and the precipitate washed well with water. There was obtained 5.50 g. (93% yield) of α,α'-bis(2-quinolyl)-*p*-xylylene-α,α'-dione (VI) m.p. 282–284°. After several recrystallizations from acetone the compound melted at 283.5–284.5°.

Anal. Calcd. for $C_{26}H_{16}N_2O_2$: C, 80.39; H, 4.15; N, 7.21. Found: C, 80.16; H, 4.37; N, 7.07.

In a similar fashion, oxidation of the diastereoisomeric diol of m.p. 179–184° yielded a white solid of m.p. 282–284°. A mixed melting point test with the analyzed sample of α,α'-bis(2-quinolyl)-*p*-xylylene-α,α'-dione (VI) obtained by the oxidation of the diol of m.p. 193–196° showed no depression. The infrared spectra, taken in potassium bromide pellets, of the two samples of the oxidation product were found to be identical.

α,α'-Bis(2-quinolyl)-p-xylylene. A mixture of 5.78 g. (0.0149 mole) of α,α'-bis(2-quinolyl)-*p*-xylylene-α,α'-dione (VI), 40 cc. of 85% hydrazine and 150 cc. of triethyleneglycol was refluxed for 2 hr. The initially white solid gradually changed to yellow. Excess hydrazine and water were evaporated by heating the mixture under an air-jet, and the temperature was raised to about 220°. Addition of about 5.0 g. of potassium hydroxide caused a vigorous reaction to ensue, one terminating in the formation of a golden brown solution. The solution was heated at 220° for 1 hr., then allowed to cool slowly to room temperature. Approximately 100 cc. of water was added to the solution causing formation of a crystalline solid. The solid, α,α'-bis(2-quinolyl)-*p*-xylylene, after filtration and drying, weighed 5.31 g. (quantitative yield) and melted at 132–135°. Recrystallization from an ethanol-water mixture yielded needle-like crystals of m.p. 135–136°.

Anal. Calcd. for $C_{26}H_{20}N_2$: C, 86.63; H, 5.59; N, 7.77. Found: C, 86.70; H, 5.50; N, 7.85.

α,α'-Bis(2-quinolyl)-p-xylylene dimethiodide (IV). A Parr bomb was charged with 1.0 g. (0.0028 mole) of α,α'-bis(2-quinolyl)-*p*-xylylene and 12 g. of methyl iodide and heated for 9 hr. at 75°. After evaporation of the excess methyl iodide under vacuum there was obtained 2.30 g. of a yellow-green solid decomposing at 273°. The solid was dissolved in boiling water and hydrogen iodide solution was added until the solution was acidic. After filtration of the hot solution, the filtrate, on cooling, yielded 1.71 g. (98% yield) of a yellow solid, α,α'-bis(2-quinolyl)-*p*-xylylene dimethiodide (IV), melting with decomposition at 289–292°.

Anal. Calcd. for $C_{28}H_{26}N_2I_2$: C, 52.19; H, 4.07; N, 4.35; I, 39.39. Found: C, 52.23; H, 4.45; N, 4.50; I, 39.22.

O,O-Dibenzoyl-α,α'-bis(2-quinolyl)-o-xylylene-α,α'-diol. The lithium salt of 1-benzoyl-1,2-dihydroquinaldonitrile was treated with phthalaldehyde in the same manner as that described for the preparation of the *p*-xylylene compounds. Addition of 20 cc. of water to the reaction mixture resulted in complete solution of the solid. The organic layer was separated, washed with three 10-cc. portions of water, dried over anhydrous magnesium sulfate, and evaporated to yield a reddish oil. Subjection of the oil to vacuum treatment for several days failed to induce complete solidification. In an attempt to recrystallize the gum (weight 10.77 g.) from 95% ethanol, a slight amount of a light yellow solid was found to be insoluble. After it had been collected by filtration, the solid weighed 1.50 g. and melted at 217–221°. Recrystallization from a benzene-petroleum ether mixture (b.p. 60–110°) yielded a solid which melted at 226.5–227.5°.

Anal. Calcd. for $C_{40}H_{28}N_2O_4$: C, 79.98; H, 4.70; N, 4.67. Found: C, 79.76; H, 4.84; N, 4.97.

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LAWRENCE, KAN.

