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The Reaction of Cyanogen Bromide with Mono- and Diamino Acids

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The principle of simultaneous activation and protection is operative in the reaction of α-amino acids with cyanogen bromide in aqueous solution at neutral pH. It is assumed that the initially formed N-cyano derivative invites participation of the carboxy function to yield a highly reactive 5-iminooxazolidone-2 which is opened in an *inter*molecular reaction by a second molecule of the α-amino acid to give in good yield N-carbamyl dipeptides. In the case of α,ω-diamino acids both α-cyano and ω-cyano derivatives are formed in ratios approaching 1:1. The α-cyano derivative yields an intermediate 5-iminooxazolidone which is opened by the ω-amino group in an *intra*molecular process to yield L-3-ureidopyrrolidone from L-α,γ-diaminobutyric acid, L-3-ureidopiperidone from L-ornithine, and L-3-ureidohomopiperidone from L-lysine. Alkaline hydrolysis and methanolysis of these ureidoheterocycles lead to open α-ureido-ω-amino acids and their methyl esters.

Cyanogen bromide in acidic medium selectively cleaves methionine peptide bonds. Studies on the primary sequence of ribonuclease[2] and myoglobin[3] have benefited from application of this new method. In the pH range 1–2 in aqueous solution, of the common amino acids only methionine and, to some minor extent, cysteine react with cyanogen bromide. A wider variety of reaction would be expected to occur in solution of higher pH. The literature contains few examples[4,5] of this type of reaction. The present investigation deals with the reaction of α-amino acids and of α,ω-diamino acids with cyanogen bromide in the neutral or slightly basic pH range.

Cyanogen bromide itself is quite stable in aqueous solution below pH 6.5. At pH values above 7 it decomposes noticeably and rapidly (*cf.* Fig. 1). Reactions of amino acids with cyanogen bromide, involving the primary amino group, start in the pH region 5.8–6.5 (*cf.* Fig. 2). For work on a preparative scale the pH range 7.4–7.8 has been found to be more convenient.

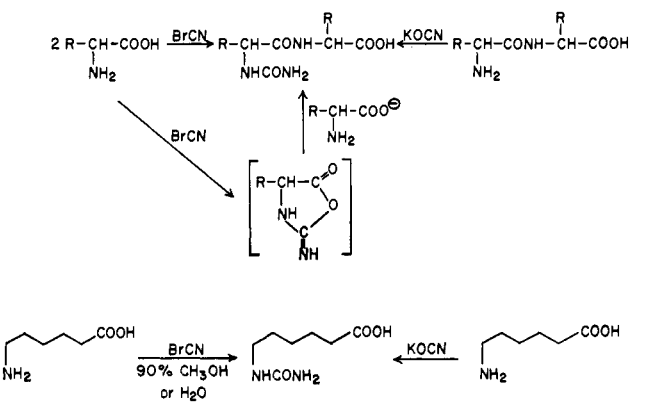
Monoamino Acids and Cyanogen Bromide.—Aqueous solutions of α-monoamino acids, such as L-alanine, L-valine, L-leucine, and L-serine, containing excess cyanogen bromide, at room temperature and constant pH of 7.6, consume slightly more than one equivalent of alkali in less than 1 hr. In each case *only one reaction product* was isolated. It contains a free carboxy group but no basic nitrogen function. Molecular weight determinations and elemental analysis gave evidence for a combination of two molecules of amino acid with one molecule of cyanogen bromide with loss of one mole of hydrogen bromide.

The optically active reaction products showed no color with ninhydrin; however, a lemon-yellow color developed with *p*-dimethylaminobenzaldehyde and HCl (Ehrlich reagent), a reaction characteristic of the presence of a $-NH-CONH_2$ grouping. The infrared spectra (solid state in KBr) showed two bands in the carbonyl region at 1740 and 1660 cm.$^{-1}$.

