2.330 to 4.940 × 10^{-6} M/min. with each value being determined by a least squares fit of 8 to 9 points.

α-N-Acetyl-L-tyrosinhydrazide.—Fifteen experiments with values of $[S]_0$ from 1.27 to 6.67 × 10^{-3} M, [E] = 0.208 mg. protein-nitrogen per ml. of Armour preparation no. 10705, total reaction time of 13 to 55 min., total extent of reaction of 5.8 to 28.5% gave 15 corrected values of v_0 of 0.735 to 3.117 × 10^{-5} M/min. with each value being determined by a least squares fit of 7 to 8 points.

α-N-Trimethylacetyl-L-tyrosinhydrazide.—Two sets of six experiments with values of $[S]_0$ from 0.494 to 1.08 × 10^{-3} M and 2.99 to 10.48 × 10^{-3} M, [E] = 0.1444 mg. protein-nitrogen per ml. of Armour preparation no. 00592, total reaction time of 32 to 60 min. and 16 to 36 min., total extent of reaction of 6.2 to 8.6% and 1.9 to 4.4% gave 12 corrected values of v_0 of 0.071 to 1.380 × 10^{-5} M/min. with each value being determined by a least squares fit of 8 to 9 points.

α-N-Dichloroacetyl-L-tyrosinhydrazide.—Ten experiments with values of $[S]_0$ from 0.909 to 3.82 × 10^{-3} M, [E] = 0.1444 mg. protein-nitrogen per ml. of Armour preparation no. 00592, total reaction time of 9 to 10 min., total extent of reaction of 10.0 to 15.0% gave 10 corrected values of v_0 of 0.145 to 0.420 × 10^{-4} M/min. with each value being determined by a least squares fit of 7 to 9 points.

α-N-Benzoyl-L-tyrosinhydrazide.—Two sets of experiments, one with 8 values of $[S]_0$ from 0.06 to 0.12 × 10^{-3} M and the other with 10 values of $[S]_0$ from 0.16 to 0.402 × 10^{-3} M, [E] = 0.208 mg. protein-nitrogen per ml. of Armour preparation no. 10705, total reaction time of 15 to 27 min. and 15 to 39 min., total extent of reaction of 57.1 to 80.1% and 53.4 to 82.7% gave 18 corrected values of v_0 of 0.290 to 1.862 × 10^{-5} M/min. with each value being determined by a least squares fit of 8 to 9 points. The conditions used in the competitive hydrolysis of α-N-acetyl- and α-N-benzoyl-L-tyrosinhydrazide are summarized in Table III.

TABLE III

α-CHYMOTRYPSIN-CATALYZED COMPETITIVE HYDROLYSIS OF α-N-ACETYL- AND α-N-BENZOYL-L-TYROSINHYDRAZIDE[a]

$[S_1]_0$ × 10^3 M	$[S_2]_0$ × 10^3 M	$[S_T]_0$ × 10^3 M	t_{max} (min.)	v_0[b] × 10^5 M/min.
0.140	0.268	0.408	14	0.696
.140	.358	.498	14	.762
.140	.447	.487	11	.918[c]

[a] In aqueous solutions at 25° and pH 7.9 and 0.02 M in the THAM component of a THAM-HCl buffer with [E] = 0.208 mg. protein-nitrogen per ml. of Armour preparation no. 10705. [b] From a least squares fit of 7 points unless otherwise noted. [c] 8 points.

α-N-Nicotinyl-L-tyrosinhydrazide.—Thirteen experiments with values of $[S]_0$ from 0.50 to 4.56 × 10^{-3} M, [E] = 0.208 mg. protein-nitrogen per ml. of Armour preparation no. 10705, total reaction time of 7 to 30 min., total extent of reaction of 13.1 to 47.8% gave 13 corrected values of v_0 of 0.102 to 0.808 × 10^{-4} M/min. with each value being determined by a least squares fit of 7 to 9 points.

