Synthesis and Biological Activity of Isomers of N-[Bis(2-chloroethyl)aminobenzoyl]glutamic Acid[1a,b]

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L and D forms of *m*- and *o*-N-[bis(2-chloroethyl)aminobenzoyl]glutamic acids and their ethyl esters were synthesized. The biological activities of these compounds, together with previously synthesized L and D *para* isomers and their ethyl esters, were demonstrated in eight microbial systems, in the KB cell culture, and in the Ehrlich ascites tumor. The *o*-N-[bis(2-chloroethyl)aminobenzoyl]-L-glutamic acid and its ester showed significant antineoplastic activity in all test systems studied.

Nitrogen mustards of glycine,[2a] alanine,[2a] and phenylalanine[2b] have been shown to possess selective cytotoxicity toward certain neoplasms. Derivatives of other naturally occurring amino acids and related metabolites, serine,[2c] threonine,[2c] tryptophan,[2d] cysteine,[2e] phenylpyruvic acid,[2f] and aminobenzoic acid[2g] were subsequently synthesized and most were found to be active against varying types of experimental neoplasms in animals. In recent studies on nitrogen mustards of dipeptides,[2h] tripeptides,[2h] the tetrapeptide[2h] and pentapeptide[2i] of phenylalanine, and dipeptides of *p*-aminobenzoic acid,[2i] their cytotoxicity observed in microbial and mammalian-cell culture systems and in experimental tumors seems to substantiate the concept of the "carrying group"[3] in relation to selective cytotoxicity. The amino acids and metabolites of the natural form (L) or peptides close to the natural form as a "carrying group" for the cytotoxic constituent enhance the activity and selectivity of the nitrogen mustards, while their enantiomorphs are less active. It has been pointed out that the activity of peptide nitrogen mustards[4] is often determined by the optical configuration of the terminal amino acid, analogous to the enzymic susceptibility of dipeptides.[5] L and D forms of N-{*p*-[bis(2-chloroethyl)amino]-benzoyl}glutamic acid have been recently synthesized.[2i] This report presents the synthesis of the *meta* and *ortho* isomers of the compound and the evaluation of their biological activities, in conjunction with the previously synthesized *para* isomer.

Experimental[6]

N-{*m*-[Bis(2-chloroethyl)amino]benzoyl}glutamic acids (L and D) were synthesized by a procedure similar to that described for the *para* isomer.[2i] Experimental modifications, yields, and melting points were as follows.

m-Aminobenzoic acid was esterified in 10 molar equiv. of absolute methanol saturated with dry HCl by the usual procedure. The ester was recrystallized twice from methanol through Darco, m.p. 37–38°.[7]

The methyl *m*-aminobenzoate was hydroxyethylated to methyl *m*-[bis(2-hydroxyethyl)amino]benzoate in 75% yield as a yellow oil. The bishydroxyethyl compound was chlorinated to methyl *m*-[bis(2-chloroethyl)amino]benzoate, yield 66%, m.p. 54–55°,[8] and then hydrolyzed to the free acid, yield 55%, m.p. 177–178°.[8] The *m*-[bis(2-chloroethyl)amino]benzoic acid was converted, with 3 molar equiv. of thionyl chloride[9] in dry benzene, to an acid chloride which was distilled repeatedly with small portions of benzene under reduced pressure to a yellow crystalline product. Condensation of the acid chloride with diethyl glutamate gave diethyl N-{*m*-[bis(2-chloroethyl)amino]-benzoyl}glutamate, yield 84%, m.p. 74–75° (for both L and D isomers). The diethyl ester was hydrolyzed to the free dipeptide nitrogen mustard, yield 90%, m.p. 22° (25° clear; for both L and D isomers).