Therefore, the reaction products must be formulated as ω-carbamido-L-alanyl-L-alanine (m.p. 193°, $[\alpha]^{20}D$ −20.0° (H_2O)), ω-carbamido L-valyl-L-valine (m.p. 208°, $[\alpha]^{20}D$ −7.3° (H_2O)), ω-carbamido-L-leucyl-L-leucine (m.p. 215°, $[\alpha]^{20}D$ −80.3° (H_2O)), and ω-carbamido-L-seryl-L-serine (m.p. 165°, $[\alpha]^{20}D$ +28.1° (H_2O)). The mechanism of formation of these N-carbamido dipeptides is indicated in Chart I.

CHART I

SIMULTANEOUS ACTIVATION AND PROTECTION WITH BrCN



The structure of these compounds has been proved in two different ways: (i) By direct comparison with the products obtained by reaction of the corresponding dipeptides with potassium cyanate to yield the N-carbamido dipeptides.[6] Only the carbamylation of L-seryl-L-serine met with some difficulties. Identity was established by m.p., mixture m.p., and by thin-layer chromatography. (ii) By cleavage of the carbamido dipeptides to the corresponding hydantoin and amino acid according to the method of Stark.[6] The hydantoins were isolated in 4 cases: L-alanylhydantoin, m.p. 173–175°; L-valinehydantoin, m.p. 144–146°; L-leucinehydantoin, m.p. 210–211°; and L-serinehydantoin, m.p. 186°. Subsequently, the hydantoins were hydrolyzed by dilute alkali to the corresponding amino acids. The amino acids obtained first by cleavage of the N-carbamido dipeptides and then by hydrolysis of the hydantoins were identified by thin-layer chromatography on silica gel (system: *t*-BuOH–HCOOH–H_2O, 4:1:1).

The rapid formation of dipeptides in neutral aqueous solution and in good yield presumably involves the highly reactive electrophilic intermediate 5-iminooxazolidone-2 (*cf.* Chart I), arising through internal addition of the carboxylate ion to the initially formed cyanamide. This intermediate could undergo further rapid reaction with a second molecule of amino acid to form the N-carbamido dipeptide. So far, attempts to isolate intermediates have failed.

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(2) E. Gross and B. Witkop, *J. Biol. Chem.*, **237**, 1856 (1962).
(3) A. B. Edmundson, *Nature*, **198**, 354 (1963).
(4) P. Pierron, *Ann.*, **11**, 361 (1919).
(5) H. E. Carter, C. C. Sweeley, E. E. Daniels, J. E. McNary, C. P. Schaffner, C. A. West, E. E. van Tamelen, J. R. Dyer, and H. A. Whaley, *J. Am. Chem. Soc.*, **83**, 4296 (1961).
(6) *Cf.* G. R. Stark and D. G. Smith, *J. Biol. Chem.*, **238**, 214 (1963).

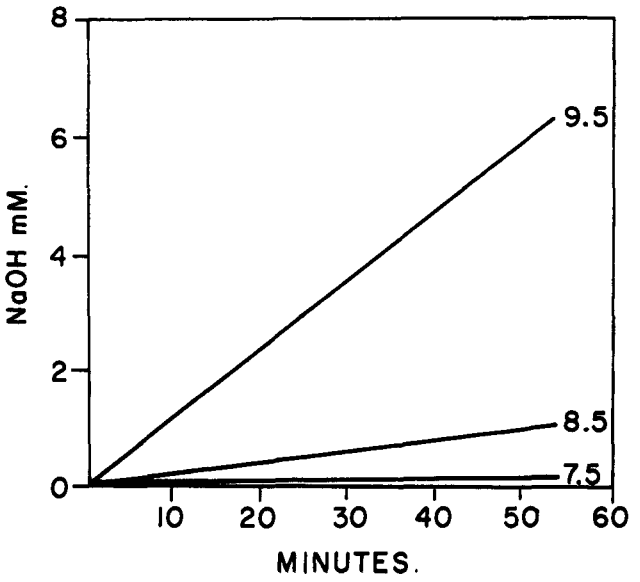


Fig. 1.—Stability of cyanogen bromide as a function of pH. The solution contained 8 mmoles of BrCN in 25 ml. of H_2O at 25°

