[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

SYNTHESIS OF SOME GUANIDINO AMINO ACIDS FROM CYANOGEN BROMIDE[1, 2]

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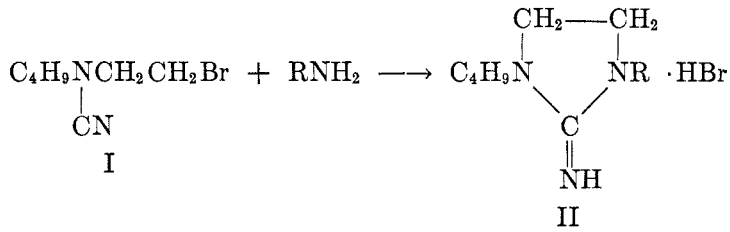
In a preceding communication (1) the preparation of representative cyclic guanidine derivatives of the type of II by the reaction of bromoalkylcyanamides with primary amines was described.



Although none of the many guanidine derivatives which have been examined as potential insulin substitutes has been found to exhibit appreciable hypoglycemic action independent of toxicity, it was felt that incorporation of amino acid units into a guanidine might be worthy of study. Guanidines containing amino acid residues have not been prepared previously. Although amino acids display no hypoglycemic activity, a recent report (2) indicates that administration of some amino acids may potentiate the hypoglycemic action of insulin. Accordingly the above reaction has been applied to the alkylation of representative amino acids.

The alkylation of aliphatic amines by β-bromoethylbutylcyanamide (I) to form cyclic guanidines takes place with explosive violence in the absence of a solvent, but smoothly and in excellent yield in boiling alcohol (1). The analogous alkylation of an amino acid is not a simple reaction. Free amino acids cannot be used in as much as they exist almost entirely as dipolar ions which are incapable of alkylation. It therefore becomes necessary to employ a derivative of the amino acid in which a free amino group is present. We have investigated the use of three classes of such derivatives in so far as their alkylation by I is concerned.

Amino acid esters provide a free amino group but suffer by virtue of their ready polymerization. Since a primary alkyl amine is about a thousand times as strong a base as an amino acid ester (3), alkylation of the ester is a much less vigorous reaction. Although a solvent is preferred, use of one, at least with

[1] The material here presented is taken from a dissertation submitted by Milton Green in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Columbia University.

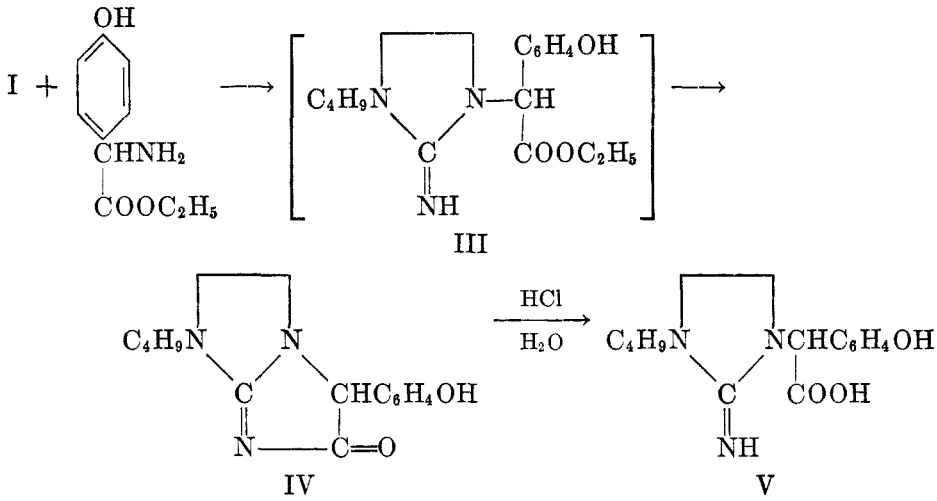
[2] We wish to acknowledge a generous grant from Eli Lilly and Co., Indianapolis, Ind., for the support of this work.

tyrosine ester, was not necessary. In either case, extensive polymerization occurred.

The amino group in a metallic salt of an amino acid is considerably more basic than that of an ester of the acid. Further, such salts are generally stable. Their applicability is limited by lack of solubility in organic solvents, and, unless conditions are carefully controlled, reaction with halides results primarily in esterification.

Finally the ω-amino group of a diamino acid may be preferentially brought into reaction by protecting the α-amino group as the copper complex with the carboxyl group. For example, arginine has been prepared in good yield (4) by treating the ornithine-copper complex with methyl isourea, and citrulline results when the orinithine-copper complex is heated with urea in a sealed tube (5).

As an example of the first type of reaction, I was condensed with tyrosine ethyl ester which was chosen because it is a solid at room temperature and reasonably stable. The reaction occurred according to the following formulas.