N-{*o*-[Bis(2-chloroethyl)amino]benzoyl}glutamic Acids.—To a suspension of methyl anthranilate (131 mmoles) in 45 ml. of 40% acetic acid, ethylene oxide (1310 mmoles) was added at 0°, under vigorous stirring for 30 min., and stirring was continued for 24 hr. at 25–30°. Two portions of ethylene oxide, 600 mmoles each, were again added at 48- and 72-hr. intervals, and stirring was continued at room temperature for 24 hr. after the last addition. The clear solution was then poured into 150 ml. water and neutralized with $NaHCO_3$ to pH 7. The light oil was separated, and the aqueous layer was extracted with 250 ml. of ethyl acetate in three portions. The oil and the ethyl acetate extracts were combined and dried (Na_2SO_4). After the solvent was evaporated under reduced pressure, a colorless oil resulted. The oil was converted to a hydrochloride by (a) dissolving it in 50 ml. of methanol saturated with dry HCl at 0°. After evaporation of the methanol, a thick oil was obtained which crystallized in 4 to 7 days at 4° over phosphorus pentoxide.

(1) (a) Presented in part before the VIIIth International Cancer Congress, Moscow, U.S.S.R., July, 1962. (b) This investigation was supported by Public Health Service Research Grants No. CY-3335 and C-6516 from the National Cancer Institute.

(2) (a) M. Ishidate, Y. Sakurai, and M. Izumi, *J. Am. Pharm. Assoc.*, **44**, 132 (1955); (b) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954); L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaja, O. S. Vasina, V. I. Troosheikina, and M. A. Novikova, *Lancet*, **2**, 169 (1955); (c) F. Bergel and R. Wade, *J. Chem. Soc.*, 941 (1959); (d) J. DeGraw and L. Goodman, *J. Org. Chem.*, **27**, 1395 (1962); (e) R. H. Iwamoto, E. A. Acton, L. O. Ross, W. A. Skinner, B. R. Baker, and L. Goodman, *J. Med. Chem.*, **6**, 43 (1963); (f) A. P. Martinez, W. A. Skinner, W. W. Lee, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 6050 (1960); F. D. Popp, *J. Org. Chem.*, **26**, 3020 (1961); (g) W. C. J. Ross, *J. Chem. Soc.*, 183 (1949); S.-C. J. Fu, Abstracts of Papers, 138th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1960, p. 8-O; R. C. Elderfield and T.-K. Laio, *J. Org. Chem.*, **26**, 4996 (1962); (h) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 3658 (1960); L. F. Larionov, *Cancer Res.*, **21**, 99 (1961); F. Bergel, I. M. Johnson, and R. Wade, *J. Chem. Soc.*, 3802 (1962); A. N. Shkodinskaja, O. S. Vasina, A. Y. Berlin, Z. P. Sofina, and L. F. Larionov, *Zh. Obshch. Khim.*, **32**, 324 (1962); L. A. Elson, A. Haddow, F. Bergel, and J. A. Stock, *Biochem. Pharmacol.*, **11**, 1079 (1962); (i) J. M. Johnson and J. A. Stock, *J. Chem. Soc.*, 3806 (1962); (j) S.-C. J. Fu, *J. Med. Pharm. Chem.*, **5**, 33 (1962).

(3) J. F. Danielli, *Nature*, **170**, 863 (1952); F. Bergel, J. A. Stock, and R. Wade in "Biological Approaches to Cancer Chemotherapy," R. C. J. Harris, Ed., Academic Press Inc., New York, N. Y., 1961, p. 125.

(4) W. C. J. Ross, "Biological Alkylating Agents," Butterworths, London, 1962, p. 162.

(5) S.-C. J. Fu, S. M. Birnbaum, and J. P. Greenstein, *J. Am. Chem. Soc.*, **76**, 6054 (1954).

(6) All melting points are corrected and, unless specified, the compounds always become clear liquid at the melting range. The yield is given for the purified compound. The elementary analyses were performed by Dr. C. K. Fitz, Needham Heights, Mass., and Scandinavian Microanalytical Laboratory, Herlev, Denmark.