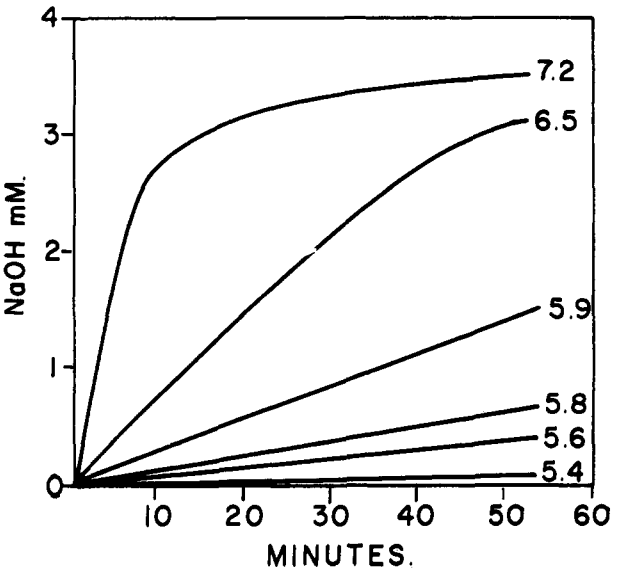
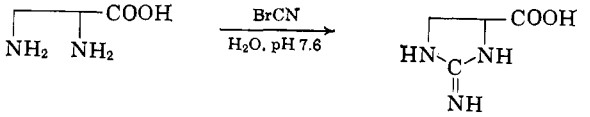


Fig. 2.—Rate of reaction of an aqueous solution of L-leucine (3 mmoles in 80 ml. of H_2O) with cyanogen bromide (6 mmoles) at 25° as reflected by consumption of base.

ϵ-Aminocaproic Acid.—Under the same conditions as mentioned before, ϵ-aminocaproic acid reacts with cyanogen bromide, consuming 2 equivalents of alkali to give ϵ-ureidocaproic acid, $C_7H_{14}N_2O_3$, m.p. 180°, isolated in 90% yield. Its structure was proved by direct comparison (m.p., mixture m.p., R_f) with ϵ-ureidocaproic acid, obtained by reaction of ϵ-aminocaproic acid and potassium cyanate. The isolation of a monomer, ϵ-ureidocaproic acid, in almost quantitative yield further supports the assumption of a reactive 5-membered cyclic intermediate in the case of α-amino acids.

α,ω-Diamino Acids.—The action of cyanogen bromide on diamino acids in neutral or slightly alkaline aqueous solution led to a complex mixture of reaction products. Separation and isolation of products was achieved by means of ion-exchange columns, *e.g.*, Dowex 1-X8 and Dowex 50-X8, 200–400 mesh. Only the products obtained in crystalline form have been investigated so far.

L-α,β-Diaminopropionic acid in an aqueous solution of pH 7.6, containing 2 equivalents of cyanogen bromide, consumed 2 equivalents of alkali at room temperature in the course of 15 min. The reaction products showed acidic as well as basic properties. One homogeneous compound, $C_4H_7O_2N_3$, was isolated by crystallization (30% yield, m.p. 268° dec., $[\alpha]^{20}D$ −37.0° (H_2O)). With ninhydrin and Ehrlich reagents the product gave no color. The infrared spectrum (solid state in KBr) showed two absorption bands at 1620, 1690 cm.$^{-1}$ and a shoulder at 1730 cm.$^{-1}$. This compound is best formulated as 2-imino-4-carboxyimidazolidine.[5] The mother liquors have not been further purified. They contained at least three more