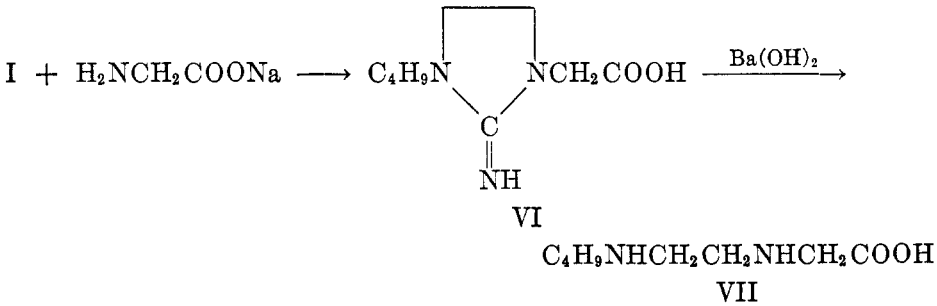
The initial product of the reaction, III, underwent cyclization by loss of alcohol to yield the hydrobromide of IV from which the free base, IV, was liberated. Hydrolysis of IV gave the desired guanidino amino acid, V.

Purification of V, as well as of the other guanidino acids prepared, was extremely tedious. Melting points were not significant and could not be used as criteria of purity. The extreme solubility of these substances in water and their insolubility in organic solvents, except occasionally ethanol, made separation from inorganic salts and recrystallization difficult. The final products were best obtained after prior purification by evaporation of an aqueous solution to dryness or by precipitation from an alcoholic solution by ether.

The free amino acids are best liberated from their hydrochlorides by passage of an aqueous solution through a column of analytical grade Amberlite IR-4B[3].

Comparable reactions with the ethyl esters of lysine and cystine gave no identifiable products.

The second type of reaction is illustrated by the alkylation of I with the sodium salt of glycine. Approximately 50% of the desired product, VI, was obtained, as well as varying amounts of the other substances shown below.

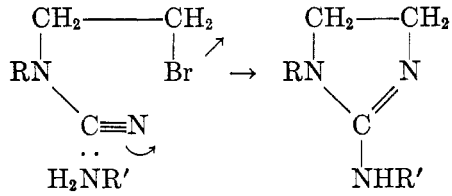


VI

$C_4H_9NHCH_2CH_2NHCH_2COOH$

VII

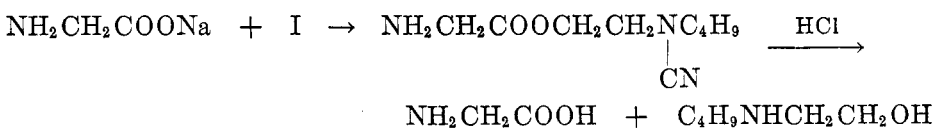
The reaction is presumed to take place by displacement of the halide ion followed by addition of the resulting secondary amine across the C≡N linkage to give the guanidine in the usual Erlenmeyer fashion. That this product is actually formed rather than the isomeric imidazoline, VIII, has been demonstrated in two instances. Alkaline hydrolysis of 1,3-di-*n*-butyl-2-iminoimidazolidine gave N,N′-dibutylethylenediamine, rather than N-butylethylenediamine which would have been expected from an imidazoline of the type of VIII (1).



VIII

Similarly, alkaline hydrolysis of VI gave a substance giving analytical data for *n*-butylaminoethylglycine (VII).

VI was separated from the products of the above reaction by taking advantage of the insolubility of its hydrochloride in acetone. Acid hydrolysis of the material remaining in the acetone after removal of the hydrochloride of VI yielded a number of products. The amount of glycine isolated indicated that ester formation was an important side reaction.

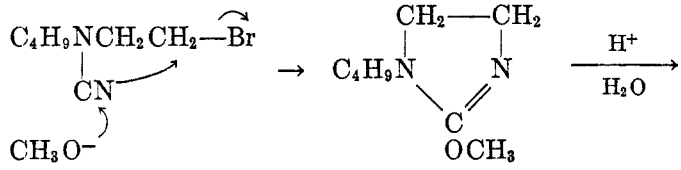


The presence of *n*-butylethanolamine was indicated by its characteristic odor, but it was not otherwise identified.

A considerable amount of the desired product, VI, which presumably had reacted further after being formed, was also obtained from this fraction. In some experiments in which not all of the glycine had dissolved and there was

present, as a result, a slight excess of sodium methoxide, the entire reaction product was soluble in acetone, and it was only after acid hydrolysis that any of the desired product was obtained.

Two other substances were isolated in very small amounts from the products of acid hydrolysis. N-(*n*-Butyl)ethylenediamine (IX) was obtained when the solution was made alkaline. Its presence is indicative of imidazoline (isourea) formation.