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

Synthesis and Biological Properties of 4-Amino-5-isopropyl-3-isoxazolidone, a Substituted Cycloserine

KYOZO HAYASHI,[1] CHARLES G. SKINNER, AND WILLIAM SHIVE

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α-Bromo-β-hydroxy-γ-methylpentanoic acid was treated with ammonium hydroxide to yield the desired α-amino acid which was then esterified in the presence of anhydrous hydrogen chloride with ethanol. The β-hydroxy grouping was subsequently replaced by chlorine and the resulting ethyl α-amino-β-chloro-γ-methylpentanoate was finally treated with hydroxylamine and cyclized to yield 4-amino-5-isopropyl-3-isoxazolidone (I). I is inhibitory to the growth of *Leuconostoc dextranicum* when this organism is grown in a medium containing D-leucine as the exogenous source of this required metabolite; however, I is essentially not toxic for *L. dextranicum* when it is grown on media containing DL-leucine. It appears that appropriately substituted cycloserine derivatives may specifically inhibit D-amino acid functions.

Cycloserine (D-4-amino-3-isoxazolidone)[2a] was originally isolated from culture filtrates of streptomycetes[2b] and its chemical structure was subsequently established.[3,4] It is an antibiotic which has been found effective in a number of microbial systems.[5] Metabolic studies with this compound have demonstrated it to be a competitive antagonist of D-alanine in preventing the incorporation of D-alanine into a uridine nucleotide intermediate

(1) Rosalie B. Hite postdoctoral fellow, 1959–1960.

(2)(a) Oxamycin, Merck and Co., Inc.

(2)(b) For references see *Antibiotics, Their Chemistry and Non-Medical Uses*, ed. H. S. Goldberg, Van Nostrand, New York, 1959, p. 71.

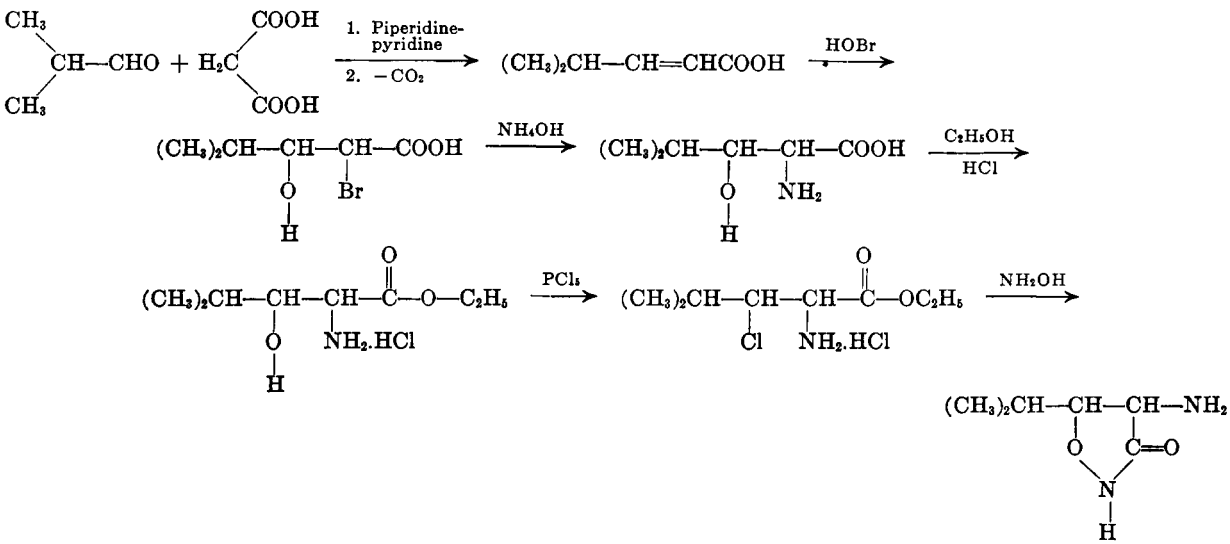
(3) F. A. Kuehl, F. J. Wolf, N. R. Trenner, R. L. Peck, E. Howe, B. D. Hunnewell, G. Dowing, E. Newstead, R. P. Buhs, I. Putter, R. Ormond, J. E. Lyons, L. Chaiet, and K. Folkers, *J. Am. Chem. Soc.*, **77**, 2344 (1955).

(4) P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Runge, H. E. Stavely, A. Pohland, H. Boaz, and H. R. Sullivan, *J. Am. Chem. Soc.*, **77**, 2345 (1955).