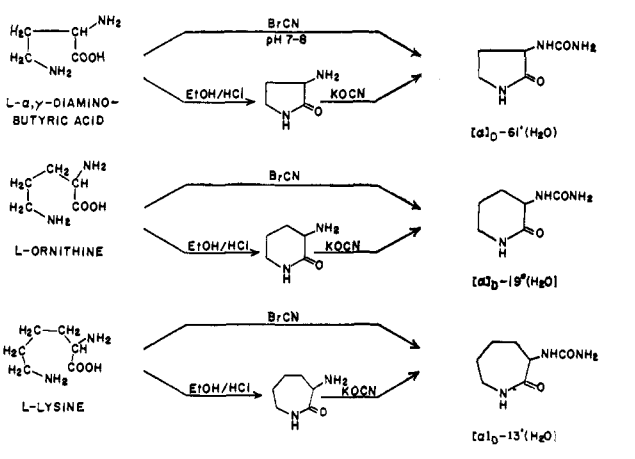
compounds, detectable by thin-layer chromatography on silica gel after exposure of the plates to iodine vapor.

L-α,γ-Diaminobutyric Acid (DAB), L-Ornithine, and L-Lysine.—These three α,ω-diamino acids on treatment with cyanogen bromide show a more or less uniform reaction pattern. The following crystalline, neutral, and nonionic compounds were isolated: from DAB: $C_5H_9O_2N_3$, m.p. 288° dec., yield 57.5%, $[\alpha]^{20}D$ −61.9° (H_2O); from L-ornithine: $C_6H_{11}O_2N_3$, m.p. 245° dec., yield 25%, $[\alpha]^{20}D$ −19.0° (H_2O); and from L-lysine: $C_7H_{13}O_2N_3$, m.p. 246° dec., 36.6%, $[\alpha]^{20}D$ −13.2° (H_2O).

A lemon-yellow color with Ehrlich reagent indicated the presence of a $-CONH_2$ group in each of these products. Molecular weight determinations and infrared spectra led to the formulas **L-3-ureido-pyrrolidone** derived from DAB; **L-3-ureido-piperidone** from L-ornithine, and **L-3-ureido-homopiperidone** from L-lysine (*cf.* Chart II). Proof of structure for these three compounds came by independent synthesis, namely by reaction of L-3-aminopyrrolidone, L-aminopiperidone ("anhydroornithine"), and L-3-aminohomopiperidone ("anhydrolysine"),[7] respectively, with potassium cyanate.[6] The corresponding ureido derivatives were found to be identical with the cyanogen bromide reaction products with respect to m.p., mixture m.p., thin-layer chromatography, and infrared spectrum (KBr) (*cf.* Chart II).

CHART II

DIAMINO ACIDS AND BrCN



(7) *Cf.* D. W. Adamson, *J. Chem. Soc.*, 39 (1943).

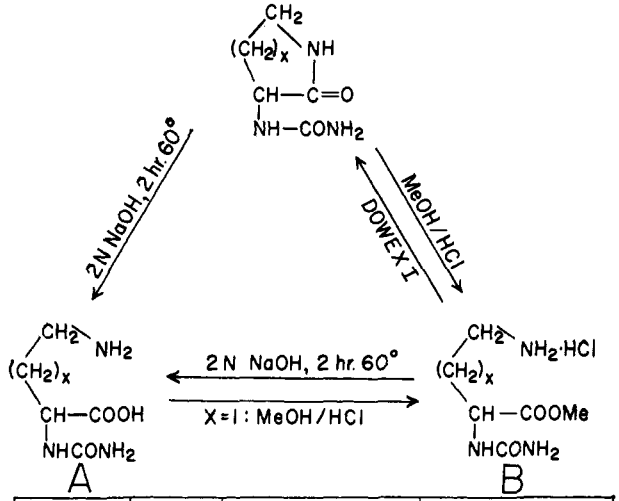
By controlled hydrolysis with 2.0 *N* sodium hydroxide for 2 hr. at 60°, the ureido heterocycles opened up to α-N-carbamido-ω-amino acids (*cf.* Chart III). There were obtained L-α-ureido-γ-aminobutyric acid (A, x = 1), yield 88%, m.p. 210°, $[\alpha]^{20}D$ +6.5° (H_2O); L-isocitrulline (A, x = 2), yield 58%, m.p. 228°, $[\alpha]^{20}D$ +27.5° (H_2O); and L-isohomocitrulline (A, x = 3), yield 54%, m.p. 216°, $[\alpha]^{20}D$ −19.8° (H_2O). In addition to the latter two compounds, two by-products were separated by repeated fractional crystallization which were both optically inactive.

