PEPTIDES OF SARCOLYSIN WITH γ-AMINOBUTYRIC ACID

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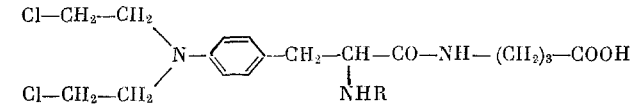
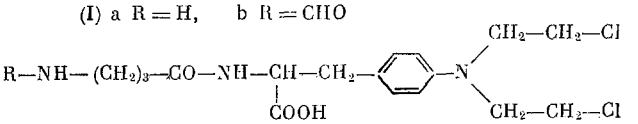
The present communication follows earlier published work [1, 2] on the synthesis of sarcolysin (p-[di-(2-chloroethyl)amino]-DL-phenylalanine) peptides containing nonprotein amino acids. The present paper describes the synthesis of sarcolysin dipeptides with γ-aminobutyric acid. An increased selectivity and an altered spectrum of antitumorous activity could be expected, as a result of the incorporation of nonprotein amino acids, in particular of β- and γ-amino acids, since cancerous cells differ from normal cells by their increased metabolism. Sarcolysin peptides containing γ-aminobutyric acid are also of interest in connection with the important role of γ-aminobutyric acid in biochemical processes.

Dipeptides were synthesized to study the effect of structure on biological properties, containing sarcolysin as the N-terminal (I) and the C-terminal (II) amino acid, and containing in each case free (Ia), (IIa) and formylated (Ib), (IIb) aminogroups, and a free carboxyl group



(I) a R = H, b R = CHO



(II) a R = H, b R = CHO

The synthesis of the peptides containing N-terminal sarcolysin (I) was achieved by the 8-hydroxyquinoline ester method [3]. Condensation of N-formylsarcolysin with 8-hydroxyquinoline in the presence of N,N'-dicyclohexylcarbodiimide (DCHC) gave the N-formylsarcolysin 8-hydroxyquinoline ester, the aminolysis of which with the γ-aminobutyric acid benzyl ester led to the benzyl ester of N-formylsarcolysyl-γ-aminobutyric acid. Hydrogenolysis of the latter in the presence of palladium black gave the N-formyldipeptide (Ib). The peptide (Ia) was synthesized by the removal of the protecting formyl group with acetyl chloride in benzyl alcohol [4], followed by catalytic hydrogenolysis of the benzyl ester.

Peptides containing the N-terminal γ-aminobutyric acid (II) were synthesized in the same way, however in this case the peptide bond was obtained by the p-nitrophenyl ester method [5]. The results of the biological tests of the substances obtained will be communicated separately.

EXPERIMENTAL

N-Formyl-γ-aminobutyric Acid. Acetic anhydride (85 ml) was added dropwise with stirring to a solution of 10.3 g γ-aminobutyric acid in 250 ml 98% formic acid, maintaining the temperature at 50-55°C. The stirring was continued for 30 min at the same temperature and for two hours at room temperature. Ice water (80 ml) was then added and the reaction mixture evaporated under vacuum. The remaining oil was recrystallized from acetonitrile. Yield 6.15 g (47%) N-formyl-γ-aminobutyric acid, mp 105-106°. Found %: C 45.57; H 7.04; $C_5H_9NO_3$. Calculated %: C 45.81; H 6.92.

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γ-Aminobutyric Acid Benzyl Ester, p-Toluenesulfonate. Obtained by the method described by Zervas et al. [6], mp 107-108° (from ethyl acetate), yield 97%. Found %: C 59.03, H 6.51; $C_{18}H_{23}NO_5S$. Calculated %: C 59.17; H 6.34.

8-Hydroxyquinoline Ester of N-Formylsarcolysin. A solution of 2.10 g DCHC in tetrahydrofuran was added with stirring to a mixture of 3.33 g N-formylsarcolysin [7] and 1.60 g 8-hydroxyquinoline in tetrahydrofuran, cooled to 0°. Cooling and stirring was continued for 6 h. A few drops of acetic acid were then added and after one hour the N,N'-dicyclohexylurea filtered off. The filtrate was evaporated under vacuum and the remaining oil dissolved in chloroform, washed with water, 0.2 N H_2SO_4, 1 N $KHCO_3$, and again with water. The solution was then dried with sodium sulfate, the chloroform removed under vacuum, and the residue recrystallized from ethyl acetate and ether. Yield 3.0 g (65%) N-formylsarcolysin 8-hydroxyquinoline ester, mp 132-134°. Found %: C 59.78, H 4.61; Cl 15.21%; $C_{23}H_{23}Cl_2N_3O_3$. Calculated %: C 59.99; H 5.04; Cl 15.40. When the above reaction was carried out in ethyl acetate, only a small quantity of unreacted N-formylsarcolysin was isolated.

p-Nitrophenyl Ester of N-Formyl-γ-aminobutyric Acid. This compound was obtained in the same way by condensation of N-formyl-γ-aminobutyric acid with p-nitrophenol in the presence of DCHC in a mixture of dimethylformamide and tetrahydrofuran (THF), mp 59-60°(after reprecipitation with petroleum ether from benzene–ethyl acetate, 1 : 1), yield 74%. Found % : C 52.69; H 5.07; $C_{11}H_{12}N_2O_5$. Calculated %: C 52.39; H 4.80.