PASADENA, CALIFORNIA

[CONTRIBUTION FROM THE CLAYTON FOUNDATION FOR RESEARCH, THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

DL-4-Oxalysine, an Inhibitory Analog of Lysine

BY TOMMY J. MCCORD, JOANNE M. RAVEL, CHARLES G. SKINNER AND WILLIAM SHIVE

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2-Amino-3-(β-aminoethoxy)-propionic acid (4-oxalysine) has been prepared through an acetamidomalonic ester condensation using 2-chloroethyl chloromethyl ether, followed by ammonolysis and hydrolysis of the intermediate, to give a mixture of products. The reaction mixture was separated into several pure components by chromatography on an alumina column. 4-Oxalysine inhibits the growth of a number of microörganisms. The reversal of this toxicity by lysine has been most extensively studied with *Leuconostoc dextranicum* 8086 and was found to be competitively reversed over a 300-fold range of concentrations with an inhibition index from 1 to 3.

The introduction of an oxygen atom in place of a methylene group in the carbon skeleton of a naturally occurring amino acid has resulted in the formation of an inhibitory analog.[1-3] In the present study, it was desired to prepare an analog of lysine by the introduction of an oxygen atom in place of the methylene group at the 4-position. Such an analog has the structural similarity and similar distance between the amino groups which would be necessary for antagonism of lysine. For example, 2,6-diaminoheptanoic acid (ε-C-methyllysine) has been reported to be an effective lysine inhibitor, but other compounds of similar structure but having amino groups different in distance apart from that of lysine are ineffective as inhibitors.[4] Accordingly, 2-amino-3-(β-aminoethoxy)-propionic acid (4-oxalysine) was prepared and found to be an effective inhibitory analog of lysine for several microorganisms.

In all of the synthetic approaches attempted, which resulted in the formation of 4-oxalysine, a mixture of products was obtained which proved difficult to separate. Further, the yield of by-products in every case exceeded that of the desired compound. The most satisfactory technique found was through an acetamidomalonic ester condensation with 2-chloroethyl chloromethyl ether. The desired product of this condensation, 2 - acetamido - 2 - (β - chloroethoxymethyl) - malonate, was always contaminated with unreacted starting material and only a 38% conversion[5] was obtained. Several attempts to improve this yield by using inverse addition, altering the temperature and/or time of addition, using other condensing agents[6] and by direct fusion of the reactants at elevated temperatures[7] failed. The substituted malonic ester derivative was treated with concentrated ammonium hydroxide at room temperature for several days to replace the terminal chlorine by an amino- group and then acid hydrolyzed; or

(1) N. H. Horowitz and A. M. Srb, *J. Biol. Chem.*, **174**, 371 (1948).

(2) M. Rabinovitz, M. E. Olson and D. M. Greenberg, THIS JOURNAL, **77**, 3109 (1955).

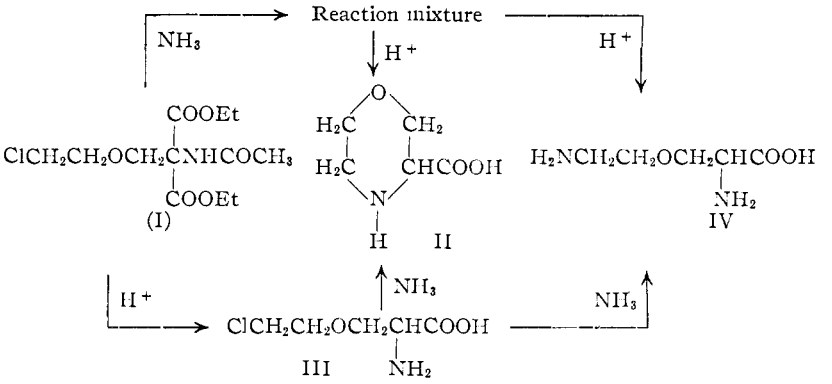
(3) C. G. Skinner, T. J. McCord, J. M. Ravel and W. Shive, *ibid.*, **78**, 2412 (1956).

(4) A. D. McLaren and C. A. Knight, *J. Biol. Chem.*, **204**, 417 (1953).

(5) This factor takes into account the recovered starting material.

(6) J. Shapira, R. Shapira and K. Dittmer, THIS JOURNAL, **75**, 3655 (1953).

(7) S. Maeda, M. Terumi and T. Suzuki, *Bull. Inst. Phys. Chem. Research (Tokyo)* **19**, 267 (1938); through *C. A.*, **34**, 6931 (1940).



alternately, the malonic ester derivative was hydrolyzed and decarboxylated, prior to the ammonia treatment, as indicated in the accompanying equations. Direct ammonolysis of the malonic ester derivative resulted in a mixture of products which were not isolated but were hydrolyzed directly and isolated through column chromatography.