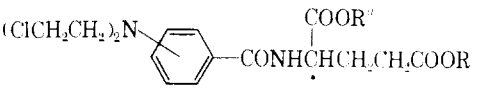
(7) H. Ulrich [*Ann.*, **332**, 196 (1904)] and J. Esterman, [*Z. physik. Chem.*, [B]**1**, 148 (1928)] reported m.p. 36–38° and 39°, respectively.

(8) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(9) Phosphorus pentachloride was used for the *para* isomer but it failed to react with the *meta* and *ortho* isomers.

Table I

Isomers of N-[Bis(2-chloroethyl)aminobenzoyl]glutamic Acid



Compd.	Isomer	R	Abs. configuration	[α]25D Abs. ethanol	[α]25D Chloroform	Found, % C	H	Cl	N
I	*p*	C_2H_5	L	−8.1	+12.6		[c]		
II	*p*	C_2H_5	D	+8.2	−12.3		[c]		
III	*m*	C_2H_5	L	−15.9	+15.6	53.7	6.2	15.9	6.3
IV	*m*	C_2H_5	D	+16.1	−15.3	53.6	6.0	15.8	6.3
V	*o*	C_2H_5	L	−19.5	−23.7	53.4	6.3	15.8	6.2
VI	*o*	C_2H_5	D	+19.8	+23.5	53.6	6.3	15.9	6.5
VII	*p*	H	L	−1.3	+7.8[d]		[c]		
VIII	*p*	H	D	+1.3	−8.0[d]		[c]		
IX	*m*	H	L	−6.8	+4.8[d]	49.0	5.5	18.1	7.1
X	*m*	H	D	+6.9	−4.4[d]	49.9	6.0	17.7	7.5
XI	*o*	H	L	−15.2	−10.5[d]	49.1	5.6	18.2	6.9
XII	*o*	H	D	+15.0	+10.8[d]	49.0	5.6	18.2	7.0

[a] Formulas for I–VI and VII–XII are $C_{20}H_{28}Cl_2N_2O_5$ and $C_{16}H_{20}Cl_2N_2O_5$, respectively. [b] *Anal.* Calcd. for I–VI: C, 53.7; H, 6.3; Cl, 15.9; N, 6.3; and for VII–XII: C, 49.1; H, 5.2; Cl, 18.1; N, 7.2, respectively. [c] *Cf.* ref. 2j. [d] Measured in purified dioxane.

Alternatively (b), the oil was dissolved in 50 ml. of anhydrous ether saturated with dry HCl at 0°. In this case, the thick oil precipitated after standing for 24 hr. at 4°. The ether was decanted, and the oil crystallized in 4 to 7 days at 4°. The methyl *o*-[bis(2-hydroxyethyl)amino]benzoate hydrochloride can be recrystallized from acetone–methanol,[10] yield 79%, m.p. 143–145° dec.[8]

Anal. Calcd. for $C_{12}H_{17}NO_4 \cdot HCl$: C, 52.4; H, 6.6; Cl, 12.8; N, 5.1. Found: C, 52.5; H, 6.8; Cl, 13.0; N, 5.1.

To the methyl ester hydrochloride (7.0 mmoles), 10 ml. of benzene and thionyl chloride (21.0 mmoles) were added and heated under reflux for 1 hr., protected from moisture. After evaporation of the benzene and excess thionyl chloride, the bis-chloroethyl ester was obtained as a yellowish oil which formed a picrate in quantitative yield, m.p. 105–106°,[8] recrystallized from methanol.

Anal. Calcd. for $C_{18}H_{18}Cl_2N_4O_9$: C, 42.8; H, 3.6; Cl, 14.1; N, 11.1. Found: C, 42.7; H, 3.7; Cl, 14.1; N, 11.0.

