal to 21,000 for the polypeptide obtained from the pentachlorophenyl ester of the tripeptide, and 7000 for the polypeptide obtained from the 2,4,5-trichlorophenyl

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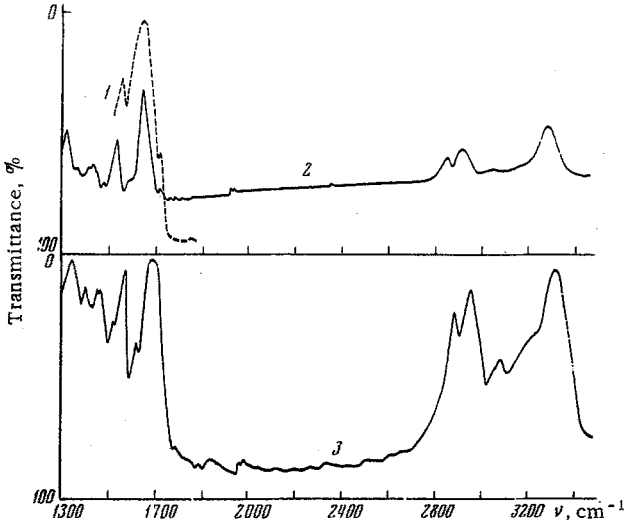

Fig. 1. Infrared spectra of polypeptides (gly-lys-(N^{ε}-tos)-lys-(N^{ε}-tos)$_n$, obtained using the 2,4,5-trichlorophenyl and pentachlorophenyl esters: 1) polytripeptide, soluble in CH_3OH, with a mol. wt. of 5500 and 7000 (Van Slyke); 2) polytripeptide, insoluble in CH_3OH, with a mol. wt. of approximately 21,000 (based on the IR spectrum); 3) polytripeptide, insoluble in CH_3OH and obtained using the pentachlorophenyl esters, with a mol. wt. of 27,000 and 21,000 (Van Slyke).

ester of the peptide, which was soluble in methanol. The molecular weight of the methanol-insoluble polymer sample was not determined by the Van Slyke method due to the poor solubility. We made an attempt to compare the molecular weights obtained by the Van Slyke method with the molecular weights that were calculated from the IR spectra [11]. We calculated the degree of polymerization and the molecular weights of the polymers by comparing the intensities of the absorption bands in the 1650 cm^{-1} region (band of amide-I) and in the 1740 cm^{-1} region (band of ester grouping) (Fig. 1). The molecular weights were equal to 27,000 for the polypeptide obtained via the pentachlorophenyl ester, 5500 for the methanol-soluble polypeptide, and 21,000 for the methanol-insoluble sample, which were obtained via the 2,4,5-trichlorophenyl ester.

EXPERIMENTAL METHOD

Lysine L-Form. The TLC was run on plates covered with a bound layer [250 mesh, silica gel–gypsum–water (3 : 0, 35 : 30)], in the systems: n-butanol–3% NH_4OH (100 : 44) (system A), n-butanol–water $-CH_3COOH$ (4 : 1 : 1) (system B), and ethanol–benzene (1 : 5) (system C). The developer was ninhydrin and iodine. The IR spectra were taken on a UR-10 spectrophotometer. The samples were prepared as KBr pellets. The concentration was 1.5/220 mg of KBr.

Pentachlorophenyl Ester of Carbobenzoxy-(N^{ε}-tosyl)lysine (I). A solution of 1.3 g of carbobenzoxy-(N^{ε}-tosyl)lysine [12] in 10 ml of absolute ethyl acetate was added to a solution of 0.6 g of DCDI in ethyl acetate. The mixture was stirred at 0-5° for 15 min, after which 0.84 g of pentachlorophenol was added. The stirring was continued at 0-5° for 3 h and then the mixture was allowed to stand overnight at room temperature. The excess DCDI was decomposed with glacial acetic acid. The obtained precipitate was filtered, while the filtrate was concentrated in vacuo. The residue was redissolved in ethyl acetate, and the insoluble portion was filtered again, and this procedure was repeated several times. The ethyl acetate solution was washed in succession with H_2O, HCl and 5% $NaHCO_3$ solution, after which it was dried over Na_2SO_4 and the solvent was removed in vacuo. A white solid residue was obtained, which was recrystallized from methanol. Ethyl acetate was used for the recrystallization and the yield was 92%; mp 151°; R_f 0.96 (system B) and 0.9 (system A). Found: N 4.00%. $C_{26}H_{23}O_6N_2Cl_5S$. Calculated: N 4.10%.

