hours with 300 ml. of commercial acetone containing 1.2% (w./v.) hydrogen chloride. The acid was removed by shaking with basic lead carbonate and the filtered solution was concentrated at reduced pressure. The residue was taken up in a small volume of acetone, centrifuged from a small amount of insoluble matter and concentrated, and the crystalline residue dried *in vacuo* over P_2O_5 and NaOH for 16 hours. Two recrystallizations from a mixture of equal volumes of *n*-butyl ether and heptane, with hot centrifugation each time from some insoluble matter, yielded 4.59 g. of material (72%); m.p. 104.5–106°, with prior sintering at 103.5°, $[\alpha]^{23}D$ −45.4° (*c* 1.9, U.S.P. chloroform).

Anal. Calcd. for $C_{12}H_{22}O_6$ (274.3): C, 56.92; H, 8.09; acetone, 42.35. Found: C, 56.81; H, 7.97; acetone, 42.5.

Hydrolysis of the diisopropylidene pinitol by refluxing for 45 minutes in 0.1 *N* hydrochloric acid regenerated the starting material; two recrystallizations, achieved by dissolving the compound in water and adding ethanol (nine volumes) gave a product of m.p. 185–187°, $[\alpha]^{26}D$ +65.0° (*c* 2.5, water) in 83% yield.

Dimethyl Diisopropylidene *d*-Inositol (IV).—Diisopropylidene pinitol (10 g.) was converted to the sodium salt with sodium sand in dry ether[12] and methylated with methyl iodide for 60 hours. After removal of the excess reagent at reduced pressure, the compound was obtained pure after three recrystallizations from *n*-pentane; further pure material was obtained from the mother liquors; total yield 8.80 g. (84%), m.p. 88–90°, $[\alpha]^{23}D$ −44.4° (*c* 1.9, U.S.P. chloroform).

Anal. Calcd. for $C_{14}H_{24}O_6$ (288.3): C, 58.32; H, 8.39; OCH_3, 21.53. Found: C, 58.46; H, 8.38; OCH_3, 21.22.

Dimethyl *d*-Inositol (V).—Six grams of dimethyl diisopropylidene *d*-inositol was refluxed for 45 minutes in 125 ml. of 0.1 *N* hydrochloric acid. The solution was then concentrated at reduced pressure and the resulting solid was recrystallized from commercial absolute ethanol to give, together with material from the mother liquor, 4.15 g. (95%), m.p. 191–193°, $[\alpha]^{23}D$ +73.0° (*c* 2, water).

Anal. Calcd. for $C_8H_{16}O_6$ (208.2): C, 46.15; H, 7.75; OCH_3, 29.81. Found: C, 46.13; H, 7.61; OCH_3, 28.9.

(12) E. Paesu and S. M. Trister, THIS JOURNAL, **61**, 2442 (1939).

Periodate titration[13]: 21.2 mg. (0.102 millimole) and 300 mg. of sodium meta-periodate (1.4 millimoles) in 50 ml. of water; moles of periodate consumed per mole of dimethyl inositol after the stated time interval were: 0.5 hour, 2.68; 1 hour, 2.74; 2 hours, 2.84; 8.5 hours, 2.99; 24 hours, 3.03; 51.5 hours, 3.05.

The tetraacetate, which crystallized on pouring the pyridine–acetic anhydride acetylation mixture into water, was recrystallized three times from *n*-butyl ether; yield 63%, m.p. 102.5–103.5°, $[\alpha]^{24}D$ −1.4° (*c* 2, U.S.P. chloroform).

Anal. Calcd. for $C_{16}H_{24}O_{10}$ (376.4): C, 51.06; H, 6.43; OCH_3, 16.49. Found: C, 51.12; H, 6.41; OCH_3, 16.51.