The ultimate reaction mixture *via* either route was separated into several components by placing it on an alumina column and eluting with various concentrations of alcohol–water. The course of this separation was followed by paper chromatography using ninhydrin, supplemented by bioassays and bioautographs to determine the position and concentration of the active principle. Four major ninhydrin active products were present in the final reaction mixture prior to column purification, and only one of these, the lower R_f material, was biologically active in the assay system used. The three inactive compounds were identified as glycine, 3-morpholinecarboxylic acid and 2-amino-3-(β-chloroethoxy)-propionic acid. Incomplete ammonolysis of the malonic ester derivate would result in the presence of the chloro compound, and cyclization of this chloro derivative through dehydrohalogenation with the amino group accounts for the morpholine compound. The presence of glycine would be expected if a trace of unreacted acetamidomalonic ester were carried through the work-up procedure.

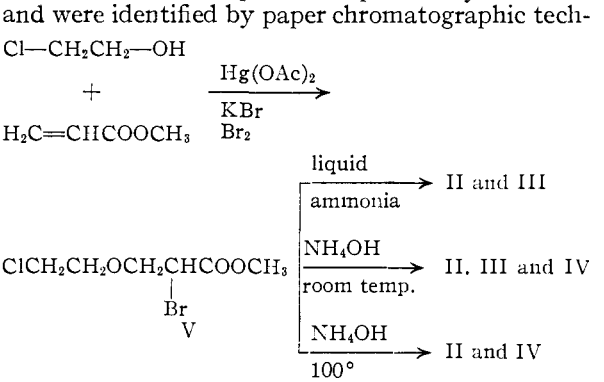
With regard to the low carbon accountability found by this procedure, it was observed in a separate experiment that acid hydrolysis of the malonic ester derivative resulted in the formation of an amount of ammonium chloride which accounted for 40% of the original nitrogen, as well as pyruvic acid, as by-products. These findings are in agreement with those observed by King[8] with a similar type malonic ester derivative.

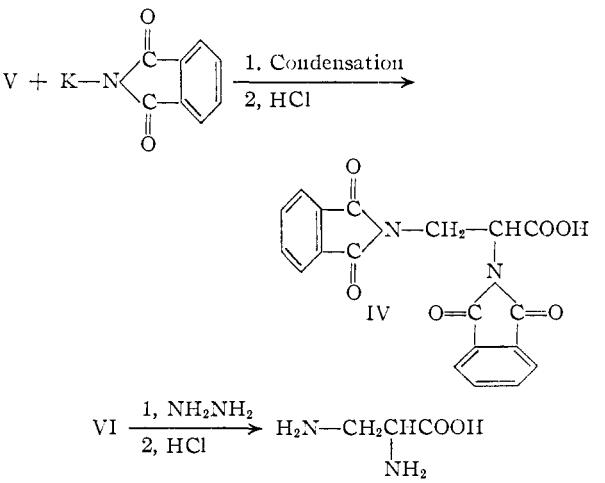
Other possible routes to 4-oxalysine which were studied involved the preparation of the appropriately substituted dihalo derivative and subsequent replacement of the halogens with an amino group. Methyl 2-bromo-3-(β-chloroethoxy)-propionate was prepared by the condensation of 2-chloroethanol and methyl acrylate in the presence of mercuric acetate, followed by bromination.[9]

(8) J. A. King, THIS JOURNAL, **69**, 2738 (1947).

(9) This procedure was patterned after the preparation of a similar type compound reported by L. R. Schiltz and H. E. Carter, *J. Biol. Chem.*, **116**, 793 (1936).

Ammonolysis of the resulting dihaloester resulted in the formation of a mixture of products similar to that previously observed *via* the acetamidomalonic ester route. Depending upon the conditions of ammonolysis there were produced varying amounts of the by-products, 3-morpholinecarboxylic acid and 2-amino-3-(β-chloroethoxy)-propionic acid, and only a small yield (*ca.* 1% based on biological activity) of the desired 4-oxalysine, as indicated in the accompanying equations. These compounds were separated by chromatography on an alumina column by the procedure previously described and were identified by paper chromatographic techniques and bioassays in the case of 4-oxalysine. In an attempt to increase the yield of the desired product using the dihaloester intermediate, a condensation with potassium phthalimide was made; however, instead of the desired compound, the material isolated from the reaction mixture proved to be 2,3-diphthalimidopropionic acid, which was subsequently hydrazinolyzed to yield α,β-diaminopropionic acid as indicated in the accompanying equations.