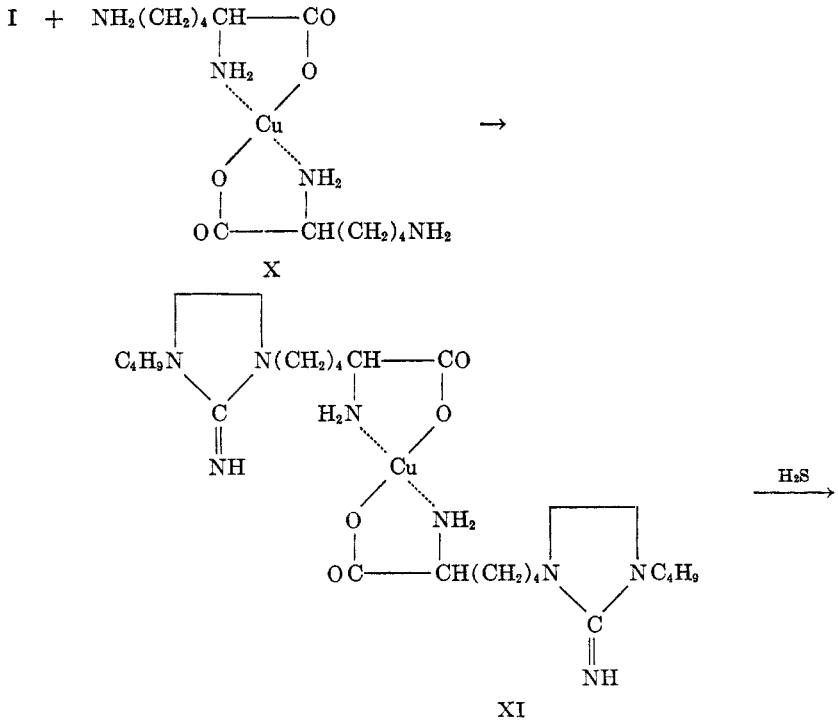
$C_4H_9NHCH_2CH_2NH_2$

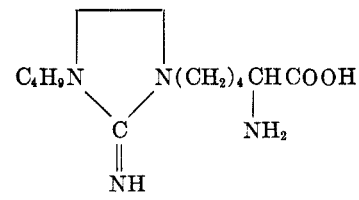
IX

I reacts with sodium ethoxide to give a basic product in excellent yield from which IX is obtained on acid hydrolysis.

A small quantity of VII which could have arisen from hydrolysis of VI was also isolated.

Despite the negative results reported by Kurtz (5) in the alkylation of the copper complex of lysine in aqueous solution, this reaction was investigated and a product was obtained in moderate yield. The sequence of reactions is illustrated by the following formulas.





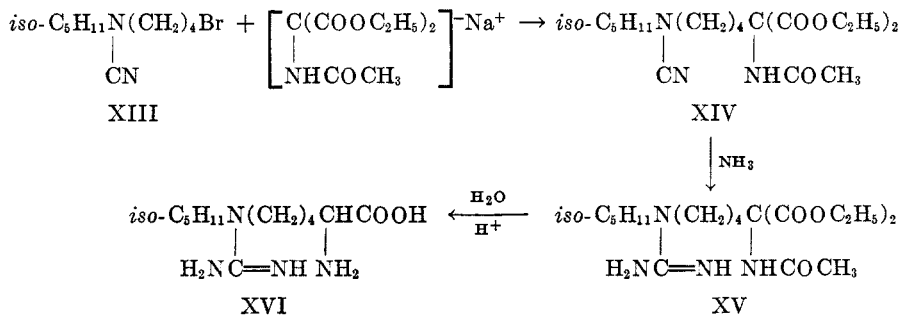
XII

Separation of the product of the reaction was accomplished either by taking advantage of the solubility of XI in absolute alcohol, in which X is insoluble, or by passage of the mixture of XII and lysine obtained after treatment of the crude reaction product with hydrogen sulfide through Amberlite IRA-400,[3] a strongly basic ion-exchange resin which retains lysine but permits passage of the guanidine.

However, alkylation of the sodium salt of lysine followed by separation of the product as the copper complex proved to be somewhat more satisfactory and gave a purer product.

The two most important methods for the preparation of guanidines—the addition of ammonia or of amines to cyanamides, and the reaction of amines with O-methylisourea or S-methylisothiourea—were investigated briefly in so far as they could be applied to the synthesis of the types of compounds under consideration. Success was only moderate.

Although cyanamide reacts readily with amines and their salts to yield guanidines, disubstituted cyanamides frequently fail to react in an analogous fashion (6). The addition of ammonia to substituted cyanamides is likewise a sluggish reaction. The following reactions were investigated.

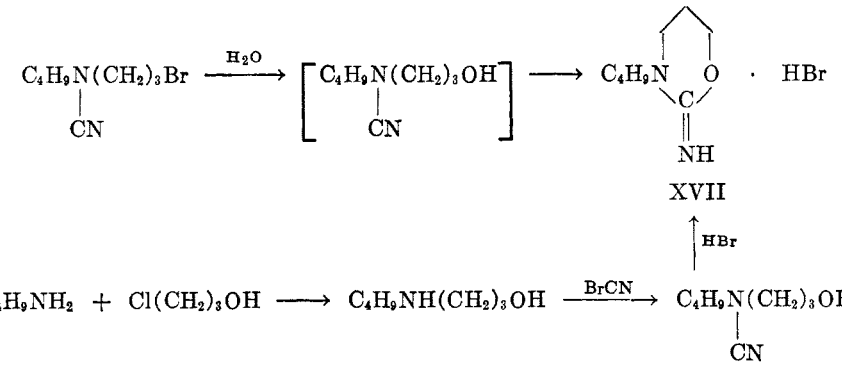


When XIV was heated at 160° in a sealed tube with a butanol solution of ammonia a substance which furnished satisfactory analytical data for XVI was obtained in poor yield. This material gave a positive color test for a guanidine with alkaline diacetyl solution (7).