Dimethyl-D-tartaric Acid Bis-(methylamide) (VI).—Dimethyl *d*-inositol (750 mg.) was dissolved in 32.6 ml. of 0.433 *N* periodic acid, and the solution was cooled in ice for a few minutes and then allowed to stand at room temperature for two hours after which it was neutralized to a *p*H of 7 with solid barium hydroxide. The precipitate was removed by filtering through Celite,[14] strontium carbonate (10 g.) and bromine (6 g. stirred until dissolved) were added to the filtrate, and the mixture allowed to stand at room temperature for 18 hours. The filtered solution was aerated to remove bromine, acidified with two ml. of 5 *N* hydrochloric acid and extracted continuously with ether for 48 hours. An excess of diazomethane in ether was added to the ether extract, the solution was concentrated at reduced pressure, and taken up in 25 ml. of methanol and saturated at 0° with methylamine. After 45 hours at room temperature the solution was concentrated *in vacuo*, methanol was added and removed *in vacuo* and this process repeated two or three times to remove all excess methylamine. The residue was taken up in 100 ml. of ethyl acetate, filtered hot and, after removal of the solvent, the crystalline residue was recrystallized twice from ethyl acetate, giving 536 mg. (73%), m.p. 209.5–210.5°, $[\alpha]^{24}D$ −134° (*c*, 1.62, water). For dimethyl-D-tartaric acid bis-(methylamide), Haworth and Jones[9] reported m.p. 205°, $[\alpha]^{17}D$ −131.8 (*c* 1.61, water).

Anal. Calcd. for $C_8H_{16}O_4N_2$ (204.2): C, 47.04; H, 7.90; N, 13.72; OCH_3, 30.39. Found: C, 47.22; H, 7.78; N, 13.62; OCH_3, 30.11.

(13) E. L. Jackson, in "Organic Reactions," Vol. II, John Wiley and Sons, New York, N. Y., 1944, p. 361.

(14) A product of the Johns-Manville Company.

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[CONTRIBUTION FROM WESTERN REGIONAL RESEARCH LABORATORY[1]]

The Free Amino Groups of Subtilin

BY JOHN F. CARSON

Deamination of the polypeptide, subtilin, with nitrous acid, followed by hydrolysis; and microbiological assay and reaction of subtilin with dinitrofluorobenzene, followed by hydrolysis and chromatographic examination of the DNP-amino acids, show that the free amino groups of subtilin are contributed by lysine and the two sulfur diamino dicarboxylic acids. Each lysine unit has its α-amino group in linkage and the ε-amino group free. The two sulfur diamino dicarboxylic acids cannot exist in subtilin with both amino groups free. They must exist with one amino group free or neither free.

The nature of the free amino groups of the polypeptide subtilin has been investigated by deamination with nitrous acid and by the dinitrofluorobenzene technique of Sanger.[2] The problem is of particular interest in the case of subtilin because of the rapid inactivation of the antibiotic in dilute alkali accompanied by an apparent decrease in amino nitrogen (Van Slyke) in isolated products.[3] The isolation of mesolanthionine[4] and a second unidentified diamino dicarboxylic sulfur acid[5] with the empirical formula $C_5H_8S(NH_2)_2(COOH)_2$ indicates that subtilin may contain free α-amino groups of a type not ordinarily encountered in proteins or polypeptides.

The polypeptide was treated with nitrous acid for different periods of time and the deaminated products isolated and hydrolyzed with acid. The hydrolysates were then assayed microbiologically for lysine, valine, leucine, isoleucine, glycine, phenylalanine and glutamic and aspartic acids.[6]

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) F. Sanger, *Biochem. J.*, **39**, 507 (1945); **40**, 261 (1946); **42**, 287 (1948).

(3) Unpublished observations.

(4) G. Alderton and H. L. Fevold, THIS JOURNAL, **73**, 463 (1951).

(5) G. Alderton, to be published.

(6) The presence of free amino groups of tryptophan or of alanine was not investigated by this method. No accurate analytical determination for the two sulfur acids was known.