Both compounds are isomeric with and show R_f values identical with those of the corresponding main products and are considered to be DL-isocitrulline and DL-isohomocitrulline. L-Isocitrulline and L-isohomocitrulline were prepared for comparison by treatment of carbobenzoxy-L-ornithine and ε-carbobenzoxy-L-lysine with potassium cyanate and subsequent removal of the protecting group by hydrogen bromide in acetic acid.

The compounds so obtained were found to be identical, respectively, with the optically active saponification products of L-3-ureidopiperidone and -homopiperidone with regard to m.p., mixture m.p., and thin-layer chromatography in two different solvent systems.

When treated with a solution of hydrogen chloride in methanol, the L-ureido heterocycles opened up to methyl ester hydrochlorides of the open α-ureido derivatives (*cf.* Chart III). Attempts to obtain the free bases by passing aqueous solutions of these ester hydrochlorides through basic ion exchange columns resulted in reconversion of the three open compounds to the cyclic ureido derivatives.

CHART III



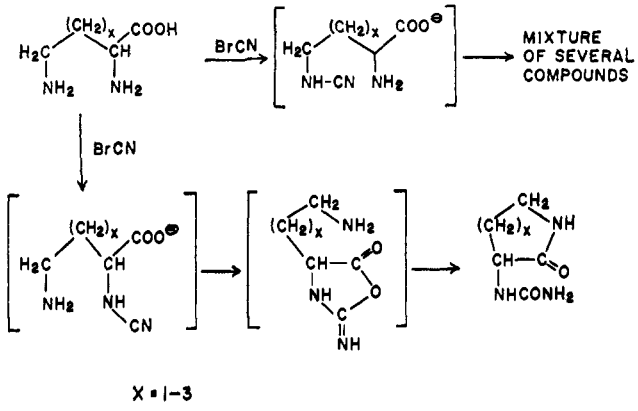
		m.p.	$[\alpha]_D^{20°}$ ($H_2O \rightarrow 1N$ HCl)	Side Product
X=1	A	210°d.	+6.5° (H_2O)	
	B	190°d.	−39.8°	
X=2	A	228°d. (amorph.)	+27.5°→−2.4°	250°d, $[\alpha]_D$=±0°
	B			
X=3	A	216°d.	−19.8°→+1.3°	170-180°, $[\alpha]_D$=±0°
	B	175°d.	−50.0°→−60.4°	

By treatment of L-α-ureido-γ-aminobutyric acid with methanol and hydrogen chloride, the same methyl ester hydrochloride (m.p. 190°, $[\alpha]^{20}D$ −39.0° (H_2O)), was obtained as by methanolysis of 3-ureidopyrrolidone.

The mechanism of the reaction of diamino acids with cyanogen bromide is analogous to the one suggested for α-amino acids (Chart IV). The formation of cyclic lactams proceeds *via* an initial α-N-cyano-ω-amino acid which then cyclizes to 5-iminooxazolidone. Subsequent intramolecular opening of this reactive intermediate by the ω-amino group leads to the ureido heterocycles. In the case of the α-amino acids the analogous intermediate is opened by an *inter*molecular reaction to yield the bimolecular products, *viz.*, ε-carbamido dipeptides.

CHART IV

MECHANISM OF BrCN WITH DIAMINO ACIDS



There is little preference in the reaction of cyanogen bromide with either the α- or ω-amino group of diamino acids. Yields of crystalline ureido heterocycles do not exceed significantly the 50% level. The 40–65% noncrystalline products arise probably through initially formed ω-cyanodiamino acids and further condensation reactions which so far have not been investigated

These model reactions on the single amino acid level are now being extended to peptides and proteins.

