CANCEROLYTIC PEPTIDES

COMMUNICATION 9. PEPTIDES OF SARCOLYSINE WITH β-ALANINE AND WITH GLYCINE*

A.A.B. Paulyukonis, K.I. Karpavichus, and I.L. Knunyants

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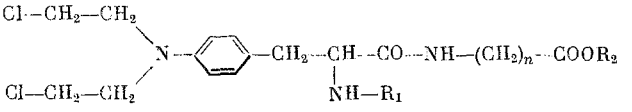
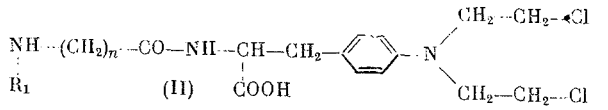
In previous communications we have published the synthesis of dipeptides of sarcolysine with DL-α-aminobutyric and DL-β-aminobutyric acids, which was undertaken in connection with the study of the biological activity of cytotoxic peptides into the structure of which nonprotein amino acids had been introduced [1, 2]. In the present work the synthesis of dipeptides of sarcolysine with β-alanine and glycine is presented. The biological activity of cytotoxic peptides containing β-alanine was also of interest due to β-alanine entering into the composition of certain biological oligopeptides (carnosine and anserine), vitamin B_3, coenzyme A, etc. Dipeptides, containing sarcolysine as N-terminal (I) and C-terminal (II) amino acids, were synthesized for the study of the effect of chemical structure on biological properties.



(I)

a $R_1 = H$, $R_2 = H$, $n = 1, 2$; b $R_1 = HCO$, $R_2 = H$, $n = 1, 2$; c $R_1 = H$, $R_2 = C_2H_5$, $n = 2$



a $R_1 = H$, $n = 1, 2$; b $R_1 = HCO$, $n = 1, 2$

In each of these cases the amino group was free (Ia) and (IIa) or formylated (Ib) and (IIb). The carboxyl group was free.

The analogous dipeptides with glycine in place of β-alanine were also synthesized in order to compare biological properties. In addition sarcolysyl-β-alanine ethyl ester (Ic) was prepared.

The synthesis of all the peptides mentioned was carried out using the carbodiimide method [3]. During the preparation of dipeptides (Ia) and (IIa) the following temporary protecting groups were used: for the amino group the o-nitrophenylsulfenyl group (NPS-) [4], and for carboxyl the benzhydryl ester (-OBzh) [5]. NPS-sarcolysine was obtained for the first time by the interaction of NPS-chlorine with sarcolysine under Schotten-Baumann reaction conditions. The NPS derivatives of β-alanine and glycine were obtained analogously. The synthesis of these has been published previously but without experimental details or elemental analytical data [6]. NPS-glycine is also known as the dicyclohexylamine salt [4]. Usually freshly prepared NPS derivatives of the amino acids were used for the condensations. The benzhydryl esters of sarcolysine and β-alanine were also prepared for the first time by the interaction of the toluene-p-sulfonates of the corresponding amino acids with diphenyldiazomethane in dimethylformamide solution [5].

When chloroform, methylene chloride, and acetonitrile were used as solvents for the condensation of NPS-sarcolysine with the ethyl and benzhydryl esters of β-alanine by the carbodiimide method, in every case there was obtained mainly side product, seemingly NPS-sarcolysyldicyclohexylurea, admixed with a little of the compound sought. Only by conducting these same reactions in tetrahydrofuran were the desired dipeptides obtained free from contamination by N-acylurea.

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TABLE 1

Number	Compound	Mp, °C	Yield, %	Solvent for crystallization	Found, %			Formula	Calculated, %		
					C	H	N		C	H	N
1	NPS-Sarc-β-Ala-OBzh	119—120	71	Ethanol	60,50	5,20	8,30	$C_{35}H_{36}Cl_2N_4O_5S$	60,43	5,22	8,05
2	NPS-β-Ala-Sarc-OBzh	88—91	65	Isopropanol	60,30	5,44	8,30	$C_{35}H_{36}Cl_2N_4O_5S$	60,43	5,22	8,05
3	NPS-Sarc-Gly-OBzh	136—138	82	"	60,08	5,17	8,50	$C_{34}H_{34}Cl_2N_4O_5S$	59,93	5,03	8,22
4	NPS-Gly-Sarc-OBzh	134—135	69	Ethanol	60,02	5,16	8,28	$C_{34}H_{34}Cl_2N_4O_5S$	59,93	5,03	8,22
5	Form-Sarc-β-Ala-OBzh	132—133	59 *	"	58,41	6,27	8,80	$C_{24}H_{29}Cl_{12}N_3O_4$	58,30	5,91	8,50
6	Form-β-Ala-Sarc-OBzh	76—80	70	$CHCl_3$ – petroleum ether	62,99	6,00	7,40	$C_{30}H_{33}Cl_2N_3O_4$	63,16	5,83	7,36
7	Form-Sarc-Gly-OBzh	122—124	72	Benzene – petroleum ether	62,86	5,83	7,45	$C_{29}H_{31}Cl_2N_3O_4$	62,62	5,62	7,55
8	Form-Gly-Sarc-OBzh	151—152	88	Benzene	62,75	5,54	7,34	$C_{29}H_{31}Cl_2N_3O_4$	62,62	5,62	7,55
9	NPS-Sarc-β-Ala-OEt	105—106	69	Isopropanol	51,93	5,17	10,00	$C_{24}H_{30}Cl_2N_4O_5S$	51,70	5,42	10,05