Hydrobromide of Pentachlorophenyl Ester of N^{ε}-tosyl-lysine (II). To a solution of 2 g of (I) in 2.6 ml of glacial acetic acid was added 2.6 ml of 37% HBr/CH_3COOH and the mixture was stirred vigorously

for 30 min. Then ether was added to precipitate the white crystalline (II); yield 69%; mp 132°; R_f 0.71 (system B).

Pentachlorophenyl Ester of Carbobenzoxy-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (III). To a solution of 1.6 g of carbobenzoxy-(N^{ε}-tosyl)lysine in 10 ml of ethyl acetate at −5° was added a solution of 0.76 g of DCDI in ethyl acetate and, after 15 min, with stirring, to the reaction mixture was added a mixture of 2.3 g of (II) and the calculated amount of $(C_2H_5)_3N$.

The mixture was stirred for 3 h at 0-5° and then allowed to stand overnight at 20°. The excess DCDI was destroyed with 0.5 ml of glacial acetic acid. The obtained precipitate of dicyclohexylurea was filtered. The ethyl acetate solution was washed in succession with water, 1 N HCl solution and 5% $NaHCO_3$ solution, after which it was dried over Na_2SO_4 and vacuum-distilled. The (III) obtained in this manner was recrystallized from methanol; yield 91%; mp 70°; R_f 0.69 (system A). $[\alpha]_D^{25}$ − 1.75 (C 1.5; CH_3OH). Found: N 6.25%. $C_{40}H_{43}O_{10}N_4S_2Cl_5$. Calculated: N 5.81%.

2,4,5-Trichlorophenyl Ester of Carbobenzoxy-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (IV). Obtained in the same manner as compound (III) from 2.7 g of carbobenzoxy-(N^{ε}-tosyl)lysine, 1.3 g of DCDI, 3.5 g of the hydrobromide of the 2,4,5-trichlorophenyl ester of N^{ε}-tosyllysine and 0.9 ml of $N(C_2H_5)_3$. The obtained (IV) was recrystallized from ethanol; yield 68%; mp 85°; R_f 0.86 (system A) and 0.86 (system C). $[\alpha]_D^{25}$ − 10.5 (C 1.5; CH_3OH). Found: N 6.6%. $C_{40}H_{45}O_{10}N_4S_2Cl_3$. Calculated: N 6.25%.

Hydrobromide of Pentachlorophenyl Ester of (N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (V). To a solution of 3.2 g of (III) in 2 ml of glacial acetic acid was added 3 ml of 37% HBr/glacial acetic acid. The reaction mixture was allowed to stand at 20° for 25 min, after which ether was added to give a pale yellow flocculent precipitate of (V), which was washed well with ether to remove any residual HBr and acetic acid, and then dried in a vacuum-desiccator over NaOH. The yield of (V) was 83%; R_f 0.83 (system B).

Hydrobromide of 2,4,5-Trichlorophenyl Ester of (N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (VI). Obtained in the same manner as (V), starting with 3.9 g of (IV), dissolved in 3 ml of glacial acetic acid, and 3.7 ml of 37% HBr/glacial acetic acid. The reaction mixture was allowed to stand at 20° for 25 min, after which ether was added to precipitate (VI), which was washed well with ether and then dried in a vacuum-desiccator over NaOH. The yield of (VI) was 68%, mp 96-98°; R_f 0.53 (system C).