Experimental

N-Carbamido-L-leucyl-L-leucine.—L-Leucine (2.62 g., 20 mmoles) was dissolved in water (250 ml.) and treated at room temperature with a solution of cyanogen bromide (4.3 g., 40 mmoles) in water (100 ml.). The solution was kept at pH 7.3 by addition of 10.0 *N* sodium hydroxide, using an automatic titrator (Radiometer, Copenhagen). The alkali consumption (2.4 ml.) ceased after 35 min. There was then added 1.0 *N* HCl (25 ml.) and the solution concentrated *in vacuo* to *ca.* 100 ml. The crystalline product was collected, washed several times with water, and dried at room temperature; yield 2.52 g. (87.5%), m.p. 215°. After recrystallization from methanol–water there was obtained 2.45 g. of colorless crystals, m.p. 217°.

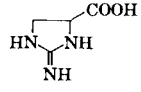
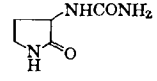
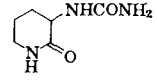
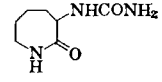
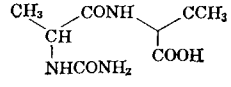
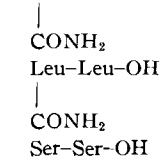
N-Carbamido-L-valyl-L-valine was prepared in the same manner as described for N-carbamido-L-leucyl-L-leucine. Crystallized from methanol–water it had m.p. 208°. For analytical data see Chart V.

N-Carbamido-L-seryl-L-serine was prepared in the same manner as described above for N-carbamido-L-leucyl-L-leucine. Recrystallized from ethanol–ether it had m.p. 165°. For analytical data see Chart V.

N-Carbamido-L-alanyl-L-alanine was prepared in the same manner as described above for N-carbamido-L-leucyl-L-leucine. The final product did not crystallize from the concentrated aqueous solution. Therefore, the solution was filtered through a column of ion-exchange resin, Dowex 1-X8, OH^- form (3 × 30 cm.) and washed with water until the eluate was neutral. The absorbed product was eluted from the column with 2.0 *N* acetic acid, evaporated *in vacuo* and crystallized from ethanol–ether; m.p. 193°. For analytical data see Chart V.

ε-Ureidocaproic Acid.—ε-Aminocaproic acid (1.31 g., 10 mmoles) was dissolved in water (25 ml.), and methanol (200 ml.) was added. A solution of cyanogen bromide (2.5 g., 25 mmoles) in methanol was added and the reaction mixture was kept at pH

CHART V

Starting material	Crystalline product: Yield, %	M.p., °C.	[α]D in H_2O or EtOH	[α]D in 1.0 *N* HCl	Formula: Structural	Empirical	Analyses, % Calculated: C	H	N	Found: C	H	N
L-α,β-Diaminopropionic acid monohydrochloride	32	268 d.	−37.0° (H_2O)	−27.7°		$C_4H_7N_3O_2$	37.21	5.46	32.55	37.69	5.16	31.96
L-α,γ-Diaminobutyric acid monohydrochloride	57.5	288 d.	−61.9 (H_2O)	−91.0 (5.0 *N* HCl)		$C_5H_9N_3O_2$	41.95	6.34	29.36	42.18	6.37	29.07
L-Ornithine monohydrochloride	25	245 d.	−19.0 (H_2O)	−35.9		$C_6H_{11}N_3O_2$	45.85	7.05	26.74	46.94	7.10	27.17
L-Lysine monohydrochloride	36.6	246 d.	−13.2	−18.3 (H_2O)		$C_7H_{13}N_3O_2$	49.11	7.65	24.55	49.86	7.58	23.42
L-Alanine	67.5	193	−20.0 (EtOH)			$C_7H_{13}N_3O_4$	41.77	6.45	20.68	41.60	6.28	20.08
L-Valine	62.5	208	−7.3 (EtOH)	−17.0		$C_{11}H_{21}N_3O_4$	50.93	8.16	16.20	50.86	8.03	16.13
L-Leucine	87.5	217	−80.3 (EtOH)			$C_{12}H_{25}N_3O_4$	54.33	8.77	14.62	53.79	9.00	13.56
L-Serine	72.5	165	+28.1 (50% EtOH)			$C_7H_{13}N_3O_6$	35.74	5.57	17.87	35.56	5.77	17.62
ε-Aminocaproic acid	67.0	178				$C_7H_{14}N_2O_3$	48.26	8.10	16.08	48.40	8.37	16.28

