pounds. The chromatographic properties of the *ortho*, *meta*, and *para* isomeric pairs, together with their melting points and ninhydrin assays, are presented in Table II.

In conclusion, all the compounds described in the previous paper[1b] should be corrected to read as α-glutamyl derivatives rather than as the corresponding γ-isomers. The condensation products with phenylisocyanate are (N-phenylcarbamyl)-α-L-glutamylaminobenzoic acids. The authentic γ-L-glutamylaminobenzoic acids have now been prepared by the mixed anhydride coupling of N-carbobenzoxy-L-glutamic acid α-benzyl ester with the appropriate methyl aminobenzoate followed by removal of the protecting groups.

Experimental[9]

Since all the γ-glutamylaminobenzoic acid derivatives herein described were prepared by similar procedures, complete experimental data are given only for the *para* isomer, and the data on the remaining analogs are presented in Table I. Comparative data for the α- and γ-glutamyl derivatives are presented in Table II.

***p*-[(N-Carbobenzoxy-α-benzyl-γ-L-glutamyl)amino]benzoic Acid Methyl Ester (*p*-IV).**—A 12.6-g. sample of N-carbobenzoxy-L-glutamic acid α-benzyl ester[7] and 8 ml. of tri-*n*-butylamine were dissolved in 80 ml. of dimethylformamide–tetrahydrofuran (1:1). the mixture was cooled in an ice bath, and 3.3 ml. of ethyl chloroformate was added dropwise to form the mixed anhydride.[8] After stirring for about 30 min. in the cold, 5.13 g. of methyl *p*-aminobenzoate in 45 ml. of the dimethylformamide–tetrahydrofuran solvent mixture was added dropwise, and the reaction mixture was allowed to come to room temperature. Stirring was continued overnight. The solvent was evaporated *in vacuo*, and the residue was dissolved in 100 ml. of ethyl acetate and extracted with three 50-ml. portions of 2 *N* HCl. The ethyl acetate phase was then evaporated *in vacuo* to a pale yellow oil which was crystallized from ethanol–water to yield 11.4 g. of solid. Recrystallization of this material from toluene produced 6.3 g. of product, m.p. 150–154°. The *meta* isomer crystallized with difficulty, and crystallization of the *ortho* isomer was sufficiently difficult that it was isolated only for elemental analysis. The reaction mixture of the *ortho* derivative was used directly without purification in the subsequent hydrogenolysis step.

***p*-(γ-L-Glutamylamino)benzoic Acid Methyl Ester (*p*-V).**—A 3.55-g. sample of *p*-IV was dissolved in 90 ml. of 90% ethanol and hydrogenolyzed at atmospheric pressure and room temperature in the presence of 0.25 g. of palladium black catalyst for about 5 hr. The reaction product precipitated on the catalyst, which was collected and extracted with 300 ml. of boiling water. Upon cooling, the aqueous filtrate yielded 0.87 g. of product, which was recrystallized from water–ethanol, m.p. 206–207°.

***p*-(γ-L-Glutamylamino)benzoic Acid (*p*-I).**—A 0.5-g. sample of *p*-V was dissolved in 175 ml. of saturated $Ba(OH)_2$ solution, and after about 15 min. the resulting crystalline precipitate was filtered, washed with water, and dried to yield 0.64 g. of crude barium salt. This was suspended in 50 ml. of water, adjusted to pH 2.5 with 0.6 *M* H_2SO_4 to precipitate the barium ions as the insoluble sulfate, taken to pH 8 with 5 *N* NaOH to solubilize the reaction product, and finally filtered. The filtrate was then adjusted to pH 5 with 0.6 *M* H_2SO_4 to precipitate 0.4 g. of product, m.p. 227–228° dec. After two recrystallizations from water, which had been adjusted to pH 3 by the addition of dilute H_2SO_4, a white solid was obtained, m.p. 258–259°. This material was chromatographically homogeneous in two different solvent systems and gave the anticipated yield of CO_2 by the quantitative ninhydrin technique.[6] The alkaline hydrolysis of each of these methyl esters was extremely rapid, and the crude barium salt was isolated only during the hydrolysis of *p*-V; for the *meta* and *ortho* isomers, the reaction mixture was processed directly without any intermediate separation of the barium salt.

