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Abstract

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CANCEROLYTIC PEPTIDES OF DIRECTED ACTION

The genetic etiology of cancer leads to the inevitable conclusion that it is necessary either to disaggregate the nuclear DNA of the cancer cell, which is difficult to achieve, or to use chemical mutagenic agents which, acting cytostatically or else causing lethal mutations, render the immediately succeeding generations of cancer cells nonviable. This view is supported by the fact that all cancerolytic preparations known up to now—sarcolysin, dopan, embichin, thioTEPA, and others—are mutagenic ([1,2]). However, these preparations are nonspecific. Their action is almost undifferentiated with respect to cancerous and normal cells ([3]), as a result of which their use, as a rule, causes damage to bone-marrow cells. Naturally, the action of the preparations is even less differentiated with respect to different strains of cancer. The nonspecificity of individual amino acids bearing a mutagenic group (for example, *p*-di-(2-chloroethyl)aminophenylalanine) may be explained by the fact that in the DNA of both healthy and cancerous cells there are loci for the sorption of any of the essential amino acids that form the basis of ordinary cancerolytic preparations.

The assumption that short peptides can be sorbed on DNA, in which the order of linkage of the amino acids will by chance correspond to the code of a definite segment of DNA, makes it probable that peptides will have a greater specificity of action than individual amino acids bearing a mutagenic group.

Biological tests of the dipeptides obtained by us earlier ([4,5]) showed that, in the absence of the toxicity inherent in previously known preparations of this group, the compounds, depending on the terminal amino acid and the structure of the carrier of the mutagenic group, possess selective action directed against various strains of malignant tumors ([6,7]).

Still greater specificity of action could be expected from tri- and tetrapeptides, since, depending on the order of linkage and the character of the amino acids, they can be endowed with the ability for specific sorption on different DNAs—on the one hand, of cancerous and healthy cells, and on the other, on the quali-

tatively distinct DNAs of different strains of cancer cells; that is, precisely the differentiation of action that is lacking in contemporary antitumor preparations.

For this purpose, by condensation of esters of di- and tripeptides with *p*-di-(2-chloroethyl)aminophenylacetic and γ-

$$*p* - di - (2 - chloroethyl)amino$$

phenylbutyric acids, tri- and tetrapeptides were obtained bearing, in the acyl residue of the N-terminal amino acid, a mutagenic group (see Table 1, compounds Nos. 1–14).

The compounds obtained showed high selectivity of action in animal experiments with respect to different strains of cancerous tumors, depending on the character of the amino acids constituting the peptide chain and on the nature of the terminal amino acid bearing the mutagenic group ([7]). In the case of peptides containing unnatural amino acids,

$$(ClCH_2CH_2)_2N{-}C_6H_4{-}(CH_2)_n{-}CO{-}NH{-}CH(R'){-}CO\cdots NH{-}CH(R^m){-}COOC_2H_5$$

Table 1

No.	*n*	R	mp, °C	Yield, %	Found C, %	Found H, %	Found N, %	Found Cl, %	Calculated C, %	Calculated H, %	Calculated N, %	Calculated Cl, %
1	1	Leucylphenylalanine ethyl ester	128–129	51	61.63	7.19		12.31	61.7	6.92		12.59
2	1	Phenylalanylleucine ethyl ester	119–122	61	61.35	6.93		11.60	61.7	6.92		12.59
3	1	Phenylalaninemethionine ethyl ester	139–140.5	57	57.76	6.10	7.37		57.73	6.36	7.22	
4	1	Leucylmethionine ethyl ester	119.5–122	34	54.80	6.98	7.39		54.74	7.12	7.66	
5	1	Phenylalanylphenylalanine ethyl ester	117–120	38	64.35	6.24		12.50	64.21	6.19		11.87

No.	n	R	mp, °C	Yield, %	Found C, %	Found H, %	Found N, %	Found Cl, %	Calculated C, %	Calculated H, %	Calculated N, %	Calculated Cl, %
6	1	Leucylleucine ethyl ester	100–104	59	58.48	7.74		13.64	58.87	7.74		13.40
7	1	Phenylalanylalanine ethyl ester	151–156	63	61.21	6.59		12.35	61.09	6.73		12.91
8	3	Phenylalanylleucine ethyl ester	143–144	40	61.92	7.28		11.28	62.84	7.26		11.99
9	3	Phenylalanylphenylalanine ethyl ester	121–125	61	65.06	6.62	6.17		65.18	6.55	6.71	
10	3	Leucylleucine ethyl ester	125–126	31	60.00	7.97		12.15	60.22	8.06		12.72
11	1	Methionylleucylphenylalanine ethyl ester	185–187	24	58.71	6.76	7.87		58.70	6.91	8.06	
12	1	Methionylphenylalanylvaline ethyl ester	184–186	24	58.42	6.57	8.33		58.15	6.75	8.22	
13	3	Methionylleucylphenylalanine ethyl ester	140–145	33	59.86	7.26	7.73		59.75	7.19	7.74	
14	3	Leucylphenylalanylmethionine ethyl ester	165–170	16	59.74	7.29	7.84		59.75	7.19	7.74	
15	1	Proline ethyl ester	81.5–82	86	56.81	6.64	6.79	17.38	56.86	6.48	6.98	17.71