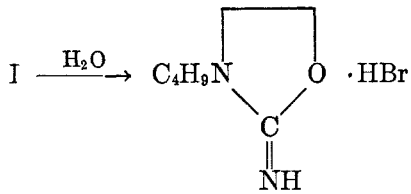
Better results were obtained when XIV was treated with potassium amide in liquid ammonia. Dialkylcyanamides are reported to react with this reagent with the probable formation of guanidines (8). From this reaction a substance giving analytical data for XVI was obtained.

[3] The Amberlite resins used in this work were obtained through the courtesy of Rohm and Haas, Inc., to whom we express our appreciation.

During the course of this work we have made some observations on the behavior of β-bromoethylbutylcyanamide (I). This is a reasonably stable substance, but, on standing for several weeks, it is gradually transformed into an ether-insoluble water-soluble compound containing ionic halogen. This compound does not separate from solution unless ether is added, but γ-bromopropylbutylcyanamide deposits white crystals of a substance which was shown to be the hydrobromide of 2-imino-3-(*n*-butyl)tetrahydro-1,3-oxazine (XVII). The formation of XVII probably takes place by the following route.



XVII was synthesized independently by passage of hydrogen bromide into an ethereal solution of γ-hydroxypropyl-*n*-butylcyanamide. Similarly the substance into which I is transformed was shown to be 2-imino-3-(*n*-butyl)oxazolidine hydrobromide (XVIII).



XVIII

In either instance the water required was presumably present in the original material or it may have been absorbed from the atmosphere. After these observations had been made, a patent in which the preparation of XVIII and similar compounds from amino alcohols and cyanogen halides appeared (9). In any event, if XVII and XVIII are regarded as isoureas, their formation by acid catalysis is not without interest.

EXPERIMENTAL[4, 5]

β-Bromoethyl-n-butylcyanamide (I). This was prepared from cyanogen bromide and *n*-butylethylenimine as described previously (1). A preferred procedure for the preparation of *n*-butylethylenimine is that of Leighton, Perkins, and Renquist (10).

[4] All melting points are corrected for stem exposure unless otherwise noted.

[5] Microanalyses by Clark Microanalytical Laboratories, Urbana, Ill., Microtech Laboratories, Skokie, Ill., or Dr. Francine Schwarzkopf, Elmhurst, N. Y.

Reaction of tyrosine ethyl ester with I. A solution of 5 g. of tyrosine ethyl ester (11) and 4.3 g. of I in 20 ml. of absolute ethanol was refluxed for 4 hours and the solvent was removed at the water pump leaving a viscous oil. Extraction of this oil with ether yielded a negligible amount of ether-soluble material which indicated substantially complete reaction. The oil could not be crystallized. It was extracted with five 50-ml. portions of water by warming on the steam-bath for 5 min. and decanting the aqueous solutions from the insoluble polymeric residue. The combined aqueous solutions were allowed to stand at room temperature for 2 hours and then decanted from a small amount of heavy oil. Addition of potassium carbonate precipitated the free base (IV) which was insoluble in ether, but was readily extracted by ethyl acetate. After removal of the solvent from the extract, 10 ml. of absolute ethanol and 20 ml. of ether were added to the residue, and dry hydrogen chloride was carefully passed into the solution. The hydrochloride of IV separated as fine white needles, m.p. 179–180° after recrystallization from alcohol-ether. The yield was 2.2 g. The same product was obtained when the reactants were heated at 125° for 15 min. without a solvent.

Anal. Calc'd for $C_{16}H_{22}ClN_3O_2$: C, 59.4; H, 6.8; N, 13.0; Cl, 10.8.
Found: C, 59.3; H, 7.1; N, 12.8; Cl, 11.0.

When the ethyl acetate solution of IV was concentrated to 50–75 ml. and cooled, white needles, m.p. 116.5–117° after treatment with decolorizing carbon and recrystallization from ethyl acetate, were obtained. If ethanol was the solvent, or if any ethanol was added to the ethyl acetate, white platelets, m.p. 139–140°, separated. Grinding converted the lower-melting into the higher-melting form, and both forms gave the same hydrochloride.

Anal. Calc'd for $C_{16}H_{21}N_3O_2$: C, 66.9; H, 7.3; N, 14.6.
Found: C, 66.9; H, 7.3; N, 14.4.