(5) E. B. Chain, *Ann. Rev. Biochem.*, **27**, 167 (1958).

essential for cell wall synthesis in *Staphylococcus aureus*.[6] Recently, the antibiotic has been reported to inhibit alanine racemase and an enzyme which catalyzes the synthesis of D-alanyl-D-alanine.[7] Cell wall synthesis in *Streptococcus faecalis* is also inhibited by cycloserine, and the toxicity is competitively reversed by D-alanine, but not by L-alanine.[8] The L-form of this antagonist is also less inhibitory than either D- or DL-cycloserine to the growth of *E. coli*.[9] Several 2-phenyl-5-aryl-cycloserine analogs have recently been synthesized, and found to be inhibitory to both gram-positive and gram-negative bacteria[10]; and more recently, the synthesis of the threonine analog, 4-amino-5-methyl-3-isoxazolidone, was reported, but without any microbiological data on its inhibitory activity.[11] In view of the fact that the biological activities of cycloserine indicate that it is an antagonist of D-alanine,[6] the synthesis of comparable analogs structurally related to other natural amino acids would seem to be desirable. Accordingly, the structural analog corresponding to leucine, 4-amino-5-isopropyl-3-oxazolidone, was prepared, and its inhibitory properties were examined in several microbial systems.

The series of reactions leading to the formation of 4-amino-5-isopropyl-3-isoxazolidone are summarized in the accompanying equations.



The synthesis of the desired intermediate, α-bromo-β-hydroxy-γ-methylpentanoic acid, was adapted from the procedure for the preparation of a corresponding α-bromo-β-hydroxy intermediate used in the synthesis of threonine.[12] During the course of this reaction, which involved bromine-water as the source of hypobromous acid, some α,β-dibromo-γ-methylpentanoic acid was also formed. The α-bromo group of the former derivative was then replaced by an amino grouping using concentrated ammonium hydroxide, and the desired hydroxyamino acid was isolated and purified using an Amberlite IR-120 column. α-Amino-β-hydroxy-γ-methylpentanoic acid was converted to the ethyl ester hydrochloride using ethanol and anhydrous hydrogen chloride, after which the hydroxyl grouping was replaced by chlorine using phosphorus pentachloride. Finally, the α-amino-β-chloro ester was treated with hydroxylamine to form the desired cycloserine analog, 4-amino-5-isopropyl-3-isoxazolidone. The latter derivative was isolated and purified on an Amberlite IR-120 column.

An alternate route to prepare the cycloserine analog through a hydantoin intermediate was also considered. α-Amino-β-hydroxy-γ-methylpentanoic acid was treated with potassium cyanate and the desired intermediate, 5-(1-hydroxy-2-methylpropyl)hydantoin, was isolated. Initial attempts to convert this hydroxy grouping to a bromo substituent failed to yield the desired product; instead, the corresponding alkylidene derivative, 5-(2-methylpropylidene)hydantoin, was isolated. The structure of this product was confirmed by reduction to the saturated analog and comparing the resulting derivative with an authentic sample of 5-(2-methylpropyl)hydantoin prepared directly from leucine by the usual procedures. In addition, the hydrogenated alkylidene product was hydrolyzed under alkaline conditions, and leucine was

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(7) J. L. Strominger, *Physiol. Rev.*, 40, 87 (1960).

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(12) H. E. Carter and C. L. Zirkle, *J. Biol. Chem.*, 178, 709 (1949).

identified as the amino acid formed by paper chromatographic techniques and by elemental analysis.

5-(1-Chloro-2-methylpropyl)hydantoin was finally prepared from the corresponding 5-(1-hydroxy-2-methylpropyl)hydantoin derivative using phosphorus pentachloride with acetyl chloride as a solvent. However, subsequent attempts to introduce the aminoxy grouping failed to yield analytically homogenous derivatives, and this potential route of synthesis was rejected in favor of the procedure initially presented in this article.