Pentachlorophenyl Ester of Carbobenzoxy-gly-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (VII). Obtained in the same manner as (IV) from 0.573 g of carbobenzoxyglycine, 0.57 g of DCDI, hydrobromide (V) and 0.4 ml of $(C_2H_5)_3N$. The reaction product was recrystallized first from an ethyl acetate–petroleum ether mixture, and then from methanol. The yield of (VII) was 71%; mp 63-64°; R_f 0.73 (system C), 0.96 (system B). $[\alpha]_D^{25}$ − 10.5 (C 1.5; CH_3OH). Found: N 7.07%. $C_{42}H_{45}O_{11}N_5S_2Cl_5$. Calculated: N 6.86%.

2,4,5-Trichlorophenyl Ester of Carbobenzoxy-gly-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (VIII). Obtained in the same manner as (III) from 0.81 g of carbobenzoxyglycine, 0.81 g of DCDI, 3.1 g of (VI) and 0.5 ml of $N(C_2H_5)_3$. The reaction product was recrystallized from ethanol. The yield of (VIII) was 60%; mp 54-56°; R_f 0.73 (system C), 0.95 (system B). $[\alpha]_D^{25}$ − 17.5 (C 1.5; CH_3OH). Found: N 7.30%. $C_{42}H_{47}O_{11}N_5S_2Cl_3$. Calculated: N 7.34%.

Hydrobromide of Pentachlorophenyl Ester of gly-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (IX). To a solution of 1 g of (VII) in 1 ml of glacial acetic acid was added 0.4 ml of 37% HBr/glacial acetic acid and the reaction mixture was allowed to stand at 20° for 25 min. The obtained (IX) was precipitated with ether. The yield of (IX) was 68%; mp 140-143°; R_f 0.7 (system B). $[\alpha]_D^{25}$ − 8.76 (C 1.5; CH_3OH). Found: N 7.63%. $C_{34}H_{39}O_9N_5S_2Cl_5Br$. Calculated: N 7.23%.

Hydrobromide of 2,4,5-Trichlorophenyl Ester of gly-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine (X). To a solution of 1 g of (VIII) in 0.7 ml of glacial acetic acid was added 0.41 ml of 37% HBr/glacial acetic acid. The obtained solution was kept, with constant stirring, at 20° for 25 min. Precipitation with ether gave the crystalline (X), which was washed with ether. The yield of (X) was 63%; mp 116-118°; R_f 0.7 (system B). $[\alpha]_D^{25}$ − 7 (C 1.5; CH_3OH). Found: N 7.92%. $C_{34}H_{41}O_9N_5Cl_3BrS_2$. Calculated: N 7.8%.

Polymerization of the Hydrobromides of the Pentachlorophenyl and 2,4,5-Trichlorophenyl Esters of gly-(N^{ε}-tosyl)-lys-(N^{ε}-tosyl)lysine. For the polymerization we took 0.5 g of the appropriate hydrobromide (IX) or (X), using a 60% concentration in DMSO solution, at 20°. At the end of five days methanol was added to the ampule and the polypeptides were precipitated as white amorphous precipitates. When preparing the polytripeptides using the 2,4,5-trichlorophenyl esters it was found that the molecular weight

depends on the purity of the starting monomer. The molecular weight of the polypeptide, obtained by the polymerization of the pentachlorophenyl ester, was 21,000, while the molecular weight of the methanol-soluble polymer obtained from the trichlorophenyl ester was 7000 (Van Slyke method); the molecular weights, calculated from the IR spectra, were respectively 27,000 and 5500; for the methanol-insoluble polypeptide the mol. wt. was 21,000.

CONCLUSIONS

1. The hydrobromides of the pentachlorophenyl and 2,4,5-trichlorophenyl esters were synthesized by the carbodiimide method.

2. The polymerization of the indicated peptides gave polypeptides of regular structure with the amino acid sequence: $[gly\text{-}lys(N^{\varepsilon}\text{-}tos)\text{-}lys(N^{\varepsilon}\text{-}tos)]_n$, with a molecular weight of 21,000 in the case of the polymerization of the tripeptide pentachlorophenyl ester, and 7000 in the case of the polymerization of the tripeptide 2,4,5-trichlorophenyl ester (Van Slyke method). Calculation of the molecular weights from the IR spectra gave values of respectively 27,000 and 5500.

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