p-DI(2-CHLOROETHYL)AMINOPHENYLALANINE (SARCOLYSINE) AND ITS DERIVATIVES

XIII. SYNTHESIS OF PEPTIDE DERIVATIVES OF N-ACETYLSARCOLYSINE BY THE MIXED-ANHYDRIDE METHOD

O. S. Vasina, N. M. Kashnikova,
K. N. Kurdyumova, L.I. Smirnova,
M. I. Chernyakhovskaya, and E. N. Shkodinskaya

UDC 615.227.3.012.1

At the present time, a considerable number of dipeptide derivatives of p-di(2-chloroethyl)aminophenylalanine (sarcolysine) of general formula I, where sarcolysine is the N-terminal amino acid, have been obtained [1-7].

$$\underset{\text{I}}{p\text{-}(ClCH_2CH_2)_2NC_6H_4CH_2CH(NHCOCH_3)CONHCHRCOOR'}$$

As has been shown previously [2, 8], the biological properties of compounds of this type depend markedly on the particular protection of the α-amino group. It has been shown that it is in fact dipeptides of N-acetylsarcolysine [3] that possess the greatest antitumoral activity while, at the same time, they act on tumors more selectively than sarcolysine or its dipeptides with an open, formylated, or benzoylated amino group.

The experimental results have subsequently been confirmed in the clinic.

The first peptide synthesized and studied in the Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences of the USSR — the ethyl ester of α-acetylsarcolysylvaline (Asalin) — has undergone successful clinical trials and has been approved for use in medical practice in the treatment of some forms of malignant neoplasms.

Hitherto, the main method for the synthesis of peptide derivatives of N-acylated sarcolysine has been that using N,N'-dicyclohexylcarbodiimide (DCHC) as condensing agent [1, 3-5]. In spite of its simplicity and the possibility of obtaining peptides with satisfactory yields, this is only a laboratory method because of the extremely high toxicity and poor availability of DCHC. Other methods using 4-[p-di(2-chloroethyl)aminobenzylidene]-2-phenyloxazol-5-one [2], 2-methyl-4-(p-nitrobenzylidene)oxazol-5-one [6], and dipeptide derivatives of N-acetyl-p-nitrophenylalanine [7], and also a recently-proposed method using the p-nitrophenyl ester of sarcolysine (obtained, in its turn, with the aid of DCHC) [9], involve several stages and are also unsuitable for the preparation of cytotoxic peptides in large amounts.

In view of the above, in the present paper a method of obtaining peptide derivatives of N-acetylsarcolysine is proposed which is based on the mixed-anhydride method [10].

The reaction of N-acetylsarcolysine with isovaleryl chloride in the presence of triethylamine in chloroform gave the mixed anhydride of N-acetylsarcolysine and isovaleric acid, which was then, without

Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow.
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 4, No. 10, pp. 25-27, October, 1970. Original article submitted January 26, 1970.

isolation, caused to react with an ester of a DL- or a L-amino acid in accordance with the equation

$$p\text{-}(ClCH_2CH_2)_2NC_6H_4CH_2\underset{\displaystyle NHCOCH_3}{CH}COOH + COClCH_2CH(CH_3)_2 \rightarrow [p\text{-}(ClCH_2CH_2)_2NC_6H_4CH_2\underset{\displaystyle NHCOCH_3}{CH}COOCOCH_2CH(CH_3)_2] \rightarrow I$$

In addition to Asalin, we have synthesized by this method the ethyl esters of dipeptide derivatives of N-acetylsarcolysine with DL- and L-methionine and with L-phenylalanine, which we have described previously, and also those with other amino acids—DL-tryptophan, DL-serine, and L- and DL-aspartic acid. The last-mentioned dipeptide was obtained in the form of diethyl and dibenzyl esters and compounds with good results. The somewhat poorer yield in the preparation of Asalin could be expected, since in this case the second amino acid was valine, the branching of which, as is well known, adversely affects the mixed-anhydride reaction [11].

In spite of this, the replacement of the difficultly accessible and highly toxic DCHC by isovaleric acid, which is manufactured in adequate amounts by the Soviet industry, makes the proposed method of obtaining cytotoxic peptides a promising one and permits its recommendation for use on the industrial scale.