4-Oxalysine inhibits the growth of a number of microörganisms. Under the testing conditions subsequently described, the inhibitory levels of this oxa analog using 7 different bacteria are recorded in Table I. The inhibitory levels vary from 0.1 γ/ml. for *Leuconostoc dextranicum* 8086

TABLE I

INHIBITORY ACTIVITY OF 4-DL-OXALYSINE AND REVERSAL BY DL-LYSINE

Microörganism	Supplements:	Inhibitory level of 4-oxalysine, γ/ml. None	DL-Lysine, 20 γ/ml.
Leuconostoc dextranicum 8086		0.1	100
Leuconostoc mesenteroides 8293		.1	100
Streptococcus lactis 8039		.3	300
Lactobacillus arabinosus 17-5		10	1000
Lactobacillus casei 7469		0.3	30
Streptococcus fecalis 8043		[a]	1000
Escherichia coli 9723		3	>1000
Escherichia coli Texas strain		3	>1000

[a] This organism requires lysine for growth.

and *Leuconostoc mesenteroides* 8293 to 10 γ/ml. for *Lactobacillus arabinosus* 17–5. In the presence of a 20 γ/ml. supplement of DL-lysine, the inhibitory level is raised to 100 γ/ml., 100 γ/ml. and 1000 γ/ml., respectively, for each organism. The reversal of 4-oxalysine toxicity by lysine has been studied more extensively with *L. dextranicum* as indicated in Table II, and this analog was found to be competitively reversed by the natural amino acid over a 300-fold range with an inhibition index from 1 to 3.

Experimental[10]

Biological Assays.—For the lactic acid bacteria a previously described amino acid medium[11] was modified by the addition of calcium pantothenate (0.2 γ/ml.) and by the omission of lysine and with the additional modifications noted for each organism. For *Lactobacillus casei* 7469 and *Streptococcus fecalis* 8043, 20 γ/ml. of L-glutamine was added, without heating, to each assay tube. For *Leuconostoc mesenteroides* 8293 and *Leuconostoc dextranicum* 8086, pantethine was added (0.02 γ/ml.) and the phosphate concentration was increased fourfold. For *L. dextranicum*, the medium was further modified by the omission of aspartic acid. All of the assays were incubated at 30° with the exception of *L. casei* which was grown at 37°. The *L. casei* and *S. fecalis* tests were allowed to incubate for about 40 hr., while the other assays were completed between 18 and 24 hr.

TABLE II

REVERSAL OF 4-DL-OXALYSINE INHIBITION BY DL-LYSINE

Test organism, *L. dextranicum* 8086, incubated 18 hours at 30°

4-DL-Oxalysine, γ/ml.	DL-Lysine, γ/ml. 0	0.1	0.3	1.0	3.0	10.0	30.0
	Galvanometer readings						
0	54	60	61	61	61	61	60
0.03	34	57	60				
0.1	1	16	57	60			
0.3		3	4	56	59		
1.0				11	52	58	
3.0				0	27	57	59
10.0					1	52	53
30.0							48
100.0							1

For *Escherichia coli* (9723 and Texas strain), a previously described inorganic salts–glucose medium[12] was employed, and the assays were incubated at 37° for 18 to 24 hr. In all assays, the amount of growth was determined turbidimetrically in terms of galvanometer readings so adjusted that in the particular instrument distilled water read 0 and an opaque object 100.

2-Chloroethyl Chloromethyl Ether.—This compound was prepared by the general procedure of Lingo and Henze[13] using aqueous formaldehyde (37%), 2-chloroethanol and hydrogen chloride. The purified product was kept for long periods of time without excessive decomposition by storage in a glass-stoppered bottle over calcium carbonate.

