CANCEROLYTIC PEPTIDES

COMMUNICATION 8. PEPTIDES OF SARCOLYSINE WITH DL-3-AMINOBUTYRIC ACID

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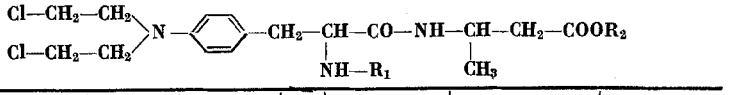
It is known that the presence of a free amino group in the N-end amino acid in sarcolysine peptides leads to the complete loss of the selectivity and specificity of antitumor action, probably as a result of the hydrolysis of the peptide to free sarcolysine [1]. There is no information on the antitumor activity of peptides of sarcolysine with β- and γ-amino acids. It may be supposed that the presence of "unusual" peptide links will lead to increase in the stability of the compounds to hydrolysis, as a result of which their biological properties should be altered considerably. In view of this it was of interest to synthesize sarcolysine peptides in which the free amino group of the N-end amino acid is not only in the α-, but also in the β-, γ-, and other positions relative to the peptide link.

In this paper we describe the synthesis of dipeptides based on sarcolysine and DL-3-aminobutyric acid in which the sarcolysine is either the N-end or the C-end amino acid, while in each case a free or formylated amino group and a free carboxy group are present. Also, in the case of N-end sarcolysine we prepared the dipeptide ethyl ester with a free amino group. All the peptides were synthesized by the p-nitrophenyl ester method [2]. As temporary protective groups we used benzyloxycarbonyl for the amino group and the benzyl ester for the carboxy group, and in all cases these were removed by catalytic hydrogenolysis. By the condensation of N-(benzyloxycarbonyl) [3] and N-formyl [4] derivatives of sarcolysine with p-nitrophenol in presence of dicyclohexylcarbodiimide (DCC) we synthesized the p-nitrophenyl esters of the corresponding N-acyl derivatives of sarcolysine. The aminolysis of the latter with benzyl or ethyl DL-3-aminobutyrate gave peptides with N-end sarcolysine (Table 1).

Peptides in which sarcolysine is the C-end amino acid were prepared by the condensation of the previously unknown p-nitrophenyl N-(benzyloxycarbonyl)- and N-formyl-DL-3-aminobutyrates. p-Nitrophenyl DL-3-[(benzyloxycarbonyl)amino]butyrate was prepared by the condensation of DL-3-[(benzyloxycarbonyl)-amino]butyric acid with p-nitrophenol in presence of DCC. Previously undescribed DL-3-formamidobutyric acid required for the preparation of the corresponding p-nitrophenyl ester could not be prepared by the formylation of DL-3-aminobutyric acid by the method described in [5]. However, this compound was prepared by the formylation of benzyl DL-3-aminobutyrate with 98% formic acid by a recently described method [6] with subsequent removal of the benzyl group by catalytic hydrogenolysis. By the condensation of

TABLE 1



Cpd. No.	R_1	R_2	mp, °C	Yield, %	Found, %		Molecular formula	Calculated, %	
					N	Cl		N	Cl
I	$C_6H_5CH_2OCO$	$CH_2C_6H_5$	98—101	81	6,99	11,31	$C_{32}H_{37}Cl_2N_3O_5$	6,84	11,53
II	H	H	194—196 *	87	10,90	17,78	$C_{17}H_{25}Cl_2N_3O_3$	10,76	18,16
III	HCO	$CH_2C_6H_5$	135—137	73	8,36	13,50	$C_{25}H_{31}Cl_2N_3O_4$	8,26	13,94
IV	HCO	H	177—178 *	93	9,98	17,00	$C_{18}H_{25}Cl_2N_3O_4$	10,04	16,95
V	$C_6H_5CH_2OCO$	C_2H_5	90—93	70	7,76	13,20	$C_{27}H_{35}Cl_2N_3O_5$	7,61	12,83
VI	H (picrate)	C_2H_5	150—152	—	13,14	11,02	$C_{25}H_{32}Cl_2N_6O_{10}$	12,98	10,95

* Melts with decomposition.