*The yield amounted to 71% when the reaction was conducted in acetonitrile.

TABLE 2

Number	Compound	Mp, °C	Yield,* %	Found, %				Formula	Calculated, %			
				C	H	Cl	N		C	H	Cl	N
1	H-Sarc-β-Ala-OH	159—160	64	51,06	6,13	18,50	11,26	$C_{16}H_{23}Cl_2N_3O_3$	51,07	6,16	18,84	11,17
2	H-β-Ala-Sarc-OH	>230	37	51,27	6,14	18,47	11,30	$C_{16}H_{23}Cl_2N_3O_3$	51,07	6,16	18,84	11,17
3	H-Sarc-Gly-OH	165—166	66	49,99	5,94	18,59	11,60	$C_{15}H_{21}Cl_2N_3O_3$	49,73	5,84	19,57	11,60
4	H-Gly-Sarc-OH	166—167	43	49,85	5,97	18,80	11,83	$C_{15}H_{21}Cl_2N_3O_3$	49,73	5,84	19,57	11,60
5	Form-Sarc-β-Ala-OH	165—166	96	50,70	6,01	17,37	10,60	$C_{17}H_{23}Cl_2N_3O_4$	50,50	5,73	17,54	10,39
6	Form-β-Ala-Sarc-OH†	144—146	94	50,80	5,71	17,60	10,40	$C_{17}H_{23}Cl_2N_3O_4$	50,50	5,73	17,54	10,39
7	Form-Sarc-Gly-OH	173—174	96	49,04	5,64	18,87	11,00	$C_{16}H_{21}Cl_2N_3O_4$	49,23	5,42	18,17	10,77
8	Form-Gly-Sarc-OH	139—141	95	49,34	5,50	17,62	10,80	$C_{16}H_{21}Cl_2N_3O_4$	49,23	5,42	18,17	10,77
9	H-Sarc-β-Ala-OEt (picrate)	174—175	--	45,34	4,91	11,81	13,13	$C_{24}H_{30}Cl_2N_6O_{10}$	45,51	4,77	11,20	13,27

*Yields of compounds 1-4 are shown after crystallization.

†The compound was also obtained by the action of 98% formic acid on Form-β-Ala-Sarc-OBzh, reaction time 3 h, yield 73%.

N-Formyl (Form) dipeptides (Ib) and (IIb) were obtained by condensing the formyl derivatives of sarcolysine, β-alanine, and glycine with benzhydryl esters (and a benzyl ester in one case) of the corresponding amino acids. This was usually carried out in tetrahydrofuran though on using acetonitrile the formation of side products was not observed. The properties of the NPS- and Form-dipeptide esters are presented in Table 1. The simultaneous removal of NPS- and OBzh protecting groups was effected for the first time by the action of 98% formic acid, the method proposed recently for the removal of N-tertiary butyloxycarbonyl [7]. Preliminary experiments with glycine benzhydryl ester showed that 3 h at room temperature was sufficient for practically complete removal of OBzh groups. The yield of glycine toluene-p-sulfonate on reacting H-Gly-OBzh toluene-p-sulfonate with formic acid, amounted to 60% after 0.5 h, 76% after 1 h, and 96% after 2 h. The NPS group, in spite of its high susceptibility towards the action of HCl in organic solvents [4], was cleaved more slowly than the OBzh group. During model experiments with NPS-Sarc-Gly-OBzh the maximal yield of H-Sarc-Gly-OH was reached with a reaction time of 10-12 h at room temperature. Yields in all cases were low (37-66% after crystallization). In addition, the reaction of NPS derivatives of amino acids with formic acid is sufficiently complex (see the analogous reaction with acetic acid [8]) and the formation of side products in the reaction,which were difficult to separate from the main product, is possible.