Ethyl 2-Acetamido-2-(β-chloroethoxymethyl)-malonate.—To a hot solution of 86.6 g. of ethyl acetamidomalonate dissolved in 300 ml. of magnesium-dried ethanol containing 9.16 g. of sodium was added 51.3 g. of freshly distilled 2-chloroethyl chloromethyl ether in small batches with vigorous shaking. Sodium chloride precipitated immediately from the highly exothermic reaction. After addition was completed, the reaction mixture was heated to reflux for 3 hr. The salt was removed, and the solvent was distilled under reduced pressure. The residue was freed of alcohol by repeated addition and distillation under reduced pressure with benzene. By adding Skellysolve G (b.p. 40–70°) to a benzene solution of the residue and chilling in the refrigerator, there was recovered a total of 42.2 g. of unreacted ethyl acetamidomalonate in several fractions. The organic solvents were then removed, and there was obtained 55.1 g. of crude material in the form of an oil. This product was continuously extracted with Skellysolve G for 48 hr. A pale yellow oil, 40 g., separated from the solvent distilling flask but failed to solidify, even in the deep freeze chest. Twenty grams of this oil was fractionally distilled *in vacuo* to yield 12.0 g. of product, b.p. 165–175° (3 mm.).

Anal. Calcd. for $C_{12}H_{20}ClNO_6$: C, 46.53; H, 6.51. Found: C, 46.58; H, 6.24.

Ammonolysis and Hydrolysis of 2-Acetamido-2-(β-chloroethoxymethyl)-malonic Ester.—A mixture of 12.0 g. of the malonic ester derivative and 150 ml. of concentrated ammonium hydroxide was shaken continuously for 72 hr. during which time complete solution was effected. After reducing to dryness under reduced pressure, the excess ammonium hydroxide was removed by repeated addition and evaporation of ethanol. The residue was dissolved in 120 ml. of 6 *N* hydrochloric acid and heated under reflux for 2.5 hr., after which the hydrochloric acid was removed under reduced pressure, with warming, and the residue was freed of residual hydrochloric acid by repeated addition and evaporation of ethanol. The resulting solid was leached with 50 ml. of ethanol, and the ammonium chloride was removed by filtration. The filtrate was then taken to dryness under reduced pressure to yield 8.0 g. of material. A paper chromatogram of this reaction mixture in 1-butanol: acetic acid:water (3:1:1) using the ascending technique, showed, on development with ninhydrin, the presence of four spots with R_f values of 0.07, 0.16, 0.30 and 0.43. A bioautograph of 100 γ of the reaction mixture using *L. dextranicum* 8086 showed a zone of inhibition corresponding only to the R_f 0.07 spot, and in a tube assay the solution was inhibitory at a concentration of 0.4 γ/ml. The other ninhydrin spots were subsequently identified as glycine, 3-morpholinecarboxylic acid and 2-amino-3-(β-chloroethoxy)-propionic acid, respectively.

The desired reaction products were separated by column chromatography and subsequent recrystallization. A 3.5 × 25 cm. column containing 80 g. of activated alumina (Alcoa) was prepared in 90% alcohol. Eight grams of the above reaction mixture was charged to this column and fractionally eluted with alcohol–water solutions of varying concentrations:

Composition of eluent	Total volume effluent	Biological activity, %
90% ethyl alcohol	400	0
75% ethyl alcohol	400	10
50% ethyl alcohol	800	60

DL-4-Oxalysine (2-Amino-3-(β-aminoethoxy)-propionic Acid) Dihydrochloride.—The 50% alcohol fraction above was evaporated to dryness under reduced pressure to yield 780 mg. of residue. A water solution of this material was acidified to *p*H 1 with dilute hydrochloric acid and then concentrated in volume under reduced pressure. The addition

(10) All melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. The authors are indebted to Messrs. J. R. Claybrook, F. D. Talbert and D. L. Ross for the chemical analyses.

(11) J. M. Ravel, L. Woods, B. Felsing and W. Shive, *J. Biol. Chem.*, **206**, 391 (1954).

(12) E. H. Anderson, *Proc. Nat. Acad. Sci.*, **32**, 120 (1946).

(13) S. P. Lingo and H. R. Henze, THIS JOURNAL, **61**, 1574 (1939).

of alcohol to this concentrated solution yielded 421 mg. of solid product, which was recrystallized from ethanol–ethyl acetate solution to give small white needles, m.p. 190–200° dec. The R_f values, using the ascending technique and development with ninhydrin, in 1-butanol:acetic acid:water (3:1:1), 65% pyridine and 95% methanol were 0.07, 0.20 and 0.15, respectively.