Subtilin also reacted with 2,4-dinitrofluorobenzene under mild conditions (in bicarbonate suspension at 3°) and the resulting DNP-subtilin[7] was hydrolyzed under various conditions. The acid hydrolysates were then separated into ether–soluble and acid-soluble–ether-insoluble fractions and each fraction submitted to partition chromatography on silica gel. Pure DNP-amino acids were synthesized and used as standards.

TABLE I

ANALYSES OF DEAMINATED SUBTILIN

Time, min.	Amino N, equiv./10^4 g.	Amino acid content (equiv./10^4 g.) Lysine	Isoleucine	Leucine	Valine	Phenylalanine	Glutamic acid	Aspartic acid	Glycine
0	13.1	8.4	4.1	10.7	2.4	2.8	8.2	2:9	5.7
7	5.07	4.2		11.0	2.3	2.8	8.2	2.8	6.2
18	1.1	0.4	3.9		1.7	2.8			6.0
30	0.93	.2	3.7	10.1	1.7	2.5	7.8	2.7	6.2

Results of deamination of subtilin recorded in Table I show that each lysine unit of subtilin has at least one free amino group, since no lysine could be detected in hydrolyzed deaminated subtilin. Glycine, leucine, isoleucine, phenylalanine, glutamic and aspartic acid contents showed no significant decrease on deamination, indicating that probably none of these contribute free amino groups to subtilin. These results were confirmed by the DNP method. The valine content was decreased by nearly 30% or approximately 0.7 equiv./10^4 g. This could result from uncertainties in the microbiological assays[8] or from a loss of material by fractionation in the recovery of the deaminated subtilin.

Chromatography of the acid-soluble fraction of hydrolyzed DNP-subtilin (which would contain any monosubstituted diamino acids) yielded only one band with several solvent combinations. The presence of ε-N-DNP-lysine in this band was indicated by mixed chromatograms and proved by isolation of the pure compound. Mixed chromatograms proved the absence of any α-N-DNP-lysine. Therefore none of the lysine groups in subtilin has the α-amino group free and the ε-amino group in linkage. The presence of additional DNP-acids in the ε-N-DNP-lysine band was suggested by the fact that mother liquors yielded traces of a second yellow crystalline component which gave a strong test for thioether. This probably consists of a mono-DNP-lanthionine or a mono DNP derivative of the unidentified sulfur amino acid.

Chromatography of the ether-soluble fraction of the hydrolysate on silica gel showed that N,N′-bis-DNP-lysine was not present, proving that none of the lysine has both amino groups free. In conjunction with the results of chromatography of the acid-soluble fraction and of nitrous acid deamination, it follows that each lysine residue has the α-amino group in linkage and the ε-amino group free. It was also proved by mixed chromatograms that none of the non-sulfur monoamino acids contribute free amino groups in subtilin. It was also shown that the unidentified sulfur amino acid, $C_5H_8S(NH_2)_2(COOH)_2$, does not exist in subtilin with both amino groups free. Chromatography of the methyl esters on silicic acid confirmed this and proved also than lanthionine in subtilin does not have both amino groups free.

In conclusion, all of the free amino groups of subtilin are contributed by lysine and the two sulfur diamino-dicarboxylic acids, two-thirds by lysine and one-third by the sulfur acids. Each lysine unit has its α-amino group in linkage and its ε-amino group free. The two sulfur amino acids do not exist with both amino groups free.

Acknowledgments.—The author gratefully acknowledges the assistance of Miss Neva Snell for microbiological assays, Mr. L. M. White and Miss Geraldine Secor and co-workers for chemical analyses, Dr. F. T. Jones for microscopic examinations and Mr. Gordon Alderton for samples of mesolanthionine and the second sulfur amino acid.