Benzyl Ester of N-Formylsarcolysyl-γ-aminobutyric Acid. A solution of 4.6 g 8-hydroxyquinoline ester in THF was kept for 24 h at room temperature. THF was then removed under vacuum, the remaining oil was dissolved in chloroform, washed with 0.5 N H_2SO_4, water, 1 N $KHCO_3$, and again with water, and dried with Na_2SO_4. Chloroform was then evaporated under vacuum and the residue recrystallized from ethanol. Yield 2.95 g (58%) benzyl ester of N-formylsarcolysyl-γ-aminobutyric acid, mp 113-114°. Found %: C 58.96; H 5.97; Cl 13.80; $C_{25}H_{31}C_{12}N_3O_4$. Calculated %: C 59.05; H 6.15, Cl 13.94.

Benzyl Ester of N-Formyl-γ-Aminobutyrylsarcolysin. Obtained in the same way by aminolysis of the p-nitrophenyl ester of N-formyl-γ-aminobutyric acid with sarcolysin benzyl ester [8], mp 99-100°, yield 85%. Found %: C 59.10, H 6.38, Cl 13.40; $C_{25}H_{31}C_{12}N_3O_4$. Calculated %: C 59.05; H 6.15; Cl 13.94.

N-Formylsarcolysyl-γ-aminobutyric Acid. A suspension of 1.27 g benzyl ester of N-formylsarcolysyl-γ-aminobutyric acid in 40 ml methanol was subjected to catalytic hydrogenolysis over palladium black until cessation of hydrogen absorption. The catalyst was filtered off, the filtrate evaporated under vacuum, and the residue recrystallyzed from acetonitrile. Yield 1.0 g (96%) N-formylsarcolysyl-γ-aminobutyric acid, mp 131-132°. Found %: C 51.72; H 6.15; Cl 17.21; $C_{18}H_{25}C_{12}N_3O_4$. Calculated %: C 51.68; H 6.02; Cl 16.95.

N-Formyl-γ-aminobutyrylsarcolysin. Obtained in the same way by hydrogenolysis of the benzyl ester of N-formyl-γ-aminobutyrylsarcolysin, mp 134-135° (from acetone), yield 89%. Found %: C 51.74, H 6.05, Cl 16.64; $C_{18}H_{25}C_{12}N_3O_4$. Calculated %: C 51.68; H 6.02; Cl 16.95.

Sarcolysyl-γ-aminobutyric Acid. A solution of 1.05 ml acetyl chloride in 10 ml benzyl alcohol was added to a solution of 3.05 g benzyl ester of N-formylsarcolysyl-γ-aminobutyric acid in 10 ml dry benzyl alcohol, and the mixture allowed to stand for 24 h at room temperature. The hydrochloride of the benzyl ester of sarcolysyl-γ-aminobutyric acid was then precipitated with anhydrous ether. The amorphous residue was then washed several times with ether, dissolved in ethanol, and evaporated to dryness. The remaining oil was suspended in ethyl acetate and 1.25 ml $(C_2H_5)_3N$ added. The precipitated $(C_2H_5)_3N{\cdot}HCl$ was filtered off and the filtrate evaporated under vacuum. The residue was dissolved in methanol and subjected to catalytic hydrogenolysis. Yield 2.13 g (91%) sarcolysyl-γ-aminobutyric acid, mp 123-124° (from ethanol – ethyl acetate 1 : 2). Found %: C 52.36; H 6.59; Cl 17.62; $C_{17}H_{25}C_{12}N_3O_3$. Calculated %: C 52.31; H 6.46; Cl 18.17% .

Benzyl Ester of γ-Aminobutyrylsarcolysin. Obtained in the same way by the reaction of the benzyl ester of N-formyl-γ-aminobutyrylsarcolysin with acetyl chloride in benzyl alcohol, mp 98-99° (from ethyl acetate – petroleum ether), yield 93%. Found %: C 59.70; H 6.36; Cl 14.61; $C_{24}H_{31}C_{12}N_3O_6$. Calculated %: C 60.00; H 6.51, Cl 14.76.

γ-Aminobutyrylsarcolysin. Obtained by hydrogenolysis of the benzyl ester of γ-aminobutyrylsarcolysin, mp 129-131° (from acetone), yield 90%. Found %: C 52.34; H 6.26; Cl 18.33; $C_{17}H_{25}C_{12}N_3O_3$. Calculated %: C 52.31; H 6.46; Cl 18.17.

CONCLUSIONS

Peptides of sarcolysin with γ-aminobutyric acid have been synthesized, containing N-terminal and C-terminal sarcolysin, as well as a free and a formylated amino group.

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