No.	n	R	mp, °C	Yield, %	Found C, %	Found H, %	Found N, %	Found Cl, %	Calculated C, %	Calculated H, %	Calculated N, %	Calculated Cl, %
16	1	Glutamic acid dimethyl ester	81–82[1]	67	52.42	5.99		16.11	52.66	6.00		16.39
17	1	β,β-Ditrifluoromethyl-α-aminopropionic acid ethyl ester (hexafluorovaline)	73–76	46	45.21	4.43	5.85		44.61	4.30	5.47	
18	1	Phenylalanine ethyl ester[4]	81–84[1]	81	62.47	6.79		14.61	62.63	6.68		14.82
19	1	Methionine ethyl ester[4]	93–95[1]	79	53.89	6.91			54.43	6.91		
20	3	Glutamic acid dimethyl ester	68–70	86	54.63	6.58			54.66	6.51		
21	3	ω-Aminocaprylic acid ethyl ester[5]	63–65	25	60.00	7.87	6.05		60.13	7.84	6.1	
22	1	Anesthesin	132.5–135[1][3]	90	59.82	5.77		16.64	59.57	5.67		16.78

No.	n	R	mp, °C	Yield, %	Found C, %	Found H, %	Found N, %	Found Cl, %	Calculated C, %	Calculated H, %	Calculated N, %	Calculated Cl, %
23	1	Treoanine (1-*n*-nitrophenyl)-2-amino-1,3-propanediol	125–128	95	53.58	5.45		14.65	53.62	5.32		15.11
24	1	6-Methoxy-8-aminoquinoline	117–118[1]	93	60.87	5.50		16.21	61.11	5.32		16.44
25	1	Indoline	161[2]	50	63.73	6.03	7.65	18.30	63.66	5.84	7.43	18.83
26	1	Tryptamine	128[3]	41	63.23	6.04	9.98	16.79	63.16	5.98	10.05	16.98
27	3	Anesthesin	116–118[1]	91	61.54	6.54	6.27	15.43	61.20	6.21	6.21	15.74

Note. For preparing analogs of tri- and tetrapeptides from intermediate *N*-formyl derivatives, the formyl group was removed by heating them for one minute with 6*N* alcoholic HCl. The solvent was removed in vacuo; the residue was dissolved in $CHCl_3$ and neutralized with a solution of ammonia in $CHCl_3$. The free base was not isolated in pure form. After removal of $CHCl_3$, the residue was acylated by the usual method with *N*-formylaminic acid—either *n*-di-(2-chloroethyl)aminophenylacetic acid or γ-[*n*-di-(2-chloroethyl)amino]phenylbutyric acid. The general method for obtaining cancerolytic acylamino acids and peptides bearing a di-(2-chloroethyl)amino group has been published previously ([4],[5]). DL-amino acids were used in the reaction. In most cases the final compound was purified by recrystallization from ethyl alcohol.

[1] Recrystallized from ethyl acetate–petroleum ether.
[2] Recrystallized from ethyl acetate.
[3] On prolonged standing in the light, it darkens.
[4] In this case, instead of *n*-di-(2-chloroethyl)aminophenylacetic acid, *n*-di-(2-chloropropyl)aminophenylacetic acid was used; the latter was obtained in 26% yield by condensation of ethyl *n*-aminophenylacetate with propylene oxide and subsequent reaction with $POCl_3$. Colorless crystals, mp 121–122° (from petroleum ether).

Found, %: C 55.27; H 6.22; Cl 22.23
$C_{14}H_{19}Cl_2NO_2$. Calculated, %: C 55.25; H 6.25; Cl 23.28

According to preliminary data of L. F. Larionov and S. S. Kyablos, *n*-di-(2-

chloropropyl)aminophenylacetic acid has high antitumor activity (in the case of sarcoma-45 the percent inhibition reaches 98.98%).

[5] As a by-product, *N*-*n*-di-(2-chloroethyl)amino-γ-phenylbutyryl-1,3-dicyclohexylurea was isolated in 38% yield.

the antitumor action is reduced or disappears. Thus, the ethyl ester of *n*-di-(2-chloroethyl)aminophenacetyl-β,β-ditrifluoromethyl-α-aminopropionic acid has no antitumor activity (compound No. 17). The percentage inhibition of sarcoma-45 in the case of the ethyl ester of *n*-di-(2-chloroethyl)aminophenacetyl-β-phenyl-β-alanine is only 51%, whereas for the ethyl ester of *n*-di-(2-chloroethyl)aminophenacetyl-β-phenyl-α-alanine it is 98.7%. Some amides of *n*-di-(2-chloroethyl)aminophenylsuccinic and γ-[*n*-di-(2-chloroethyl)amino]phenylbutyric acids (compounds Nos. 22 and 24) also possess antitumor action.

In order to study the influence of the mobility of halogen atoms located in the mutagenic group on the antitumor activity of the compounds, the action of similarly constructed peptides bearing di-(2-chloroethyl)- and di-(2-chloropropyl)amino groups (compounds Nos. 18 and 19) was compared. In the case of the latter, the antitumor action is reduced*. Comparison of the antitumor activity of peptides containing residues of *n*-di-(2-chloroethyl)amino-DL-phenylalanine (sarcolysin) with peptides bearing residues of *n*-di-(2-chloroethyl)aminophenylsuccinic and γ-[*n*-di-2-(chloroethyl)amino]phenylbutyric acids shows that, in the latter case, the spectrum of antitumor action has become broader, and the antitumor activity (in experiments on animals) not only is not reduced, but for certain strains of cancer tumors exceeds that of peptides containing sarcolysin residues.

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* Preliminary data of the Lithuanian Oncology Institute.

Note: Figure translations are in progress. See original paper for figures.

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