Anal. Calcd. for $C_5H_{14}Cl_2N_2O_3$: C, 27.16; H, 6.38; N, 12.67. Found: C, 26.95; H, 6.46; N, 12.87.

2-Amino-3-(β-chloroethoxy)-propionic Acid.—After standing in the cold overnight, a white crystalline solid precipitated in the initial 100 ml. of 90% alcohol effluent from the alumina column above. It was filtered, washed with cold ethanol and dried under vacuum over anhydrous calcium chloride to yield 250 mg. of product, m.p. 178–180°. This material was biologically inactive in the assay system and was characterized by chemical analysis and by subsequent conversion to 4-oxalysine with concentrated ammonium hydroxide. The R_f values, using the ascending technique and development with ninhydrin, in 1-butanol:acetic acid:water (3:1:1), 65% pyridine and 95% methanol were 0.43, 0.70 and 0.56, respectively.

Anal. Calcd. for $C_5H_{10}ClNO_3$: C, 35.83; H, 6.01. Found: C, 35.90; H, 6.02.

In a separate experiment, (2-chloroethoxymethyl)-acetamidomalonic ester (12.0 g.) was acid hydrolyzed, without prior ammonolysis, using 150 ml. of 6 *N* hydrochloric acid for 4 hr. A small aliquot of the reaction mixture was neutralized with concentrated sodium hydroxide and, after treatment with ammonium hydroxide, followed by sodium nitroprusside solution, developed a green color indicating the presence of pyruvic acid.[14] The main body of the reaction mixture was then reduced to dryness *in vacuo*, and the residue was extracted with hot ethanol. The insoluble fraction (0.9 g.) was ammonium chloride. The alcoholic solution was adjusted to *p*H 6 with concentrated ammonium hydroxide, and 1.8 g. of white solid precipitated. A paper chromatogram of this material in several solvents indicated the presence of only two ninhydrin active components, glycine and 2-amino-3-(β-chloroethoxy)-propionic acid. After several recrystallizations from isopropyl alcohol–water, there was recovered 0.5 g. of an analytically pure sample of the latter compound. The occurrence of ammonium chloride and pyruvic acid as degradation products are in agreement with the data observed by King on a similar type malonic ester derivative.[15]

3-Morpholinecarboxylic Acid.—The 75% alcohol fraction from the alumina column above was reduced to dryness *in vacuo* to yield an oil. Several attempts to crystallize this oil from various solvents failed to yield a solid product. The oil was finally dissolved in water and taken to *p*H 1 with dilute hydrochloric acid. When the water was removed *in vacuo*, there remained a white solid which was taken up in a very small amount of water, and then 6 volumes of alcohol were added. After standing overnight in the refrigerator, 200 mg. of material precipitated, m.p. 245–250° dec. R_f values, by the ascending technique after development with ninhydrin, in 1-butanol:acetic acid:water (3:1:1), 65% pyridine and 95% methanol were 0.30, 0.60 and 0.46, respectively.

Anal. Calcd. for $C_5H_9NO_3 \cdot H_2O$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.46; H, 7.80; N, 9.49.

Methyl 2-Bromo-3-(β-chloroethoxy)-propionate.—A mixture of 23.1 g. of methyl acrylate, 79.4 g. of mercuric acetate and 200 ml. of 2-chloroethanol was shaken at room temperature for 48 hr. To the cooled reaction mixture, 29.75 g. of potassium bromide, dissolved in 300 ml. of water, was added slowly with stirring, and the resulting oil was recovered. The aqueous phase was extracted with chloroform, and the combined organic phase was dried over magnesium sulfate. After decantation from the drying agent, 40.0 g. of bromine was added dropwise, over a period of 1 hr., to the chloroform solution illuminated with a 250 watt photoflood bulb, while maintaining a reaction temperature of 50–55° with occasional cooling. Upon refrigeration of the reaction mixture, 68 g. of mercuric bromide precipitated, which was filtered, and the filtrate was distilled under reduced pressure to yield a forerun of 19.0 g. of material, b.p. 30–90° (3 mm.). The resulting residue yielded additional mercuric bromide which was removed prior to continued distillation of the dark oil. There was recovered 35 g. of crude dihaloester which was fractionally redistilled to yield 23.4 g. of material, b.p. 96–102° (3 mm.), $n^{25}D$ 1.4783.