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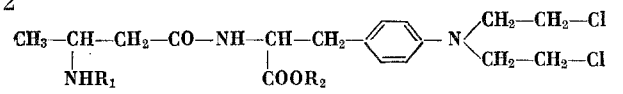
TABLE 2



Cpd. No.	R_1	R_2	mp, °C	Yield, %	Found, %		Molecular formula	Calculate, %	
					N	Cl		N	Cl
VII	$C_6H_5CH_2OCO$	$CH_2C_6H_5$	146—148	80	6,89	12,10	$C_{32}H_{37}Cl_2N_3O_5$	6,84	11,53
VIII	H	H	209—211 *	74	10,72	17,90	$C_{17}H_{25}Cl_2N_3O_3$	10,76	18,16
IX	HCO	$CH_2C_6H_5$	133—135	67	8,34	13,76	$C_{25}H_{31}Cl_2N_3O_4$	8,26	13,94
X	HCO	H	123—126 *	91	10,19	16,60	$C_{18}H_{25}Cl_2N_3O_4$	10,04	16,95

*Melts with decomposition.

DL-3-formamidobutyric acid with p-nitrophenol by the carbodiimide method we obtained p-nitrophenyl DL-3-formamidobutyrate. This ester was synthesized also by another method: by the removal of the benzyloxycarbonyl group from p-nitrophenyl DL-3-[(benzyloxycarbonyl)amino]butyrate with a solution of HBr in glacial acetic acid we obtained p-nitrophenyl DL-3-aminobutyrate hydrobromide. This compound, which contains an activated ester group, we succeeded in formylating by the method described in [6]. In both cases p-nitrophenyl DL-3-aminobutyrate was obtained in high yield. By the aminolysis of the p-nitrophenyl DL-3-(acylamino)butyrates with sarcolysine benzyl ester we obtained peptides with C-end sarcolysine (Table 2).

EXPERIMENTAL

DL-3-Aminobutyric acid was prepared by the addition of ammonia to crotonic acid by the method described in [7].

Benzyl DL-3-(p-Tosylamino)butyrate was prepared by the method described in [8]; mp 92-95°C (ethyl acetate). Found: C 59.30; H 6.30; N 3.62%. $C_{18}H_{23}NO_5S$. Calculated: C 59.17; H 6.34; N 3.83%.

N-(Benzyloxycarbonyl)sarcolysine p-Nitrophenyl Ester. A suspension of 4.39 g of N-(benzylozycarbonyl)sarcolysine [3] in ethyl acetate was cooled to 0°C, and 1.67 g of p-nitrophenol and 2.07 g of DCC were added. The mixture was stirred at 0°C for 30 min and then left overnight at room temperature. The precipitated 1,3-dicyclohexylurea was filtered off, the filtrate was vacuum-evaporated, and the residue was recrystallized from ethanol. We obtained 4.85 g (84.5%) of yellow crystals of N-(benzyloxycarbonyl)sarcolysine p-nitrophenyl ester, mp 118-119°C. Found: C 57.88; H 4.73; Cl 12.78%. $C_{27}H_{27}Cl_2N_3O_6$. Calculated: C 57.86; H 4.86; Cl 12.65%.

Benzyl DL-3-[[N-(Benzyloxycarbonyl)sarcolysyl]amino]butyrate (I). A solution of 3.71 g of N-(benzyloxycarbonyl)sarcolysine p-nitrophenyl ester and an equivalent amount (1.28 g) of benzyl DL-3-aminobutyrate in 12 ml of tetrahydrofuran (THF) containing 2-3 drops of glacial acetic acid was left for 24-30 h at room temperature, after which THF was vacuum-evaporated and the oily residue was dissolved in chloroform. The chloroform solution was washed successively with 0.2 N HCl, water, 5% $NaHCO_3$ solution (4-6 times), and again water, and it was dried over Na_2SO_4. Chloroform was vacuum-evaporated, and the residue was crystallized from ethanol and washed with ether. We obtained 3.30 g (81%) of (I), mp 98-101°C.