The growth inhibitory properties of 4-amino-5-isopropyl-3-isoxazolidone were examined in a variety of microörganisms including *Streptococcus lactis* 8039, *Lactobacillus arabinosus* 17-5, *Streptococcus faecalis* 8043, *Leuconostoc dextranicum* 8086, *Pediococcus cerevisiae* 8081, and *Escherichia coli* 9723, but no general toxicity was observed up to a level of 200 γ/ml. The media used contained a minimal amount of valine, isoleucine and leucine, all of which were in the DL-configuration. Under comparable assay conditions, cycloserine was inhibitory to the growth of these organisms at levels of 60, 200, 20, 60, >200, and <6 γ/ml., respectively. Since D-leucine may not be required by these organisms for essential metabolic roles, it was of interest to determine whether the isopropyl-cycloserine analog would inhibit utilization of D-leucine as a substitute for L-leucine in the usual assay media. For *L. dextranicum*, D-leucine is utilized about one-tenth as effectively as the L-isomer; a supplement of 10 γ/ml. of the D-form gives near maximal growth. Under these latter testing conditions with *L. dextranicum*, using an amino acid medium containing L- or DL-amino acids in every case except that D-leucine was the sole exogenous source of this amino acid, the isopropylcycloserine derivative was inhibitory to growth, and was reversed in a competitive manner over a four-fold range of increasing concentrations of D-leucine (Table I). The inhibition index (ratio of inhibitor to substrate necessary for maximal inhibition) is about 20.

TABLE I

INHIBITION OF D-LEUCINE UTILIZATION BY 4-AMINO-5-ISOPROPYL-3-ISOXAZOLIDONE IN *Leuconostoc dextranicum*[a]

4-Amino-5-isopropyl-3-isoxazolidone, γ/ml.	Supplement, D-leucine, γ/ml. 5	10	20
	Galvanometer readings		
0	50	55	51
10	49		
20	49	51	
50	36	48	48
100	13	34	50
200		7	21
500			8

[a] Media described in ref. 14; except that the DL-isoleucine and DL-valine were decreased to 10 γ/ml.; the DL-glutamic acid was increased to 100 γ/ml.; the DL-leucine was omitted, and D-leucine was added as indicated.

Since it is probable that D-leucine does not perform an essential role in the metabolic processes of *L. dextranicum*, the inhibition of the racemization and/or utilization of D-leucine by isopropyl-cycloserine is of no consequence to the organism grown on L-leucine. In contrast, D-alanine performs an essential role in several metabolic functions and thus an inhibitor of this amino acid, cycloserine, is an effective antimetabolite. Since there are other D-amino acids which have been demonstrated to have essential roles in microörganisms, the corresponding substituted-cycloserine analogs of these metabolites may be expected to have growth inhibitory activities similar to cycloserine.

EXPERIMENTAL[13]

Biological assays. For the survey of microbial toxicity with lactic acid bacteria a previously described amino acid medium[14] was modified by the addition of calcium pantothenate (0.2 γ/ml.) and by decreasing the concentrations of DL-leucine, DL-isoleucine and DL-valine to 10 γ/ml.; by increasing the concentration of DL-glutamic acid to 100 γ/ml.; and with the additional modifications noted for each organism. The media was used without further modifications for *Streptococcus lactis* 8039, *Lactobacillus arabinosus* 17-5 and *Streptococcus faecalis* 8043. For *Leuconostoc dextranicum* 8086 pantethine was added (0.02 γ/ml.) and the phosphate concentration was increased four-fold. For *Pediococcus cerevisiae* 8081 the phosphate concentration also increased four-fold. All of the assays were incubated at 30° with the exception of *P. cerevisiae* which was incubated at 37°. The *S. faecalis* assay was allowed to grow for about 40 hr., while the other assays were completed in about 20 hr.

For *Escherichia coli* 9723 a previously described inorganic salts-glucose medium[15] was used, and the assays were incubated at 37° for about 16 hr.

For the assays reported in Table I utilizing D-leucine as the source of this amino acid in the medium, the basal media previously described for *L. dextranicum* was used except that DL-leucine was omitted, and D-leucine was added as indicated in the Table. 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.

α-Bromo-β-hydroxy-γ-methylpentanoic acid[16]. 4-Methyl-2-pentenoic acid was prepared by the condensation of 72 g. of isobutyraldehyde and 104 g. of malonic acid followed by decarboxylation to yield 101 g. of product, b.p. 115–118° (24 mm.).[17] A well stirred solution of 300 g. of 4-methyl-2-pentenoic acid in 5 l. of water was cooled to 0–5° in an ice bath, and 270 g. of bromine mixed with air was bubbled through the reaction mixture at such a rate that only a slight yellowish bromine color was evident during the addition reaction. The bromine-air mixture was obtained by bubbling dry air through the liquid bromine in a suitable vessel, and about 5 hr. was required to utilize all of the

(13) All melting points are uncorrected. The authors are indebted to Dr. J. M. Ravel and Mrs. Jean Humphries for assistance with the microbial testing. The analyses were carried out by Dr. Alfred Bernhardt, Mülheim, Germany.