Experimental

Subtilin was prepared as described elsewhere[9] from submerged cultures of *B. subtilis*. The polypeptide was deashed by passage of a 5% aqueous solution through the cation exchanger, IR-100[10] (hydrogen cycle), and recovered by lyophilization of the effluent. The lyophilized material had the analyses in terms of equiv./10^4 on the dry basis: Kjeldahl nitrogen, 113; sulfur, 15.0; amino nitrogen (Van Slyke), 13.1; amide nitrogen, 15.7[11]; and carboxyl, 6–7.[11]

Deamination with Nitrous Acid.—In a typical experiment 1.75 g. of dry subtilin was dissolved in 75 ml. of 8 *M* acetic acid. Seventy-five ml. of 3 *M* aqueous sodium nitrite was added and the mixture was stirred for 30 minutes at 26 ± 2°. A precipitate formed after about one minute of reaction. At the expiration of 30 minutes, the mixture was cooled to 10° by the addition of ice, and an excess of glycine (20 g.) was added in small portions to destroy excess nitrite. After standing two days at 3°, the suspension was filtered and washed seven times with 100-ml. portions of aqueous acetic acid–potassium acetate (0.5 *M*) and several times each with 5% acetic acid in 95% ethanol, 95% ethanol, and finally with ether. The dry product weighed 1.3 g. (74%) and had the Van Slyke amino nitrogen, 0.13% or 0.93 equiv./10^4 g.

Hydrolysis of Deaminated Subtilin.—The dry deaminated subtilin (141 mg.) in 4 ml. of 10 *N* hydrochloric acid was heated at 100° for one hour, 3 ml. of water was added, and the solution was refluxed for 22 hours. The hydrolysate was then taken to dryness *in vacuo* and assayed for amino acids microbiologically by the procedure described by J. C. Lewis, *et al.*[12]

Preparation of DNP-Subtilin.—A solution of 2.26 g. of subtilin in 75 ml. of water was cooled to 3°, then 150 ml. of ethanol, 10 g. of sodium bicarbonate and 4 ml. of 2,4-dinitrofluorobenzene were added. The suspension was stirred for 46 hours at 2–4°.[13] The suspension was then diluted with 200 ml. of water, the excess bicarbonate was destroyed with hydrochloric acid, and the yellow solid isolated by filtration on sintered glass followed by washing with cold 0.5% aqueous hydrochloric acid, distilled water and finally ether; yield 2.89 g. (theoretical yield assuming complete reaction, 2.76 g.). Van Slyke amino nitrogen, 0.25% or 1.56 equiv./10^4 g. A second treatment of this material

(7) DNP will be used as an abbreviation for 2,4-dinitrophenyl-.

(8) The decrease in valine is most likely the result of uncertainties in the microbiological assay. The results of valine assays of hydrolyzed deaminated subtilin showed a much greater variation than has been experienced with the valine assays of hydrolyzed subtilin or proteins.

(9) J. A. Garibaldi and R. E. Feeney, *Ind. Eng. Chem.*, **41**, 432 (1949); H. L. Fevold, *et al.*, *ibid.*, **18**, 27 (1948).

(10) Resinous products and Chemical Company, Philadelphia, Pennsylvania.

(11) These analyses were obtained by procedures previously described, J. F. Carson, E. F. Jansen and J. C. Lewis, THIS JOURNAL, **71**, 2318 (1949).

(12) J. C. Lewis, N. S. Snell, D. J. Hirschmann and Heinz Fraenkel-Conrat, *J. Biol. Chem.*, **186**, 23 (1950).

(13) The low temperature was employed to minimize subtilin decomposition. Subtilin was found to be stable in bicarbonate suspension at low temperature for several days.

with dinitrofluorobenzene under the same conditions gave the product with amino nitrogen, 0.12% or 0.7 equiv./10^4 g.