Acid hydrolysis of IV. (V). A solution of 0.5 g. of IV in 60 ml. of 20% hydrochloric acid was refluxed for 3 hours. The solution was evaporated to dryness under reduced pressure, 20 ml. of water was added, and the evaporation was repeated. The residue was taken up in 75 ml. of water and the solution was run during 2 hours through a column of 25 ml. of analytical grade Amberlite IR-4B in a 50-ml. burette. The column was washed with 50 ml. of water. The aqueous solution, now chloride-free, was extracted with ethyl acetate in order to remove any unreacted starting material. The aqueous solution was concentrated to dryness under reduced pressure, and the last traces of water were removed by azeotropic distillation with absolute ethanol and benzene. The residue was extracted with several small portions of ethyl acetate, and the insoluble portion was dissolved in 25 ml. of butanol. Anhydrous ether was added to the solution to incipient precipitation and the solution was allowed to stand for 6 hours. The small amount of hygroscopic material which separated was removed by centrifugation and discarded. Anhydrous ether was added to the supernatant liquid, with scratching, until precipitation began. After refrigerating overnight, 240 mg. of a somewhat hygroscopic, microcrystalline product (V) was obtained. It began to soften at about 115° with gas evolution, and was not completely liquid until 180°.

Anal. Calc'd for $C_{16}H_{23}N_3O_3$: C, 62.9; H, 7.5; N, 13.8.
Found: C, 62.5; H, 7.8; N, 13.9.

Reaction of the sodium salt of glycine with I. (VI). To a solution of 1.15 g. of sodium in 70 ml. of absolute methanol was added 4.15 g. of glycine (10% excess) and the suspension was refluxed for 20 min. After addition of 2.2 g. of I, the solution was refluxed for 4 hours. After removal of the solvent, the residue was extracted with ether to remove unreacted I (10–15% was usually recovered). The yellow-orange, gummy residue was taken up in 100 ml. of absolute ethanol and filtered from unreacted glycine. Hydrochloric acid was added to the solution until it was distinctly acid. After addition of 50 ml. of ether, the mixture was refrigerated overnight to completely precipitate sodium halides. The filtered solution was concentrated to dryness under reduced pressure, 100 ml. of acetone was added to the residue, and the suspension was shaken for an hour. Part of the material gradually dissolved to give a yellow-orange solution, leaving a white crystalline residue of the hydrohalides of the desired guanidino acid, 1-(*n*-butyl)-2-imino-3-carboxymethylimidazolidine (VI). The crystalline residue was collected, washed with acetone, and dissolved in 50 ml.

of warm butanol. After cooling, the solution was filtered from traces of sodium chloride, and again taken to dryness under reduced pressure. The residue was dissolved in 200 ml. of water. Passage of this solution through 45 ml. of Amberlite IR-4B freed the solution of halide ion and gave a mildly basic effluent (*p*H 8–8.5). Evaporation to dryness at the water pump, extraction of the residue with absolute ethanol, filtration from a small amount of insoluble material, and removal of the solvent gave a 44% yield of the guanidino acid (VI). The somewhat hygroscopic acid was recrystallized from butanol-dioxane-ether. The hydrochloride was obtained as lustrous plates, m.p. 194–194.5°, by suspending the solid acid in 100 times its weight of acetone, acidification with concentrated hydrochloric acid, addition of ether to turbidity, and allowing the mixture to stand for several hours. The hydrochloride may be recrystallized from butanol-ether, but if it is heated in butanol for too long a time, it cannot be obtained in crystalline form.

Anal. Calc'd for $C_9H_{18}ClN_3O_2$: C, 45.9; H, 7.7; N, 17.9.
Found: C, 46.1; H, 7.7; N, 17.8.

A portion of the acetone-soluble material obtained as above was refluxed with 10 volumes of 20% hydrochloric acid, and the resulting solution was concentrated to dryness under reduced pressure. Part of the residue was made alkaline. In addition to a strong odor of ammonia the characteristic odor of *n*-butylethanolamine was present. The alkaline solution was extracted with ether, and, after drying over magnesium sulfate, dry hydrogen chloride was passed into the ether extract. The hydrochloride of *n*-butylethanolamine, which is a hygroscopic salt somewhat soluble in ether, did not precipitate. Instead a small amount of precipitate was obtained. This gave small lustrous leaflets, m.p. 233–233.5°, after recrystallization from acetone-dioxane. Analytical data corresponded to those for the *dihydrochloride of N-butylethylenediamine.*

Anal. Calc'd for $C_6H_{18}Cl_2N_2$: C, 38.1; H, 9.5; N, 14.8.
Found: C, 38.3; H, 9.5; N, 14.6.

King and McMillan (12) report m.p. 231–232° for this compound. An authentic sample was prepared for comparison by refluxing an alcoholic solution of *n*-butyl bromide with four times the theoretical amount of ethylenediamine. After removal of the solvent through a Vigreux column, the residue was poured into a concentrated potassium hydroxide solution and the upper layer was separated. After drying over potassium hydroxide, the product was distilled through a 2-ft. helix-packed column. The fraction boiling at 171–172° was collected. The hydrochloride of this material melted at 231–232° and the m.p. was not depressed on admixture of it with the hydrochloride obtained above.