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

(15) E. H. Anderson, *Proc. Natl. Acad. Sci.*, **32**, 120 (1946).

(16) This preparative procedure was patterned after that of H. E. Carter and C. L. Zirkle (10) for the synthesis of α-bromo-β-hydroxybutyric acid.

(17) R. P. Linstead, *J. Chem. Soc.*, 2498 (1929).

halogen. After all of the bromine was added, air was bubbled through the solution to expel any excess bromine gas. During the bromine addition, a crystalline precipitate formed which was recovered and is characterized below as α,β-dibromo-γ-methylpentanoic acid. The resulting reaction mixture was extracted with 500 ml. of petroleum ether (b.p. 60–68°); the aqueous phase was then saturated with sodium chloride and finally extracted three times with 200-ml. portions of ether. The combined organic phase was dried over sodium sulfate, and the solvents were then removed *in vacuo*. There was recovered 300 g. of product which was recrystallized from petroleum ether, m.p. 126–127°.

Anal. Calcd. for $C_6H_{11}BrO_3$: C, 34.14; H, 5.25. Found: C, 34.11; H, 5.57.

α,β-Dibromo-γ-methylpentanoic acid. The crystalline precipitate formed during the course of the reaction above for the synthesis of α-bromo-β-methylpentanoic acid was filtered to yield 27 g. of product. The material was recrystallized from petroleum ether, m.p. 124–125°.

Anal. Calcd. for $C_6H_{10}Br_2O_2$: C, 26.30; H, 3.65. Found: C, 26.66; H, 3.84.

α-Amino-β-hydroxy-γ-methylpentanoic acid. A sample of 40 g. of α-bromo-β-hydroxy-γ-methylpentanoic acid was dissolved in 400 ml. of concd. ammonium hydroxide and allowed to stand at room temperature for 4 days. The reaction mixture was then reduced in volume *in vacuo* to yield a residue containing the desired amino acid derivative and ammonium bromide. The residue was taken up in 200 ml. of water and charged to an Amberlite IR-120 column (35 mm. × 300 mm.). The column was washed with distilled water until the effluent gave a negative test for bromide ion. The amino acid was then eluted with 1000 ml. of 0.5*N* ammonium hydroxide, and after the combined ninhydrin-positive eluates were concentrated to a small volume *in vacuo*, ethyl alcohol was added to induce crystallization. The resulting product was then recrystallized from alcohol-water to yield 16 g. of material, m.p. 237–238°.

Anal. Calcd. for $C_6H_{13}NO_3$: C, 49.10; H, 8.85; N, 9.52. Found: C, 49.01; H, 9.13; N, 9.43.

5-(1-Hydroxy-2-methylpropyl)hydantoin. A solution containing 12 g. of α-amino-β-hydroxy-γ-methylpropanoic acid and 6.5 g. of potassium cyanate in 75 ml. of water was heated at 65° for 2 hr. with stirring. The resulting reaction mixture was then treated with 50 ml. of 48% hydrobromic acid and heated an additional 2 hr. at 90°. Finally, the solution was reduced to dryness *in vacuo* and the residue was crystallized from ethanol-water to yield 7 g. of product, m.p. 223–225°.

Anal. Calcd. for $C_7H_{12}N_2O_3$: C, 48.83; H, 6.98; N, 16.30. Found: C, 48.86; H, 6.99; N, 16.20.

5-(2-Methylpropyledene)hydantoin. Several attempts were made to convert the hydroxyl group of 5-(1-hydroxy-2-methylpropyl)hydantoin (I) to the corresponding bromo derivative; however, in each instance the product isolated was an unsaturated compound which gave a negative test for halogen. (1) A solution of 5 g. of I in 50 ml. of 48% hydrobomic acid was heated at 90–95° for 2 hr., the reaction mixture was reduced to dryness *in vacuo* to yield 6.5 g. of white needles. The product was recrystallized from ethanol-water, m.p. 194–195° (II). (2) A mixture of 1.2 g. of I suspended in 10 ml. of carbon tetrachloride was treated with 5 g. of phosphorus tribromide and heated at 60–70° for 2 hr. After reduction in volume *in vacuo* the residue was crystallized from ethanol-water to yield the same product as indicated above, 5-(2-methylpropyledene) hydantoin, II.

Anal. Calcd. for $C_7H_{10}N_2O_2$: C, 54.53; H, 6.54; N, 18.10. Found: C, 54.64; H, 6.71; N, 17.62.