The free methyl *o*-[bis(2-chloroethyl)amino]benzoate was hydrolyzed in 20 molar equiv. of boiling concentrated HCl for 1 hr. The brown reaction mixture was evaporated under reduced pressure and repeatedly distilled with small portions of benzene. The last trace of water was removed by benzene extraction in a liquid–liquid extractor. In an anhydrous system, the *o*-[bis(2-chloroethyl)amino]benzoic acid was extracted into the benzene layer and the compound was recovered after evaporation of the solvent. The acid was obtained by recrystallization from benzene–hexane, yield 54%, m.p. 85–87°.[8]

Anal. Calcd. for $C_{11}H_{13}Cl_2NO_2$: C, 50.4; H, 5.0; Cl, 27.0; N, 5.4. Found: C, 50.5; H, 5.0; Cl, 27.0; N, 5.3.

The *ortho* acid was converted to an acid chloride with thionyl chloride[9] as described for the *meta* isomer. The formation, in $NaHCO_3$, and isolation of diethyl N-{*o*-[bis(2-chloroethyl)-amino]benzoyl}glutamate (75% yield, oil) were carried out by the procedure previously described.[2j] However, the hydrolysis of the ester to free *ortho* dipeptide nitrogen mustard with 2 molar equiv. of 0.5 *N* NaOH in 50% methanol required 2.5 hr. at a strictly controlled temperature of 25°, yield 56%, m.p. 27–30° (35° clear; for both L and D isomers), from chloroform–ethanol–hexane (Table I).

Biological Activities.[11] **Antimicrobial Assay.**—The dipeptide nitrogen mustards and their ethyl esters were tested in the eight microbial systems currently in use in Laboratories of Microbiology of this foundation. The assay methods have been described in detail elsewhere,[12] and the results are summarized in Table II.

Inhibitory Activity in KB[13] Cell Culture.—The dipeptide nitrogen mustards and their ethyl esters were assayed for inhibitory activity in KB cell culture as described previously.[14] The results are summarized in Table III.

Mouse Tumor Assay against Ehrlich Ascites Carcinoma.—The Ehrlich ascites (tetraploid) carcinoma has been maintained in CAF/JAX mice by transplanting 0.1 ml. of 1:7 dilution of ascites fluid in sterile Locke's solution at 10-day intervals. For treated and control groups 5 mice and 10 mice were used, respectively. In order to observe any effect of the vehicle in which the nitrogen mustards were suspended, solutions of 12% ethanol in 10% Tween-80[15] were utilized as diluent controls.

Injections were given intraperitoneally once daily, starting 24 hr. after inoculation of the tumor, and were continued until death of the last experimental animal. The solutions were prepared fresh daily. The results are shown in Tables III and IV. Animals were autopsied at death, and gross observations, including weight of the ascites fluid, were made. Tissue samples were taken for detailed microscopic examination.[16]

Results and Discussion[17]

In the synthesis of the N-{*p*-[bis(2-chloroethyl)-amino]benzoyl}glutamic acid, two independent routes were employed.[2j] The esters of N-{*p*-[bis(2-chloro-ethyl)amino]benzoyl}glutamic acid were obtained by hydroxyethylation followed by chlorination of the esters of *p*-aminobenzoylglutamic acid, and alternatively by condensation of *p*-[bis(2-chloroethyl)amino]-benzoic acid with esters of glutamic acid. The synthesis of the *meta* and *ortho* isomers was made possible by adaptation of the latter route. The exhaustive hydroxyethylation was found to be essential for the

(10) In most of our preparations, a pure compound was obtained without recrystallization.

(11) The antimicrobial assay and the assay in KB cell culture were performed in the Microbiological Laboratories, C.C.R.F., under the direction of Dr. G. E. Foley. The mouse tumor assay was performed in the Rodent Bioassay Laboratories, C.C.R.F., under the direction of Dr. C. L. Maddock.

(12) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N. Y. Acad. Sci.*, **76**, 413 (1958).

(13) H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **89**, 362 (1955).