Anal. Calcd. for $C_5H_{10}BrClO_3$: C, 29.35; H, 4.11. Found: C, 29.46; H, 4.36.

Ammonolysis and Hydrolysis of Methyl 2-Bromo-3-(β-chloroethoxy)-propionate.—A. The ester was placed in a 150 × 25 mm. glass tube and immersed in a Dry Ice–acetone-bath, following which an equal volume of liquid ammonia was condensed in the cold section. The bomb was then sealed and allowed to stand at room temperature for 12 hr., after which time a precipitate of ammonium halide was observed. The reaction mixture was cooled, the bomb opened, and the excess ammonia was allowed to evaporate at room temperature. The residue was extracted with hot ethanol and subsequently taken up in water to yield a reaction mixture which proved to be biologically inactive. The major products were identified as 3-morpholinecarboxylic acid and 2-amino-3-(β-chloroethoxy)-propionic acid.

B. A sample of the ester was placed in concentrated ammonium hydroxide and allowed to stand at room temperature for several days to effect complete solution of the oil phase. After removal of the solvent, the residue proved to contain only a small amount of 4-oxalysine, as evidenced by paper chromatography and bioassay, along with some 3-morpholinecarboxylic acid and 2-amino-3-(β-chloroethoxy)-propionic acid.

C. The above conditions were repeated except that the reaction mixture was heated at 100° in a steel bomb for 24 hr. The solvent was removed, and the residue was placed on an alumina column and eluted with alcohol–water solutions, as previously described, to yield 4-oxalysine (1% yield) and 3-morpholinecarboxylic acid.

Methyl 2,3-Diphthalimidopropionate.—To a solution of 12.3 g. of methyl 2-bromo-3-(β-chloroethoxy)-propionate dissolved in 100 ml. of dimethylformamide was added 18.5 g. of potassium phthalimide. The reaction mixture was heated to 100° for 1 hr. with frequent shaking; 150 ml. of chloroform was then added, and the resulting mixture was poured into 450 ml. of water. The chloroform layer was separated, the aqueous phase was extracted with fresh chloroform and the combined organic phase was then washed with cold 0.1 *N* sodium hydroxide and dried over magnesium sulfate. After removal of the solvent *in vacuo*, trituration of the residue with ether gave 14 g. of crude material, which was recrystallized from benzene–ether to yield a pure product, m.p. 205–207°.

Anal. Calcd. for $C_{20}H_{14}N_2O_6$: C, 63.49; H, 3.73; N, 7.40. Found: C, 63.54; H, 3.77; N, 7.54.

2,3-Diaminopropionic Acid Hydrochloride.—A mixture of 4.2 g. of methyl 2,3-diphthalimidopropionate suspended in 50 ml. of methanol containing one ml. of 98% hydrazine was heated under reflux for 1 hr. After addition of 25 ml. of water, the methanol was removed *in vacuo*, and the aqueous residue was treated with 25 ml. of concentrated hydrochloric acid and heated under reflux for an additional hour. After filtering, to remove the insoluble phthalhydrazide, the filtrate was taken to dryness under reduced pressure. The resulting solid material was extracted twice with 25-ml. portions of hot ethanol and the solution concentrated to a small volume and, finally, treated with pyridine to yield a very fine white solid. The solid was collected, washed with cold ethanol followed by ether and dried over phosphorus pentoxide *in vacuo* to give 0.7 g. of product which was subsequently recrystallized from ethanol–ether, m.p. 226–227°.[16]

Anal. Calcd. for $C_3H_9ClN_2O_2$: C, 25.63; H, 6.45; N, 19.93. Found: C, 25.71; H, 6.81; N, 19.60.

AUSTIN, TEXAS

(14) L. Simon, *Compt. rend.*, **125**, 534 (1897).

(15) J. A. King, THIS JOURNAL, **69**, 2738 (1947).

(16) The reported m.p. is 225° dec.; E. Klebs, *Z. physiol. Chem.*, **19**, 316 (1894).