A 0.3-g. sample of II was dissolved in 30 ml. of ethanol and hydrogenated in the presence of 0.1 g. of platinum oxide at atmospheric pressure for 2 hr. The catalyst was filtered, the filtrate was reduced to dryness *in vacuo*, and the residue was crystallized from ethanol-water to yield 250 mg. of compound, m.p. 209–211°. The saturated product isolated did not depress the melting point of an authentic sample of 5-(2-methylpropyl)hydantoin prepared from DL-leucine by the usual procedure with potassium cyanate under acidic conditions.

The hydrogenated hydantoin isolated above from II was finally hydrolyzed in the presence of barium hydroxide to yield the amino acid DL-leucine which was identified by elemental analyses, and by paper chromatography in several solvent systems.

5-(1-Chloro-2-methylpropyl)hydantoin. A sample of 1.5 g. of 5-(1-hydroxy-2-methylpropyl)hydantoin was suspended in 30 ml. of acetyl chloride and cooled to 0–5°, after which, 2.5 g. of phosphorus pentachloride was added and the reaction was allowed to continue for about 2 hr. The resulting precipitate was filtered, and the filter was washed with carbon tetrachloride and finally water. The solid was crystallized from ethanol-water to yield 0.85 g. of product, m.p. 175–177°.

Anal. Calcd. for $C_7H_{11}N_2O_2Cl$: C, 44.10; H, 5.81; N, 14.70. Found: C, 44.58; H, 5.82; N, 14.75.

Ethyl α-amino-β-hydroxy-γ-methylpentanoate hydrochloride. Dry hydrogen chloride gas was bubbled through a suspension of 8.3 g. of α-amino-β-hydroxy-γ-methylpentanoic acid in 50 ml. of ethanol while the reaction mixture was maintained at reflux temperature. After about 5 hr., the resulting solution was cooled and concentrated to a small volume *in vacuo*. The residue was finally crystallized from ethanol-ethyl acetate to yield 9 g. of product, m.p. 124–125°.

Anal. Calcd. for $C_8H_{18}NO_3Cl$: C, 45.38; H, 8.54; N, 6.62. Found: C, 44.77; H, 8.55; N, 6.57.

Ethyl α-amino-β-chloro-γ-methylpentanoate hydrochloride. To a suspension of 2 g. of ethyl α-amino-β-hydroxy-γ-methylpentanoate hydrochloride in 20 ml. of acetyl chloride was added 3 g. of phosphorus pentachloride with continuous stirring and external cooling. The solid reactant dissolved after about 30 min., and stirring was continued for an additional 2 hr. The reaction mixture was then concentrated to a small volume *in vacuo*, and ether was then added plus a small amount of petroleum ether to induce turbidity. After the solution was kept in the refrigerator overnight, there was recovered 2 g. of white needles, m.p. 132–133°.

Anal. Calcd. for $C_8H_{17}NO_2Cl_2$: C, 41.75; H, 7.44; N, 6.09. Found: C, 42.14; H, 7.61; N, 6.16.

DL-4-Amino-5-isopropyl-3-isoxazolidone. A solution of 5.8 g. of sodium hydroxide in 12 ml. of water was cooled to −5° and 3.4 g. of hydroxylamine hydrochloride was added with stirring. To this cold solution was then added 6 g. of ethyl α-amino-β-chloro-γ-methylpentanoate hydrochloride, and the temperature was maintained at −5° with stirring for about 1 hr. The temperature was then allowed to rise to 25° and stirring was continued an additional 2 hr. The reaction mixture was then cooled to −10° and the *p*H of the solution was adjusted to 6 with concentrated hydrochloric acid, after which it was concentrated *in vacuo* to a viscous residue. The residue was extracted with ethanol, the alcohol was removed, and the resulting residue was finally taken up in 200 ml. of water. This aqueous solution was then charged to an Amberlite IR-120 column (15 mm. × 150 mm.), the column was washed with 250 ml. of water, and then eluted with a total of 250 ml. of 0.2*N* ammonium hydroxide. The ammonia eluate was concentrated *in vacuo* to give a precipitate which was subsequently recrystallized from water to yield 0.7 g. of product, m.p. 183–184° dec.

Anal. Calcd. for $C_6H_{12}N_2O_2$: C, 49.98; H, 8.39; N, 19.43. Found: C, 49.93; H, 8.27; N, 19.61.

AUSTIN, TEX.