$$\mathrm{NPS{-}NH{-}\underset{\displaystyle R}{\underset{|}{CH}}{-}COOH \xrightarrow{CH_3COOH} NH_2{-}\underset{\displaystyle R}{\underset{|}{CH}}{-}COOH + [NPS{-}OH]}$$

$$[NPS{-}OH] \rightarrow (NPS)_2 + NPS{-}SO_2{-}C_6H_4{-}NO_2 + o\text{-}NO_2{-}C_6H_4{-}SO_2H + o\text{-}NO_2{-}C_6H_4{-}SO_3H$$

In the present case the dipeptides were easily crystallized from dimethylformamide and it was shown that one crystallization was sufficient to obtain an analytically pure compound. The OBzh group of the Form-dipeptides was removed by catalytic hydrogenation. A more impure product in lower yield was obtained by cleavage with formic acid for 3 h. The NPS group of the dipeptide NPS-Sarc-α-Ala-OEt was removed by the action of HCl in organic solvents. The properties of the dipeptides containing free or formylated amino groups and a free carboxyl group are presented in Table 2. The results of the biological testing of the compounds obtained will be published separately.

EXPERIMENTAL METHOD

NPS-Sarcolysine. To a solution of 30.5 g sarcolysine base in 50 ml 2 N NaOH and 125 ml dioxane was added simultaneously, with vigorous stirring and ice cooling, 19.0 g NPS-chloride and 50 ml 2 N NaOH in small portions over 15-20 min such that the pH of the reaction mixture was kept weakly alkaline at all times. The stirring was continued for a further 5-10 min after which 500 ml water was added to the reaction mixture which was then acidified with 1 N H_2SO_4. An amorphous mass of NPS-sarcolysine separated which crystallized on subsequent stirring for 1-2 h. Yellow crystals of NPS-sarcolysine (45.6 g) of mp 117-119° (with decomposition) were obtained, yield 99%. After recrystallization from 90 ml benzene 30.8 g product was obtained having mp 123-124°C (with decomposition), yield 67%. Found: C 49.70; H 4.48; N 9.36%. $C_{13}H_{21}Cl_2N_3O_4S$. Calculated: C 49.77; H 4.62; N 9.16%.

NPS-β-Alanine. β-Alanine (4.45 g) was dissolved in a mixture of 25 ml 2 N NaOH and 65 ml dioxane and to this was added, in small portions with vigorous stirring and ice cooling 10.45 g NPS-chloride and 27.5 ml 2 N NaOH such that the pH of the reaction mixture was kept weakly alkaline. Stirring was continued for a further 5-10 min after which the reaction mixture was diluted with 500 ml water, filtered, the filtrate acidified with 1 N H_2SO_4 and NPS-β-alanine, which separated in form of an oil was extracted two times with ethyl acetate. The ethyl acetate layer was washed with water, dried over Na_2SO_4, evaporated in vacuum, and petroleum ether added to the residue until initiation of crystallization. NPS-β-alanine (10.65 g) of mp 84-85°C was obtained, yield 88%. After recrystallization from ethyl acetate—petroleum ether (or nitromethane) it had mp 85-86°C. According to data in [6]: mp 85°. Found: C 44.81; H 4.41; N 11.84%. $C_9H_{10}N_2O_4S$. Calculated: C 44.62; H 4.16; N 11.56%.

NPS-Glycine. Obtained analogously to NPS-β-alanine. After acidification of the reaction mixture NPS-glycine separated in crystalline form with mp 125-126° (with decomposition: from ethyl acetate—

petroleum ether), yield 84%. According to the data of [6]: mp 147°C. Found: C 41.99; H 3.64; N 12.50%. $C_8H_8N_2O_4S$. Calculated: C 42.10; H 3.53; N 12.27%.

Sarcolysine Toluene-p-sulphonate. To a suspension of 36.6 g sarcolysine base in 360 ml acetone was added 24.0 g toluene-p-sulfonic acid (monohydrate) and the mixture stirred for 10-15 min, then filtered, the filtrate evaporated in vacuum, and the residue recrystallized from acetonitrile. Sarcolysine toluene-p-sulfonate (40.0 g) of mp 155-157°C was obtained, yield 70%. Found: C 50.52; H 5.33; N 6.01%. $C_{20}H_{26}Cl_2N_2O_5S$. Calculated: C 50.31; H 5.49; N 5.87%.