(14) H. Eagle and G. E. Foley, *Cancer Res.*, **18**, 1017 (1958).

(15) Inoculation of 0.1 ml. of 12% ethanol in 10% Tween-80 per AKD2F1 brown mouse produced intense intoxication of the mice. The mice went to sleep 2–3 min. after injection and slept for 2–3 hr. without other apparent ill effect. However, the CAF JAX mice received the same dose of ethanol and Tween-80 mixture without sign of intoxication or other effects.

(16) The pathological findings will be reported elsewhere.

(17) A preliminary account of the inhibition of the L isomers was previously discussed.[2g]

(18) S.-C. J. Fu, H. Terzian, and C. L. Maddock, *Proc. Intern. Cancer Congr., 8th, Moscow*, **6**, 67 (1965); Russian Ed., *Acta Unio Intern. Contra Cancrum*, **20**, 87 (1964).

TABLE II

INHIBITORY EFFECT ON EIGHT MICROBIOLOGICAL SYSTEMS, ID_{50}, γ/ML.[a]

Compd.	1 (a)	1 (b)	2 (a)	2 (b)	3 (a)	3 (b)	4 (a)	4 (b)	5 (a)	5 (b)	6 (a)	6 (b)	7	8
I	83	40	I	I	I	I	I	I	30	30	70	50	125	80
II	160	240	I	I	I	I	I	I	45	35	100	100	45	35
III	50	100	I	I	I	I	I	I	30	7	250	55	40	180
IV	61	220	18	28	30	60	34	29	38	35	I	I	38	33
V	I	I	I	I	I	I	I	I	55	42	I	I	I	I
VI	54	90	28	20	18	50	26	32	68	48	I	I	31	33
VII	700	600	I	I	I	I	I	900	I	I	230	200	I	I
VIII	I	225	I	I	I	400	I	I	I	I	I	700	I	I
IX	350	180	I	I	I	550	I	700	550	280	40	I	350	350
X	71	52	35	25	13	45	29	32	360	430	I	I	27	33
XI	160	90	18	18	400	38	I	I	210	60	I	I	100	300
XII	100	35	27	7	13	53	32	29	45	320	I	I	28	31

[a] I (inactive) = $ID_{50} \geq 1000$ γ/ml. The numerals in the heading represent a microorganism; the letters represent the culture medium: 1, *Streptococcus faecalis*—PGA, (a) 0.01 γ/ml. and (b) 0.001 γ/ml.; 2, *Lactobacillus arabinosus*—nicotinic acid, (a) 0.1 γ/ml. and (b) 0.01 γ/ml.; 3, *Lactobacillus arabinosus*—pantothenate, (a) 0.1 γ/ml. and (b) 0.01 γ/ml.; 4, *Pediococcus cervisiae*—citrovorum factor, (a) 0.01 γ/ml. and (b) 0.001 γ/ml.; 5, *Lactobacillus fermenti*—thiamine, (a) 0.1 γ/ml. and (b) 0.01 γ/ml.; 6, *Lactobacillus casei*—riboflavin, (a) 0.1 γ/ml. and (b) 0.01 γ/ml.; 7, *Escherichia coli*—synthetic medium; 8, *Candida albicans*—semisynthetic medium.

TABLE III

RESULTS ON KB CELL CULTURE AND EHRLICH ASCITES TUMOR (4N) BEARING MICE

Compd.	KB mammalian cell culture[a] ID_{50}, γ/ml.	Ehrlich ascites tumor bearers ($T/C - 1$) $\times 100$[b]	Dose, mg./kg.[c]
I	45	+35	100
II	550	+13	100
III	65	+40	100
IV	38	+43	400
V	100	+129	25
VI	34	+99	100
VII	150	+35	100
VIII	100	+17	6.25
IX	65	+86	100
X	48	+39	100
XI	55	+129	100
XII	160	+45	100

[a] *Cf.* ref. 13 and 14. [b] T/C = treated mice/control mice. [c] Daily effective dose given i.p.

synthesis of the ethyl *o*-[bis(2-hydroxyethyl)amino]-benzoate. With a low proportion of ethylene oxide to methyl anthranilate, Everett, *et al.*,[8] reported that the monohydroxyethylaminobenzoate or, under drastic conditions, a hydroxyethyloxazepine derivative was obtained. The ethyl esters were converted to the free dipeptide nitrogen mustards by aqueous alkaline hydrolysis. The chemicals synthesized, and their physical properties, are listed in Table I.