The remainder of the acid hydrolysate, about 15 g., was dissolved in 400 ml. of water and stirred for 30 min. with 12 g. of silver oxide. After filtering, silver ion was precipitated with hydrogen sulfide. The filtrate from the silver sulfide was evaporated to dryness under reduced pressure, finally with benzene for removal of traces of water. The residue was boiled for 20 min. with 200 ml. of absolute alcohol and filtered. The filtrate deposited 1.2 g. of glycine on cooling. Identification was by m.p. 204.5–206°, and mixture m.p's. of the acetyl derivative with a known sample of acetylglycine.

The alcoholic solution remaining after removal of the glycine was evaporated to dryness, 150 ml. of acetone was added to the residue, and the mixture was acidified with hydrochloric acid. After shaking for 30 min., the white precipitate was filtered off and warmed with 60 ml. of butanol. Most of the solid dissolved. To the filtered solution anhydrous ether was added until appearance of turbidity. After refrigeration overnight, 6.8 g. of crystalline hydrochloride of VI, m.p. 193–195°, separated.

Anal. Found: C, 45.7; H, 7.8; N, 17.8.

The butanol-insoluble material (0.8 g.) obtained above was dissolved in 15 ml. of water and the solution was made strongly acid with hydrochloric acid. After addition of an equal volume of ethanol and refrigeration, lustrous white crystals, m.p. 215–217°, separated. The analytical data corresponded to those for the dihydrochloride of N-(*n*-butylamino)-ethylglycine (VII).

Anal. Calc'd for $C_8H_{20}Cl_2N_2O_2$: C, 38.9; H, 8.1; N, 11.3.
Found: C, 38.8; H, 8.2; N, 11.0.

VII was also prepared by alkaline hydrolysis of VI. One gram of VI was refluxed with 50 ml. of saturated barium hydroxide solution for 18 hours. Barium was precipitated by a slight excess of sulfuric acid and sulfate ion was removed by passage through a 30-ml. column of Amberlite IR-4B. The solution was acidified with hydrochloric acid and taken to dryness. After recrystallization from 95% ethanol, the residue melted at 214–215°.

Anal. Found: C, 39.0; H, 8.3; N, 11.5.

Alkylation of the copper complex of lysine (XII). The copper hydroxide used was prepared by making a 5% copper sulfate solution slightly alkaline with 10% sodium hydroxide and washing the precipitate by intermittent decantation for 2 days.

A solution of 3.0 g. of lysine monohydrochloride in 75 ml. of water was stirred for 15 min. with 3 g. of silver oxide, filtered, treated with hydrogen sulfide, and again filtered. After evaporation to half its volume, about 40 ml. of a copper hydroxide suspension was added, the mixture was warmed on the steam-bath for 15 min., and then stirred for a half-hour. The solution, now a deep blue color, was filtered from excess copper hydroxide and evaporated to 40 ml. under reduced pressure. Addition of 80 ml. of 95% ethanol precipitated the greater part of the copper complex. After addition of 3.5 g. of I the mixture was refluxed. After 5 hours it had turned a greenish blue and most of the solid had gone into solution. Refluxing was continued for 16 hours when the solution was sea green.

The alcohol was distilled off and the solution was extracted with ether which removed 0.6 g. of unreacted I. The solution was then taken to dryness under reduced pressure and the residue was extracted with five 50-ml. portions of absolute ethanol in which the copper complex of lysine is insoluble. The alcohol was removed from the deep green extracts, 100 ml. of water and 5 ml. of 10% hydrochloric acid were added to the residue, and the copper was precipitated with hydrogen sulfide. After evaporation to half the volume, 40 ml. of 20% hydrochloric acid was added and the solution was refluxed for 3 hours. After removal of as much acid as possible by evaporation to dryness, a solution of the residue in 150 ml. of water was run during a 3 hour period through 50 ml. of Amberlite IRA-400 which quantitatively adsorbs lysine and less basic amino acids but which allows passage of amino acids more basic than lysine. The column was washed with 50 ml. of water and the combined effluents were taken to dryness. The yellow orange, viscous residue was extracted with several small portions of acetone, and the insoluble part was dissolved in 25 ml. of absolute ethanol. The solution was treated with charcoal, acidified with hydrochloric acid, again charcoaled, and evaporated to dryness finally with benzene and alcohol to remove the last traces of water. The residue was allowed to stand with 100 ml. of dry acetone for 6 hours and the solution was decanted. After addition of ether to the acetone solution and standing for a week the solid was centrifuged and dried at 80° *in vacuo*. The product, somewhat off-white in color, was extremely hygroscopic and it was difficult to obtain a completely satisfactory analysis. A better product was obtained by the following procedure.