N-DNP-Glycine, -alanine, -valine, -leucine, -isoleucine, -proline, -tryptophan, -glutamic acid, -aspartic acid, -phenylalanine and N,N'-bis-DNP-lysine were prepared as the D,L-compounds by reaction of the corresponding amino acids with dinitrofluorobenzene in 80% ethanol in the presence of sodium bicarbonate as described by Porter and Sanger.[14]

ε-N-DNP-Lysine was prepared by reaction of 2,4-dinitrofluorobenzene with the copper complex of L-lysine.[14]

α-N-DNP-Lysine was prepared by the sequence of steps: lysine copper complex → ε-N-benzoyllysine → ε-N-benzoyl-α-N-DNP-lysine → α-N-DNP-lysine as described by Sanger.[2]

N,N'-Bis-DNP-mesolanthionine.—A suspension of 338 mg. of mesolanthionine, 4.0 g. of 2,4-dinitrochlorobenzene, 6.0 g. of sodium bicarbonate and 100 ml. of 75% ethanol was boiled under reflux for four hours. The deep orange mixture was concentrated to dryness *in vacuo*. After extraction of the dry solid several times with ether, 50 ml. of water was added and the mixture acidified with 3 *N* hydrochloric acid. The precipitated brown amorphous solid was isolated by centrifugation and washed repeatedly with normal hydrochloric acid followed by water, yield 658 mg. The product was recrystallized five times from solution in 50 parts of acetic acid–35 parts of water, yield 338 mg. *Anal.* Calcd. for $C_{18}H_{16}N_6O_{12}S$: N, 15.32; S, 5.85. Found: N, 14.8; S, 5.70. This derivative could not be prepared in satisfactory yields using dinitrofluorobenzene. It usually separated from aqueous acetic acid as an oil which later crystallized.

N,N'-Bis-DNP-mesolanthionine Dimethyl Ester.—Bis-DNP-mesolanthionine, 350 mg., was dissolved in 50 ml. of 3 *N* methanolic hydrogen chloride and allowed to stand at room temperature for 18 hours. As esterification proceeded, crystals of the dimethyl ester separated. The mixture was concentrated *in vacuo* to dryness, fresh methanol was added, and the suspension again concentrated to dryness. The crystals were then suspended in 50 ml. of methanol, cooled to 0°, and filtered, yield 283 mg. Additional product could be obtained by re-esterifying the mother liquor. The derivative was unusual in that it was practically insoluble in methanol. It was recrystallized from acetone–methanol (1:2) as yellow needles, softens at 110–140°, resolidifies and melts sharply at 157–158°.

Anal. Calcd. for $C_{20}H_{20}N_6O_{12}S$: N, 14.79; S, 5.64. Found: N, 14.3; S, 5.50.

N,N'-Bis-DNP-derivative of the second sulfur amino acid was prepared by shaking for 18 hours a suspension of 200 mg. of the compound in 30 ml. of 80% ethanol, containing 2 g. of dinitrofluorobenzene and 2.5 g. of sodium bicarbonate. The product was isolated in the same manner as the corresponding lanthionine derivative, but could not be obtained crystalline. It was purified by precipitation of the acid by slow acidification of aqueous solutions of the sodium salt; $[\alpha]^{25}D$ +15° (*l* 1, *c* 2.13) in 2% sodium bicarbonate; $[\alpha]^{25}D$ −66° (*l* 1, *c* 2.46) in ethyl acetate.

Anal. Calcd. for $C_{19}H_{18}N_6O_{12}S$: N, 15.16; S, 5.77; C, 41.08; H, 3.27. Found: N, 15.5; S, 6.13; C, 40.8; H, 3.47.

Hydrolysis of DNP-Subtilin.—A solution of 163 mg. of DNP-subtilin in 10 ml. of acetic acid plus 10 ml. of 10 *N* hydrochloric acid was refluxed for 29 hours and then taken to dryness *in vacuo*. The residue was dissolved in 40 ml. of normal hydrochloric acid, filtered and extracted 5 times with 25-ml. portions of ether, most of the color remaining in the acid phase. The acid-soluble fraction was concentrated *in vacuo* to dryness and brought to a volume of 10 ml. in 0.5 *N* hydrochloric acid and the ether-soluble fraction after drying over sodium sulfate was concentrated to dryness and dissolved in 10 ml. of acetone to be used as a stock solution. It was found that hydrolyzing for periods of 18 to 30 hours in acetic acid–hydrochloric acid or in 6 *N* hydrochloric acid gave essentially the same results on chromatography. The former solvent combination dissolved the derivative more rapidly and yielded less insoluble material. In testing for the presence of DNP-proline, DNP-subtilin was hydrolyzed for 2 and 5 hours.