DL-3-(Sarcolysylamino)butyric Acid (II). (I) (3.08 g) was hydrogenated as a suspension in a mixture of 75 ml of methanol and 5.0 ml of glacial acetic acid in presence of 0.1 g of palladium black until no more hydrogen was absorbed. The clear solution was filtered from the catalyst and vacuum-evaporated. The oily residue was dissolved in absolute ethanol, and the reaction product was precipitated with ether. We obtained 1.70 g (87%) of (II), mp 194-196°C (dimethylformamide; blackens at 171°C).

N-Formylsarcolysine p-Nitrophenyl Ester. This was prepared by the condensation of N-formylsarcolysine [4] with p-nitrophenol by the method described above. In this case the product was precipitated together with 1,3-dicyclohexylurea and was separated by subsequent extraction with acetone, which was then removed in a vacuum. N-Formylsarcolysine p-nitrophenyl ester had mp 128-129°C (ethanol). Yield 66%. Found: C 52.63; H 4.86; Cl 15.72%. $C_{20}H_{21}Cl_2N_3O_5$. Calculated: C 52.87; H 4.66; Cl 15.60%.

Benzyl DL-3-[N-Formylsarcolysyl)amino]butyrate (III) was prepared by the aminolysis of N-formylsarcolysine p-nitrophenyl ester with benzyl DL-3-aminobutyrate in THF analogously to (I): mp 135-137°C; yield 73%.

DL-3-[(N-Formylsarcolysyl)amino]butyric Acid (IV) was prepared analogously to (II) by the catalytic hydrogenolysis of (III). After recrystallization from ethanol it had mp 177-178°C (decomposition); yield 93%.

Ethyl DL-3-[[N-(Benzyloxycarbonyl)sarcolysyl]amino]butyrate (V) was prepared analogously to (I) by the condensation of N-(benzyloxycarbonyl)sarcolysine with ethyl DL-3-aminobutyrate [9]; mp 90-93°C; yield 70%.

Ethyl DL-3-(Sarcolysylamino)butyrate (VI). (V) (2.76 g) was reduced in 75 ml of absolute ethanol over palladium back until no more hydrogen was absorbed. The solution was vacuum-concentrated, the residue was dissolved in dry ether, and the equivalent amount of HCl in dry ether was added. The amorphous hydrochloride of (VI) was precipitated. An analytical sample was isolated as the picrate, mp 150-152°C.

p-Nitrophenyl DL-3-[(Benzyloxycarbonyl)amino]butyrate was prepared analogously to N-(benzyloxycarbonyl)sarcolysine p-nitrophenyl ester by the condensation of DL-3-[(benzyloxycarbonyl)amino]butyric acid [10] with p-nitrophenol in presence of DCC; mp 92-93°C; yield 78%. Found: C 62.75; H 5.30; N 8.21%. $C_{18}H_{18}N_2O_6$. Calculated: C 63.16; H 5.30; N 8.18%.

N-(DL-3-[(Benzyloxycarbonyl)amino]butyryl]sarcolysine Benzyl Ester (VII) was prepared analogously to (I) by the aminolysis of p-nitrophenyl DL-3-[(benzyloxycarbonyl)amino]butyrate with sarcolysine benzyl ester [3]; mp 146-148°C; yield 80%.

N-(DL-3-Aminobutyryl)sarcolysine (VIII). (VII) (2.46 g) was reduced as a suspension in 60 ml of methanol containing a few drops of glacial acetic acid over palladium back until no more hydrogen was absorbed. The precipitated product was filtered off together with catalyst and was precipitated from glacial acetic acid with ether. We obtained 1.55 g (74%) of (VIII), mp 209-211°C (darkens at 181°C).