The identity and purity of the *para* isomers were proven by quantitative ultraviolet and infrared absorption spectra, rotatory dispersion, and neutralization titration.[2i] During aqueous hydrolysis of the ethyl esters to the free dipeptides, no cyclization took place,[18] while N-methyl-N-bis(2-chloroethyl)amine (HN2) and other nitrogen mustards give rise to cyclic intermediates. The *m*- and *o*-N-[bis(2-chloroethyl)aminobenzoyl]glutamic acids are likewise of correct identity and high purity.

Data on the inhibitory activity observed in eight microbial systems are given in Table II. These studies indicate that most compounds are moderately active, ID_{50} 50–200 γ/ml., in all of the microbial systems and are most effective in *Streptococcus faecalis* and *Lactobacillus fermenti*. In the *para* isomers, the L compound seems to be slightly more active than the D. The ethyl esters are more active than the free dipeptides, and the ethyl ester of the *meta* L dipeptide is also more active than the dipeptide. Both the *meta* dipeptides and their esters are slightly more active than the corresponding *para* compounds. The *ortho* isomers, however, show reversed relationship between the dipeptides and their ethyl esters. The *ortho* compounds appear to have the highest activities. The D compounds and the ethyl esters of *meta* and *ortho* dipeptide nitrogen mustards (IV, VI, X, and XII) showed activities in all testing systems employed, except in *L. casei*. Both enantiomorphism and position isomerism modifications of *p*-aminobenzoyl-L-glutamic acid nitrogen mustard appear to effect growth interference in these microbial systems.

In KB mammalian cell culture, inhibitory activity appears to be uniform. With the exception of *para* D dipeptide ethyl ester, the indices are confined in a range of ID_{50} 50–100 γ/ml., as shown in Table III. The L compound of the *para* isomers showed substantially higher activity than the D compound. The difference in activity between the L and D forms in the *meta* and *ortho* isomers is less pronounced and without a fixed pattern.

The antineoplastic activity of the 12 compounds against Ehrlich ascites tumor (tetraploid) in CAF/JAX mice is also shown in Table III. The *ortho* L dipeptide and its ethyl ester showed the highest activity with increases in survival, at 25–100 mg./kg. dose level, beyond 100% of the control mice survival. For comparison, the detailed experimental data for V, XI, VI, XII, the parent compounds (nitrogen mustards of *p*-, *m*-, and *o*-aminobenzoic acids), HN2, and the phenylalanine nitrogen mustard (Sarcolysin) are shown in Table IV. The biological activity of the *ortho* L isomer compares well with that of HN2 and Sarcolysin, but at higher effective and toxic dose levels. Coincidentally, the *ortho* isomer of the phenylalanine nitrogen mustard also showed higher biological activity than the *para* and *meta* isomers.[19] In contrast to the variation of activities in microbial and cell-culture systems observed between L and D compounds of *meta* and *ortho*

(19) F. R. White, *Cancer Chemotherapy Rept.*, **No. 24**, 61 (1962).