Chromatography of the Acid-soluble Fraction.—Columns of silica gel,[15] 11 mm. × 120 mm., were prepared as described by Sanger.[2] The solvent combinations, 30% butanol–chloroform–water, 40% butanol–chloroform–water and 66% methyl ethyl ketone–ether–water were used. Aliquots of the acid-soluble fraction (evaporated to dryness and dissolved in the appropriate solvent) moved as a single band with 30% butanol–chloroform, 40% butanol–chloroform and 66% methyl ethyl ketone–ether with the respective *R* values[16] of 0.15, 0.27–0.30 and 0.15. ε-DNP-lysine had approximately the same *R* values and when mixed with the acid-soluble fraction, migrated as one band on each column. α-DNP-lysine moved with 30% butanol–chloroform, 40% butanol–chloroform, and methyl ethyl ketone–ether with the *R* values of 0.07, 0.12 and 0.10, respectively. When mixed with the acid-soluble fraction, two bands were observed with each solvent combination.

Isolation of ε-N-DNP-Lysine.—A solution of 2.70 g. of DNP-subtilin (air dry) in 50 ml. of acetic acid and 40 ml. of 10 *N* hydrochloric acid was heated in an oil-bath maintained at 114±2° for 18 hours. The hydrolysate was then concentrated *in vacuo* to dryness, taken up in 75 ml. of normal hydrochloric acid and extracted 5 times with 50-ml. portions of ether. The acid-soluble fraction was filtered with a small amount of carbon, the filtrate concentrated to dryness and the yellow residue redissolved in 15 ml. of 0.1 *N* hydrochloric acid. After standing several days at 0°, a yellow crystalline material separated. The crystals were recrystallized from 10 ml. of 0.1 *N* hydrochloric acid to yield 170 mg. (23%) of ε-N-DNP-lysine hydrochloride monohydrate identified by microscopic examination of the optical properties and comparison with an authentic sample. The mother liquor yielded additional impure ε-N-DNP-lysine which gave a strong test for sulfide by the potassium iodoplatinate test[17] and contained a second yellow crystalline material indicating the probable presence of mono-DNP derivatives of the sulfur acids in this fraction. Attempts to separate the mono-DNP-sulfur acids from ε-N-DNP-lysine by chromatography have not been successful.

Chromatography of the Ether-soluble Fraction.—The absence of any DNP derivatives of the known amino acids of subtilin, with the exception of lanthionine, in this fraction was demonstrated by chromatography with chloroform–water and ethylene glycol–benzene. When chromatographed on a column of silica gel with chloroform–water, the ether-soluble fraction moved as an extremely fast, faint band. Addition of DNP-tryptophan yielded a second band. Similarly, chromatography with 17% butanol–chloroform–water eliminated the presence of the DNP derivatives of glutamic and aspartic acids.

With ethylene glycol–benzene (glycol, the stationary phase) the ether-soluble fraction moved as two very faint fast bands with the approximate *R* values of 1.0 and 2.0. Addition of the DNP derivatives of glycine, alanine, phenyl alanine, valine, leucine, isoleucine, proline and the bis-DNP derivative of the unidentified sulfur amino acid in each case yielded a separate band. N,N'-Bis-DNP-lanthionine was not sufficiently soluble in any of the solvent combinations except 17% butanol chloroform–water with which it moved too fast for identification. The absence of this derivative was shown by chromatography of the dimethyl ester on silicic acid.