(9) All melting points are corrected, and were determined by the capillary technique using a well-stirred liquid bath. The paper chromatograms were prepared by the ascending procedure using the indicated solvent systems on Whatman No. 1 paper, and were subsequently developed with ninhydrin reagent.

Synthesis of N,N-Bis(2-haloethyl) Aliphatic Amides

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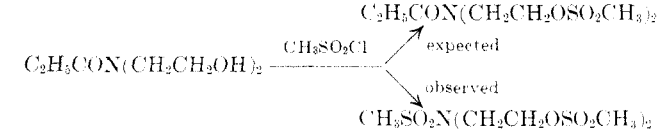
With the purpose of contributing to the series of the N,N-bis(2-chloroethyl) aliphatic amides[1] with potential cytotoxic activity, four amides were prepared by acylation of bis(2-chloroethyl)amine with acid chlorides in pyridine–chloroform solution (Table I).

In order to obtain the iodo analogs by means of the corresponding methanesulfonates (mesylates),[2] N,N-bis(2-hydroxyethyl)propionamide was obtained by ester aminolysis with diethanolamine, and this compound was mesylated with methanesulfonyl chloride in pyridine–chloroform solution at 3–5°. Methanesulfonyl chloride, besides esterifying the hydroxyl groups, displaced the acyl by the sulfonyl group. The same results



were obtained at different temperature and with or without a solvent. The N,N-bis(2-hydroxyethyl)-acetamide behaved like the propionamide.

The N,N-bis(2-mesylethyl)methanesulfonamide was also synthesized from diethanolamine. The diiodo derivative was obtained from the mesylate by displacement with sodium iodide. The structure of the compounds was confirmed by their infrared spectra.

$$CH_3SO_2N(CH_2CH_2OSO_2CH_3)_2 \xrightarrow{NaI} CH_3SO_2N(CH_2CH_2I)_2$$

Biological Results.—The drugs were tested in rats with transplanted Sarcoma 180 and Ehrlich ascitic carcinoma. The results are shown Table II.

Experimental

N,N-Bis(2-chloroethyl)amides.—The acid chloride (0.04 mole) in dry chloroform was added slowly to a stirred solution of bis(2-chloroethyl)amine prepared from the hydrochloride (0.045 mole) and pyridine (0.05 mole) in chloroform. Stirring was continued for 1 hr. and the mixture then was poured into water. Distillation of the solvent from the dried solution yielded thick oils which solidified by cooling in the case of caproamide (see Table I).

N,N-Bis(2-hydroxyethyl)propionamide.—Equimolar quantities of ethyl propionate and diethanolamine were mixed and refluxed for 10 hr. Excess solvent was removed by vacuum distillation and the product distilled at less than 1 mm.; yield 60%; colorless oil, b.p. 134–135° (0.35 mm.).

Anal. Calcd. for $C_7H_{15}NO_3$: C, 52.1; H, 9.31; N, 8.70. Found: C, 51.8; H, 9.42; N, 8.92.

Attempt to Obtain N,N-Bis(2-mesylethyl)propionamide.—Methanesulfonyl chloride (0.18 mole) in chloroform (10 ml.)

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TABLE I
AMIDES
$RCON(CH_2CH_2Cl)_2$

R	B.p. (mm.), °C.	Form	Yield, %	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found
C_2H_5	124–126 (3)	Oil	65	$C_7H_{13}Cl_2NO$	42.4	42.1	6.57	6.61
C_3H_7	136–138 (3)	Oil	60	$C_8H_{15}Cl_2NO$	45.2	44.6	7.07	7.10
C_4H_9	147–146 (3)	Oil	58	$C_9H_{17}Cl_2NO$	47.8	47.3	7.52	7.55
C_5H_{11}	M.p. 60–62	Wax	58	$C_{10}H_{19}Cl_2NO$	49.9	49.6	7.91	7.93