Sarcolysine Benzhydryl Ester Toluene-p-sulfonate. To a solution of 35.75 g sarcolysine toluene-p-sulfonate in 60 ml dimethylformamide was added dropwise with stirring a solution of 20.4 g (1.4 equivalent) diphenyldiazomethane in 40 ml dimethylformamide such that the temperature of the reaction mixture did not exceed 55°C. The mixture was then cooled and the reaction product precipitated with ether. Sarcolysine benzhydryl ester toluene-p-sulfonate (40.2 g) was obtained having mp 173-174°C, yield 83%. After crystallization from methanol it had mp 174-175°C. Found: C 61.31; H 5.91; N 4.56%. $C_{33}H_{36}Cl_2N_2O_5S$. Calculated: C 61.59; H 5.64; N 4.35%.

β-Alanine Toluene-p-sulfonate. Obtained by the method in [5]; mp 148-149°C (from ethanol – ether), yield 94%. Found: C 45.93; H 6.01; N 5.41%. $C_{10}H_{15}NO_5S$. Calculated: C 45.96; H 5.79; N 5.36%.

β-Alanine Benzhydryl Ester Toluene-p-sulfonate. The reaction was carried out analogously to the preparation of the corresponding sarcolysine ester but in this case the dimethylformamide was removed in vacuum, the residual oil dissolved in ethyl acetate, and ether added to incipient crystallization: mp 108-114°C, yield 85%. After recrystallization from ethyl acetate (great loss) mp 136-139°C. Found: C 64.81; H 6.20; N 3.46%. $C_{23}H_{25}NO_5S$. Calculated: C 64.63; H 5.89; N 3.28%.

General Method for Preparing OBzh and OBzl Esters of NPS- and Form-Dipeptides. To a mixture (cooled to 0°C) of 5 mmole amino acid ester hydrochloride, 5 mmole triethylamine, and 5 mmole N-acyl-amino acid in tetrahydrofuran was added with stirring a solution of 5.2 mmole N,N'-dicyclohexylcarbodiimide in tetrahydrofuran. Cooling and stirring were continued for 4-7 h further, after which 3-5 drops glacial acetic acid were added, the mixture stirred 1 h more, and dicyclohexylurea filtered off. The filtrate was evaporated in vacuum, the residual oil dissolved in a small volume of ethyl acetate, and after 1 h an additional quantity of dicyclohexylurea, which had separated, was filtered off. The filtrate was once again evaporated in vacuum, the residue dissolved in chloroform and washed in turn with water, 0.2 N H_2SO_4, 1 N $KHCO_3$, with water once again, and dried over Na_2SO_4. After this the chloroform was removed in vacuum and the residue crystallized. Compounds obtained are shown in Table 1.

General Method of Removal of NPS- and OBzh Protecting Groups. NPS-Dipeptide OBzh ester (1 mmole) (see Table 1, compounds 1-4) was dissolved in 98% formic acid and the solution kept 12 h at room temperature. The mixture was then evaporated in vacuum, the residue dissolved in ethanol and evaporated once more, after which it was dissolved once again in ethanol and the reaction product precipitated with ether. After crystallization from dimethylformamide sufficiently pure dipeptides were obtained (see Table 2, compounds 1-4).

General Method for Hydrogenolysis of Benzyl and Benzhydryl Esters. Benzhydryl or benzyl ester of Form-dipeptide (5 mmole) (see Table 1, compounds 5-8) was dissolved in 75 ml methanol and subjected to catalytic hydrogenolysis over palladium black until hydrogen uptake ceased, which usually required 2-3 h. The catalyst was filtered off, the filtrate evaporated in vacuum, and the residue crystallized from acetonitrile (for β-alanine) or ethanol (for glycine). The compounds obtained are presented in Table 2 (compounds 5-8).

Sarcolysyl-β-Alanine Ethyl Ester. To a solution of 1.12 g NPS-β-Ala-OEt (see Table 1, compound 9) in ethyl acetate was added 2.5 equivalents of ethereal HCl solution. The very hygroscopic ethyl ester hydrochloride of sarcolysyl-β-alanine was precipitated. An analytical specimen was isolated as the picrate (from isopropanol, see Table 2, compound 9).

CONCLUSIONS

1. New key compounds of sarcolysine viz. NPS-sarc-OH and H-sarc-OBzh, and of β-alanine viz. H-β-Ala-OBzh have been obtained.

2. New dipeptides of sarcolysine with β-alanine and glycine have been prepared.

3. A method has been proposed for the simultaneous removal of protecting NPS- and OBzh groups from dipeptides.

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