Alkylation of the sodium salt of lysine. To a solution of 1.15 g. of sodium in 50 ml. of absolute ethanol was added 4.57 g. of lysine monohydrochloride. After addition of 5.1 g. of I and 15 ml. of ethanol the solution was refluxed for 4 hours. Undissolved solids were centrifuged, the solvent was removed *in vacuo*, and the viscous residue was extracted with three 50-ml. portions of ether. The solution of the residue in 75 ml. of absolute ethanol was acidified with hydrochloric acid, and, after refrigeration, the inorganic precipitate was filtered off. After removal of the solvent, the solution of the residue in 75 ml. of butanol was filtered from additional inorganic salts and again taken to dryness. The solution of the residue in 100 ml. of water was stirred for a half-hour with excess copper hydroxide suspension, filtered, and taken to dryness. The residue was worked up as described above. In this case treatment with Amberlite IRA-400 gave a colorless effluent, but the characteristics of the final product were about the same as those of the substance obtained above. The yield was low (10–15%). The substance must be very carefully dried just prior to analysis. It melts at 110–140° (dec.)

Anal. Calc'd for $C_{13}H_{28}Cl_2N_2O_2$: C, 45.5; H, 8.2; N, 16.3.

Found: C, 45.1; H, 7.9; N, 16.5.

N-Isoamyl-N-(ε-carboxy-ε-amino-n-amyl)guanidine. XVI. (*a*) *1-Isoamylpyrrolidine* was

prepared by warming a mixture of 150 g. of 1,4-dichlorobutane and 220 g. of isoamylamine on the steam-bath for 16 hours. Heat of the reaction kept the solution refluxing vigorously for an hour after which the reaction slowly subsided. After adding excess 10% sodium hydroxide solution, the upper layer was separated, dried over potassium hydroxide, and distilled yielding 102 g. (61.5%) of material, b.p. 168–170°. Reported, b.p. 166–167° (13).

(*b*) *ω-Bromobutylisoamylcyanamide* (XIII) was prepared by the action of cyanogen bromide on isoamylpyrrolidine by the method described in the preceding paper (14). A crude yield of 83% was obtained, but only about a half of this distilled, the remainder resinifying in the flask. The b.p. was 140° (0.5 mm.).

To a solution of 2.3 g. of sodium in 100 ml. of absolute ethanol (dried over magnesium ethoxide) was added 21.7 g. of acetamidomalonic ester, and, after solution was complete, 28 g. of XIII. The solution was refluxed for 4 hours, cooled, and filtered from salt. The solvent was removed *in vacuo*, 100 ml. of 10% hydrochloric acid was added to the residue, and the mixture was extracted with four 50-ml. portions of ether. The combined extracts were dried over magnesium sulfate, and the solvent removed leaving 32.2 g. (84%) of XIV as a reddish-orange viscous oil. An acid-soluble fraction of 7 g. was discarded.

A mixture of 4 g. of crude XVI and 50 ml. of butanol containing 4 g. of ammonia was heated in a sealed tube at 150° for 14 hours. The resulting acid-soluble material (XV) was refluxed for 6 hours with 50 ml. of 10% hydrochloric acid and the solution was taken to dryness. A solution of the residue in 100 ml. of water was passed through 30 ml. of Amberlite IRA-400 and the column was washed with 150 ml. of water. Evaporation of the solution to dryness and extraction with absolute alcohol left a white, crystalline residue (0.24 g.) which melted at 260–264° (dec.) after recrystallization from 90% ethanol. The analytical data agreed with those for the *monohydrochloride of XVI*.

Anal. Calc'd for $C_{11}H_{25}ClN_2O_2$: C, 52.3; H, 10.0; N, 11.1.

Found: C, 52.2; H, 9.7; N, 11.1.

XVI was obtained in better yield as follows. Approximately 150 ml. of liquid ammonia was added to 3.9 g. of potassium and 1 sq. cm. of rusty wire gauze in a 200 ml. 3-necked flask fitted with a stirrer and air condenser equipped with a drying tube. After 15 min., when the blue color of the potassium had disappeared, 8 g. of XIII was added dropwise. Stirring was continued for 4 hours with no cooling other than that provided by the evaporation of the ammonia after which 5.3 g. of ammonium chloride was added. When evaporation of the ammonia was complete, the residue was extracted with 200 ml. of absolute alcohol. The extract gave a strong color test for guanidines with diacetyl. After removal of the solvent, the residue was refluxed for 8 hours with 100 ml. of 20% hydrochloric acid, the solution was evaporated to dryness. A solution of the residue in 250 ml. of water was run through 100 ml. of Amberlite IRA-400 followed by 250 ml. of water. The combined effluents were evaporated to dryness leaving a basic oil. This was redissolved in 125 ml. of water and the solution was passed through 30 ml. of Amberlite IRA-400. The column was washed with 250 ml. of water. The washings were taken to dryness separately from the original effluent and yielded 0.38 g. of a white solid which decomposed on rapid heating at 50–80°. Its analysis corresponded to that of the desired guanidine (XVI) with perhaps a slight contamination by the corresponding urea.

Anal. Calc'd for $C_{12}H_{26}N_4O_2$: C, 55.8; H, 10.1; N, 21.7.

Found: C, 55.7; H, 10.0; N, 20.9.