7.5 by automatic addition of 10.0 *N* NaOH. The consumption of alkali ceased after 45 min. and the addition of slightly more than 2 equivalents. The solution was then concentrated *in vacuo* to a volume of 20 ml. and poured onto a column of Dowex 1-X8, OH^- form (4 × 10 cm.). The column was washed with water until the effluent was neutral. The retained product was eluted from the column with 2.0 *N* acetic acid, evaporated *in vacuo*, and crystallized from methanol–water. There was obtained 1.00 g. of colorless crystals, m.p. 173–175°; recrystallized from the same solvent, m.p. 177–178°; for analytical data see Chart V.

L-2-Imino-4-carboxyimidazolidone.—A solution of L-α,β-diaminopropionic acid monohydrochloride (5.6 g., 40 mmoles) in water (2000 ml.) was mixed at room temperature with a solution of cyanogen bromide (8.5 g., ~80 mmoles) in water (150 ml.). The pH of the solution was adjusted to 7.6 and maintained by automatic addition of 10.0 *N* NaOH. After consumption of 1 equivalent of NaOH (30 min.), the reaction was stopped by removal of excess cyanogen bromide *in vacuo*. The solution was then poured onto a column of Dowex 1-X8, OH^- form (4.5 × 15 cm.) and the column washed with water until the effluent was neutral. The retained product was eluted from the column with 2.0 *N* acetic acid and the solvent removed *in vacuo* to leave a residue of 4.6 g. Crystallization from ethanol–water yielded 1.85 g. of colorless crystals, m.p. 265° dec., and, after recrystallization, m.p. 268° dec. (1.78 g.); for analytical data see Chart V.

The mother liquors were discarded; they contained at least 3 more substances detectable on thin layer silica gel plates in the solvent system *t*-butyl alcohol–formic acid–water, 4:1:1. The spots were made visible by exposure to iodine vapor.

L-3-Ureidopyrrolidone.—L-α,γ-Diaminobutyric acid monohydrochloride (3.08 g., 20 mmoles) was dissolved in water (1000 ml.) and mixed with a solution of cyanogen bromide (4.4 g., 40 mmoles) in water (100 ml.). The solution was adjusted to, and maintained at, pH 7.5 by automatic addition of 2.94 *N* sodium hydroxide. After consumption of 2 equivalents of alkali (50 min.), the solution was concentrated *in vacuo* to about 700 ml. and then brought on the top of two connected columns of ion-exchange resin. The first column contained Dowex 1-X8 OH^- form (3.5 × 15 cm.); the second column contained Dowex 50-X8, H^+ form (2.5 × 25 cm.). The columns were then washed with water (2000 ml.) and the collected effluent was evaporated *in vacuo* to dryness. The neutral residue (1.7 g.) was crystallized from methanol–water and recrystallized from ethanol–water to leave 1.495 g. of colorless crystals, m.p. 288° dec.; for analytical data see Chart V.

Further elution of the Dowex 1 column with 2.0 *N* acetic acid gave noncrystallizable material (1.10 g.) which was not further investigated.

L-3-Ureidopiperidone.—In analogy to the procedure described above for diaminobutyric acid, L-ornithine monohydrochloride (6.75 g., 40 mmoles) in H_2O (2 l.), was treated with cyanogen bromide (8.8 g., 80 mmoles) at pH 7.5. The alkali consumption was 81 mmoles. The neutral product weighed 3.19 g. After crystallization and recrystallization from ethanol–water there was obtained 1.69 g. of colorless crystals, m.p. 240° dec. For analytical data see Chart V. Further elution from the column of Dowex 1 with 2.0 *N* acetic acid yielded 1.80 g. of material.