Table II
BIOLOGICAL RESULTS

Drugs	Dose/day, mg./kg.	Days	LD_{50}, mg./kg.	Ascitic carcinoma	Sarcoma 180
$C_5H_{11}CON(CH_2CH_2Cl)_2$	100	6	...	Only reduction of ascitic fluid	No effect
$CH_3SO_2N(CH_2CH_2OSO_2CH_3)_2$	5	6	300	No effect	Some inhibition, life extension 10 days
$CH_3SO_2N(CH_2CH_2I)_2$	100	8	2700[a]	Some inhibition, life extension 7 days	Inhibition, life extension 25 days

[a] By oral administration.

was added slowly to a stirred solution of N,N-bis(2-hydroxyethyl)-propionamide (0.06 mole) and pyridine (30 ml.) in chloroform (30 ml.) in an ice bath. After 2 hr., addition of 200 ml. of 1 *N* HCl gave 2.4 g. (12%) of N,N-bis(mesylethyl)methanesulfonamide as a crystalline solid, m.p. 112–114°. A mixture melting point with N,N-bis(mesylethyl)methanesulfonamide synthesized from diethanolamine gave no depression.

N,N-Bis(2-mesylethyl)methanesulfonamide.—Methanesulfonyl chloride (0.45 mole) in chloroform (10 ml.) was added slowly to a stirred solution of diethanolamine (0.20 mole), pyridine (70 ml.), and chloroform (40 ml.) in an ice bath. Stirring was continued for 2 hr. and the product was crystallized by addition of 200 ml. of 1 *N* HCl and recrystallized from water; yield 25%; white needles, m.p. 112–114°. The infrared spectrum showed maxima at the following fequencies: 3000, 2980, 1450, 1340, 1260, 1180, 1150, 1120, 1020, 1000, 975, 950, 930, 900, 807, 775, 735, and 725 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{17}NO_8S_3$,: N 4.13; S, 28.39. Found: N, 3.98; S, 28.05.

N,N-Bis(2-iodethyl)methanesulfonamide.—Sodium iodide (0.02 mole) was added to a solution of N,N-bis(2-mesylethyl)-methanesulfonamide (0.008 mole) in acetone (60 ml.) and stirred at 37° for 45 hr. The solid was removed by filtration, half of the acetone was evaporated, and the crude product crystallized by adding water. Recrystallization from petroleum ether (b.p. 70–80°) gave white needles, m.p. 96–98°, yield 66%. The infrared spectrum showed maxima at the following frequencies: 3000, 2980, 1450, 1340, 1230, 1180, 1150, 1110, 1050, 1020, 970, 930, 800, 780, 735, and 715 cm.$^{-1}$.

Anal. Calcd. for $C_5H_{11}I_2NO_2S$: I, 62.90; N, 3.47. Found: I, 62.73; N, 3.50.

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New Compounds

Agents Affecting Lipid Metabolism. XVI. The Synthesis of Analogs of *trans*-1,4-Bis(2-chlorobenzylaminomethyl)cyclohexane[1]

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Previous reports from this laboratory[2] have described the effects of *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane on various aspects of lipid metabolism. The synthesis of this compound and of a series of related compounds containing the cyclohexane-1,4-bis(methylamine) moiety has recently been described.[3] In this communication, the synthesis of a series of related compounds is reported, wherein the cyclohexane-1,4-bis(methylamine) moiety is replaced by others of different electron density, bulk, shape, and inter-nitrogen distance. They are shown in Table I and some of the intermediates used in their preparation are in Table II.

Experimental

Melting points were taken on a Thomas–Hoover apparatus and are corrected. Analyses were done by Mr. W. Turnbull and Staff of our laboratories. The compounds described in Table I were prepared, as indicated, by methods A or B.[3] Some of the required starting materials were obtained from commercial sources and others by published procedures, *viz.*, *trans*-2,5-bis-(aminomethyl)-1,4-dioxane,[4] *trans*-1,4-bis(2-aminoethyl)cyclohexane,[5] 2,5-*bis*(aminomethyl)spiro[3.3]heptane,[6] *d-cis*-1,3-bis-(aminomethyl)camphocean,[7] *trans*-cyclohexane-1,4-diacetic acid,[5]

(1) For Part XV of this series see M. L. Givner and D. Dvornik, *Biochem. Pharmacol.*, in press.

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