Isourea formation (*Acid-catalyzed*). *2-Imino-3-(n-butyl)tetrahydro-1,3-oxazine* (*XVII*) *and 2-imino-3-(n-butyl)oxazolidine* (XVIII). The discovery of ionic halogen in a sample of I that had been standing for several months led to an investigation of this substance. Upon addition of ether, about half the material separated as a water-soluble gummy solid. However, γ-bromopropylbutylcyanamide deposited white cubic crystals on standing; therefore it was investigated first. The crystals were powdered, washed with ether to remove occluded cyanamide, and recrystallized from 9:1 dioxane-ethanol to yield a white crystalline product, m.p. 156–157°. When an aqueous solution of this substance was made alkaline, a basic oil separated which was extracted with ether. Passage of hydrogen bromide into this ethereal solution gave the same hydrobromide of XVII, m.p. 156–157°.

Anal. Calc'd for $C_8H_{17}BrN_2O$: C, 40.5; H, 7.2; N, 11.8; Br, 33.8.
Found: C, 40.6; H, 7.1; N, 11.9; Br, 34.1.

The *oxalate* prepared from the above tetrahydrooxazine melted at 126–128°. When XVII was refluxed with 20% sodium hydroxide for 12 hours and the resulting solution was extracted with ether, an oxalate of a base was precipitated from the ether extract. This oxalate, m.p. 145–146°, gave no depression of m.p. on admixture with an authentic sample of the oxalate of *n*-butylaminopropanol prepared as described below.

Anal. Calc'd for $C_9H_{19}NO_5$: C, 48.9; H, 8.6; N, 6.3.
Found: C, 49.0; H, 8.7; N, 6.2.

n-Butylaminopropanol was prepared by warming 2 moles of *n*-butylamine with 1 mole of trimethylenechlorohydrin on the steam-bath. The vigorous reaction subsided after 10 min. After 2 hours an excess of 40% sodium hydroxide was added, the alkaline solution was extracted with ether, and the extract was dried over potassium carbonate. After removal of the solvent, the residue was distilled yielding *n*-butylaminopropanol, b.p. 115–120° (20 mm.).

A solution of 5.2 g. of *n*-butylaminopropanol in 50 ml. of absolute ethanol was added to 4.3 g. of cyanogen bromide in 50 ml. of absolute ethanol, and the solution was allowed to stand for 15 min. without cooling although considerable heat was evolved. The solvent was distilled off and the residue was extracted with ether to remove unreacted material. The insoluble residue was treated with aqueous alkali, extracted with 30 ml. of ether, and the ether extract was dried over potassium carbonate. To the dry ether solution 25 ml. of dioxane was added and dry hydrogen bromide was passed over the surface of the solution. Immediate precipitation occurred. The crystalline material was recrystallized from dioxane-ethanol giving 6.3 g. of the hydrobromide of XVII, m.p. 157–157.5°; mixture m.p. with the substance obtained above was 156.5–157.5°.

In the same way the material obtained from I was recrystallized from dioxane-ethanol giving small white, cubic crystals, m.p. 130–131°. Extraction of the base and reprecipitation as the hydrochloride gave crystals, m.p. 145.5–147°. Abramovitch (9) reports m.p. 145–147° for the hydrochloride of 2-(*n*-butyl)-3-iminoxazolidine. The mixture m.p. of this material with a known sample prepared from *n*-butylaminoethanol and cyanogen bromide was not depressed.

SUMMARY

1. Cyclic guanidine derivatives of amino acids have been prepared.
2. Applications of guanidine syntheses to dialkylcyanamides have been explored.

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REFERENCES

(1) ELDERFIELD AND HAGEMAN, *J. Org. Chem.*, **14**, 605 (1949).
(2) JOHLIN, *Proc. Soc. Exptl. Biol. Med.*, **70**, 425 (1949).
(3) EDSALL AND BLANCHARD, *J. Am. Chem. Soc.*, **55**, 2337 (1933).
(4) TURBA AND SCHUSTER, *Z. physiol. Chem.*, **283**, 27 (1948).
(5) KURTZ, *J. Biol. Chem.*, **180**, 1253 (1949).
(6) LECHER AND DEMMLER, *Z. physiol. Chem.*, **167**, 163 (1927).
(7) HARDEN AND NORRIS, *J. Physiol. (London)*, **42**, 332 (1911).
(8) FRANKLIN, *J. Am. Chem. Soc.*, **55**, 4912 (1933).
(9) ABRAMOVITCH, U. S. Patent 2,443,062 [*Chem. Abstr.*, **43**, 252 (1949)].
(10) LEIGHTON, PERKINS, AND RENQUIST, *J. Am. Chem. Soc.*, **69**, 1540 (1947).
(11) FISCHER, *Ber.*, **39**, 540 (1906).
(12) KING AND MCMILLAN, *J. Am. Chem. Soc.*, **68**, 1774 (1946).
(13) RUBINSTEIN AND MENSHIKOV, *J. Gen. Chem. (U.S.S.R.)*, **14**, 161 (1944).
(14) ELDERFIELD AND GREEN, *J. Org. Chem.*, **17**, preceding article.