TABLE 1. Dipeptide Derivatives of Sarcolysin (I)

R	R′	Optical form of the second amino acid	Yield (in %)	mp (in degrees)	Found (in %) C	Found (in %) H	Found (in %) Cl	Found (in %) N	Empirical formula	Calculated (in %) C	Calculated (in %) H	Calculated (in %) Cl	Calculated (in %) N
$—CH(CH_3)_2$	$—C_2H_5$	DL	48	143—5	55,69	6,86	14,9	8,86	$C_{22}H_{33}Cl_2N_3O_4$	55,93	5,88	14,68	8,85
$—CH_2C_6H_5$	C_2H_5	L	65	123—6	59,77	6,25	13,59	8,23	$C_{26}H_{33}Cl_2N_3O_4$	59,82	6,3	13,57	8,28
$—CH_2CH_2SCH_3$	C_2H_5	DL	70	141—3	52,08	6,62	13,86	8,30	$C_{22}H_{33}Cl_2N_3O_4S$	52,14	6,52	14,03	8,31
$—CH_2CH_2SCH_3$	C_2H_5	L	71,6	133—8	52,09	6,57	13,92	8,29	$C_{22}H_{33}Cl_2N_3O_4S$	52,14	6,52	14,03	8,31
Indol-3-ylmethyl	C_2H_5	DL	61,5	178—80	60,13	6,29	11,80	10,15	$C_{28}H_{34}Cl_2N_4O_4$	60,0	6,10	12,6	9,98
$—CH_2OH$	C_2H_5	DL	40,8	127—31	52,2	6,6	14,83	9,33	$C_{20}H_{29}Cl_2N_3O_5$	52,10	6,3	15,2	9,08
$—CH_2COOC_2H_5$	C_2H_5	DL	58	116—8	53,76	5,27	13,76	8,16	$C_{23}H_{33}Cl_2N_3O_6$	53,8	5,46	13,84	8,18
$—CH_2COOC_2H_5$	C_2H_5	L	67	118—22	53,68	5,62	13,79	8,17	$C_{23}H_{33}Cl_2N_3O_6$	53,8	5,46	13,84	8,18
$CH_2COOCH_2C_6H_5$	$—CH_2C_6H_5$	DL	54,0	122—7	61,79	5,67	—	6,73	$C_{33}H_{37}Cl_2N_3O_6$	61,68	5,80	—	5,53
$—CH_2COOH$	—H	DL	65,5	115 (decomp.)	49,39	5,63	15,07	9,01	$C_{19}H_{25}Cl_2N_3O_6$	49,36	5,45	15,34	9,08

EXPERIMENTAL

General Method for Obtaining Peptide Derivatives of N-Acetylsarcolysine. A solution of 0.4 mole of anhydrous triethylamine in 50 ml of chloroform was added dropwise over 20-25 min to a suspension of 0.4 mole of N-acetylsarcolysine in 600 ml of dry chloroform at a temperature of from 5 to −7°C. Then the resulting solution was treated at the same temperature with a solution of 0.4 mole of isovaleryl chloride in 50 ml of chloroform over 30-40 min. After 2 h, a solution of 0.45 mole of the ester of the amino acid in 500 ml of chloroform was added to the reaction mixture at such a rate that the temperature did not rise above −5°C (30-40 min), and then stirring was carried out at this temperature for 2 h and (except in the preparation of Asalin) the resulting mixture was left overnight (at from −5 to 0°C).

The chloroform solution so obtained was washed successively with water (3 × 300 ml), 0.5 N sodium bicarbonate solution (3 × 350 ml), and again with water. After drying with sodium sulfate, the solvent was distilled off in vacuum (35-40°C), and the residue was recrystallized from ethanol.

The yields and constants of the substances obtained are given in Table 1.

N-Acetylsarcolysylaspartic Acid. A solution of 14.5 g of the dibenzyl ester of N-acetylsarcolysylaspartic acid in a mixture of 150 ml of ethanol and 30 ml of ethyl acetate was hydrogenated in the presence of 2 g of 5% palladized carbon for 2 h. After the removal of the catalyst and the distillation of the solvent, 10.33 g of a substance was obtained which was recrystallized from ethanol, giving 6.13 g (65.5%) of the dipeptide, decomp. 115°C.

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