Table IV

Antineoplastic Activity of Some N-[Bis(2-chloroethyl)aminobenzoyl]glutamic Acids, Related Compounds, and Sarcolysin against Ehrlich Ascites Tumor (4N) in CAF/JAX Mice

Compd.	Dose,[a] mg./kg.	Av. wt. change, g./day T/C[b]	Survival: Mean days T/C[b]	Survival: Increase, %	Eval.[c]
V	6.25	+0.43/+0.20	16.6/13.3	+25	±[d]
	25	+0.09/+0.20	30.4/13.3	+129	++
	100	−0.27/+0.20	27.0/13.3	+103	++
	400	−0.46/+0.20	18.8/13.3	−32	±
XI	6.25	+0.26/+0.20	16.6/13.3	+25	±[d]
	25	+0.25/+0.20	26.0/13.3	+95	+
	100	+0.04/+0.20	30.4/13.3	+129	++
	400	−0.63/+0.20	9.0/13.3	−32	Toxic
VI	6.25	+0.04/+0.30	20.2/16.8	+20	±[d]
	25	+0.41/+0.30	23.0/16.8	+37	±
	100	+0.22/+0.30	33.4/16.8	+99	+
	400	−0.14/+0.30	29.2/16.8	+74	+[d]
XII	6.25	+0.36/+0.30	16.6/16.8	−1	−
	25	+0.57/+0.30	18.2/16.8	+8	−
	100	+0.11/+0.30	24.4/16.8	+45	±
	400	−0.55/+0.30	5.6/16.8	−67	Toxic
p-Bis(2-chloroethyl)-aminobenzoic acid	6.25	+0.44/+0.49	15.8/15.8	±0	−
	25	+0.67/+0.49	18.4/15.8	+16	−
	100	+0.35/+0.49	22.4/15.8	+42	±
	400	−0.57/+0.49	2.8/15.8	−82	Toxic
m-Bis(2-chloroethyl)-aminobenzoic acid	6.25	+0.36/+0.49	22.0/15.8	+39	±
	25	+0.11/+0.49	27.4/15.8	+73	+
	100	−0.34/+0.49	23.8/15.8	+51	+
	400	−0.22/+0.49	3.2/15.8	−80	Toxic
o-Bis(2-chloroethyl)-aminobenzoic acid	0.156	+0.71/+0.68	17.8/15.6	+14	−
	0.625	+0.41/+0.68	23.8/15.6	+53	+
	2.5	−0.37/+0.68	16.6/15.6	+6	−
	10	−0.71/+0.68	10.2/15.6	−35	Toxic
HN2	0.125	+0.18/+0.45	28.0/15.4	+82	+
	0.5	−0.27/+0.45	24.6/15.4	+60	+
	2	−0.97/+0.45	9.0/15.4	−42	Toxic
	8	−1.02/+0.45	6.2/15.4	−60	Toxic
Sarcolysin	0.313[e]	+0.16/+0.49	36.5/19.3	+89	+
	0.625[e]	+0.26/+0.49	40.4/19.3	+109	++
	1.25[e]	+0.10/+0.39	47.4/19.1	+148	++
	2.5[e]	−0.03/+0.39	40.9/19.1	+114	++
	5[e]	−0.16/+0.39	34.9/19.1	+83	+

[a] Administered intraperitoneally in 12% ethanol in 10% Tween-80. [b] Treated mice/control mice. [c] Survival: +50% or less = ±, +51 to 100% = +, +101 to 150% = ++. [d] Not statistically significant due to wide range spread on the survival time. [e] Given every 4th day after the initial injection at 24 hr. after the tumor implantation.

isomers, the L compounds of the position isomers in all cases showed higher activity against Ehrlich ascites tumor in mice than their corresponding enantiomorphs.

The stereospecificity observed in this group of dipeptide nitrogen mustards warrants further investigation, particularly as to the selective cytotoxicity in relation to the enzymic susceptibility of the peptide "carrying group." The biological activity of the *ortho* L isomer is outstanding. Elucidation is necessary to determine whether it is due to (1) an effective binding of the nitrogen mustards to nucleic acids, proteins, etc., (2) the configuration of the molecule which might permit slow and continuous reaction of the nitrogen mustard *in vivo*, or (3) other reason(s) presently unapparent.

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