L-3-Ureidohomopiperidone.—In analogy to the procedure described above for L-diaminobutyric acid, L-lysine monohydrochloride (3.64 g., 20 mmoles) in H_2O (1 l.), was treated with cyanogen bromide (4.5 g., ~40 mmoles) at pH 7.7. The alkali consumption was 40 mmoles. The neutral product weighed 1.86 g. After crystallization and recrystallization from ethanol–water there was obtained 1.33 g. of colorless crystals, m.p. 246–248° dec.; for analytical data see Chart V. Further elution from the column of Dowex 1 with 2.0 *N* acetic acid produced 1.46 g. of material.

L-α-Ureido-γ-aminobutyric Acid.—L-3-Ureidopyrrolidone (400 mg.) was dissolved in 1.0 *N* sodium hydroxide (10 ml.) and warmed for 2 hr. at 62°. The solution was then cooled, brought onto a column of Dowex 1, OH^- form (2 × 12 cm.), and the column washed with water until the effluent was neutral. The retained product was eluted from the column with 2.0 *N* acetic acid, the solvent evaporated *in vacuo*, and the residue crystallized from ethanol–water: 350 mg. of colorless crystals, m.p. 210° dec., $[\alpha]^{20}D$ +6.5° (H_2O).

Anal. Calcd. for $C_5H_{11}N_3O_3$: C, 37.26; H, 6.88; N, 26.08. Found: C, 37.40; H, 6.85; N, 25.97.

DL-Isocitrulline.—L-3-Ureidopiperidone (500 mg.) was dissolved in 1.0 *N* NaOH, saponified, and worked up as described above. Crystallization from ethanol–water yielded a first crop of crystals (293 mg.), m.p. 250° dec., $[\alpha]^{20}D$ ±0°.

Anal. Calcd. for $C_6H_{13}N_3O_3$: C, 41.13; H, 7.48; N, 23.99. Found: C, 41.22; H, 7.48; N, 23.87.

L-Isocitrulline.—A second crop (211 mg.) had m.p. 228° dec., $[\alpha]^{20}D$ +27.5° (H_2O).

Anal. Calcd. for $C_6H_{13}N_3O_3$: C, 41.13; H, 7.48; N, 23.99. Found: C, 41.10; H, 7.60; N, 23.94.

L-Isohomocitrulline.—L-3-Ureidohomopiperidone (1.0 g.) was dissolved in 1.0 *N* sodium hydroxide, saponified, and worked up as described above. Crystallization from ethanol–water yielded a first crop of crystals; 540 mg., m.p. 216° dec., $[\alpha]^{20}D$ −19.8°.

DL-Isohomocitrulline.—A second crop (408 mg.) had m.p. 170–180° dec. and $[\alpha]^{20}D$ ±0°.

Anal. Calcd. for $C_7H_{15}N_3O_3$: C, 44.43; H, 7.99; N, 22.21. Found: C, 44.26; H, 7.99; N, 21.94.

L-α-Ureido-γ-aminobutyric Acid Methyl Ester Hydrochloride.—L-3-Ureidopyrrolidone (400 mg.) was dissolved in absolute methanol (20 ml.). The solution was cooled in an ice bath and saturated with hydrogen chloride. After standing for 15 hr. at room temperature, the solvent was evaporated *in vacuo* and the residue crystallized from ethanol. There was obtained 0.386 mg. of colorless crystals, m.p. 195°. A second crop (72 mg., m.p. 185–190°) was obtained from mother liquors.

Anal. Calcd. for $C_6H_{13}N_3O_3 \cdot HCl$: C, 34.5; H, 6.6; N, 19.9. Found: C, 35.1; H, 6.8; N, 18.9.