Benzyl DL-3-Formamidobutyrate. With cooling with ice and stirring 5.46 g of 98% formic acid was added gradually to a solution of 20.85 g of benzyl DL-3-aminobutyrate (see above) and 24.5 g of DCC in chloroform. Cooling and stirring were continued further for 1 h, and then the reaction mixture was left overnight in a refrigerator. We obtained 21.5 g (90%) of benzyl DL-3-formamidobutyrate, mp 59-60°C. After precipitation with hexane from acetone it had mp 60-60.5°C. Found: C 65.56; H 6.87; N 6.07%. $C_{12}H_{15}NO_3$. Calculated: C 65.15; H 6.83; N 6.33%.

DL-3-Formamidobutyric Acid. Benzyl DL-3-formamidobutyrate (16.6 g) was subjected to hydrogenolysis as a solution in methanol in presence of palladium black until no more hydrogen was absorbed. The solution was vacuum-evaporated, and the oily residue was crystallized from ethyl acetate. We obtained 8.9 g (90.5%) of DL-3-formamidobutyric acid, mp 67-69°C. Found: C 45.86; H 7.05; N 10.86%. $C_5H_9NO_3$. Calculated: C 45.81; H 6.92; N 10.68%.

p-Nitrophenyl DL-3-Aminobutyrate Hydrobromide. p-Nitrophenyl DL-3-[(benzyloxycarbonyl)amino]-butyrate was dissolved in a 3 N solution of HBr in glacial acetic acid, and the solution was left for 1 h at room temperature. The hydrobromide was precipitated with ether. After recrystallization from acetone it had mp 165-166°C; yield 89%. Found: C 39.20; H 4.29; N 9.15; Br 26.20%. $C_{10}H_{13}BrN_2O_4$. Calculated: C 39.36; H 4.29; N 9.18; Br 26.19%.

p-Nitrophenyl DL-3-Formamidobutyrate was prepared analogously to N-(benzyloxycarbonyl)sarcolysine p-nitrophenyl ester by the condensation of DL-3-formamidobutyric acid with p-nitrophenol in presence of DCC. The oily residue remaining after the removal of solvent crystallized rapidly when it was under ether. After precipitation with hexane from ethyl acetate it had mp 74-75°C. Yield 78%. Found: C 52.45; H 4.73; N 11.15%. $C_{11}H_{12}N_2O_5$. Calculated: C 52.39; H 4.80; N 11.11%.

With cooling with ice and stirring 0.76 g of 98% formic acid was added gradually to a mixture of 4.58 g of p-nitrophenyl DL-3-aminobutyrate hydrobromide, 2.07 ml of triethylamine, and 3.40 g of DCC. Stirring and cooling were continued further for 1 h, after which the reaction mixture was left overnight in a refrigerator. The precipitated 1,3-dicyclohexylurea was filtered off, and the filtrate was washed successively with 0.5 N HCl, water, 5% $NaHCO_3$ solution, and again water. It was dried over Na_2SO_4, and after the removal of solvent the product was isolated in the usual way. We obtained 3.20 g (85%) of the p-nitrophenyl ester, mp 74-75°C. A mixture with the product of the preceding experiment melted without depression.

N-(DL-3-Formamidobutyryl)sarcolysine Benzyl Ester (IX) was prepared analogously to (I) by the condensation of p-nitrophenyl DL-3-formamidobutyrate with sarcolysine benzyl ester; mp 133-135°C; yield 67%.

N-(DL-3-Formamidobutyryl)sarcolysine (X) was prepared analogously to (IV) by the catalytic hydrogenolysis of N-(DL-3-formamidobutyryl)sarcolysine benzyl ester; mp 123-126°C (decomposition); yield 91%.

CONCLUSIONS

1. The p-nitrophenyl ester of N-(benzyloxycarbonyl) and N-formyl derivatives of sarcolysine and DL-3-aminobutyric acid were synthesized.

2. The p-nitrophenyl esters obtained were used for the synthesis of dipeptides based on sarcolysine and DL-3-aminobutyric acid.

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