Chromatography of the Methyl Esters of the Ether-Soluble Fraction and of the Bis-DNP-sulfur Acids.—The dimethyl esters of the bis-DNP derivatives of lanthionine and of the second sulfur amino acid were both adsorbed on silicic acid[18] from solution in acetone–hexane (1:6) and on development with hexane–ethyl acetate (3:1) moved slowly at rates too close to be separated. When the ether-soluble fraction of the hydrolysate was treated with methanol-hydrochloric acid and the product chromatographed, two faint bands were obtained, each different from the methyl esters of the two DNP-sulfur acids as shown by mixed chromatograms. The stability of the two DNP derivatives of lanthionine and of the other sulfur acid to acid hydrolysis was shown by adding each separately to DNP subtilin, hydrolyzing, fractionating into ether- and acid-soluble frac-

(14) R. R. Porter and F. Sanger, *Biochem. J.*, **42**, 287 (1948).

(15) 50–200 mesh "Silica for Chromatographic Columns," G. Frederick Smith Chemical Company, Columbus, Ohio.

(16) The *R* value is defined as the ratio, movement of the center of band/movement of solvent above column.

(17) H. M. Winegard, G. Toennies and R. J. Block, *Science*, **108**, 506 (1948); J. W. Sease, T. Lee, G. Holzman, E. H. Swift and C. Niemann, *Anal. Chem.*, **20**, 431 (1948).

(18) Silicic acid, 100 mesh "Analytical Reagent," Mallinckrodt Chemical Works, St. Louis, Missouri.

tions, esterifying with methanol–hydrochloric acid, and chromatographing on silicic acid. In each case a band corresponding to the methyl ester was obtained.

ALBANY 6, CALIFORNIA RECEIVED SEPTEMBER 7, 1951

[CONTRIBUTION FROM THE WM. H. CHANDLER CHEMISTRY LABORATORY, LEHIGH UNIVERSITY]

Some Substituted Biguanides and s-Triazines[1]

BY W. K. DETWEILER AND E. D. AMSTUTZ

This paper discusses the products arising from the fusion of dicyandiamide with pyrrolidine hydrochloride. The reactions of cycloaliphatic amines and primary heterocyclic amines of the amidine or guanidine type with 2-chloro-4,6-diamino-s-triazine are presented. The preparation of several 2,4,6-trisubstituted-amino-s-triazines by the use of cyanuric chloride are also given.

The purpose of this work was to synthesize a few selected compounds containing the amidine or guanidine structure for pharmacological testing.

The standard method of fusing an amine hydrochloride with dicyandiamide appeared to offer an attractive method for the preparation of a variety of substituted biguanide hydrochlorides. Thus, the fusion of pyrrolidine hydrochloride with dicyandiamide at approximately 130° for 24 hours permitted the isolation of 1,1-tetramethylenebiguanide hydrochloride in acceptable (44%) yield. Occasionally however, and under apparently the identical conditions, the same reactants have been found to evolve ammonia rapidly (6.5 hr.) and produce 1,1-tetramethyleneguanidine hydrochloride and 2-(1-pyrrolidyl)-4,6-diamino-s-triazine in addition to a very poor yield of 1,1-tetramethylenebiguanide hydrochloride.

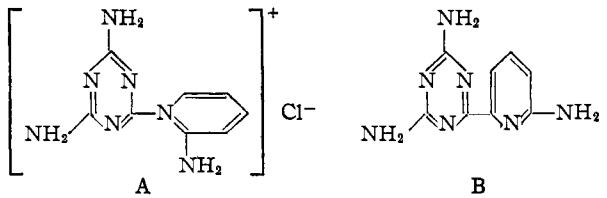
Although this method of preparation of substituted biguanides appears to be general and suitable for aliphatic, cycloaliphatic and aromatic amine hydrochlorides, it does not appear to be satisfactory for heterocyclic primary amine salts which contain a guanidine or amidine structure.[2]

Potassium dicyanoguanidine has been reported[3] to react with aliphatic, cycloaliphatic and aromatic secondary amine salts in aqueous solution to form N,N-disubstituted melamines. We have successfully treated pyrrolidine with potassium dicyanoguanidine in an acidic solution to produce a 14% yield of 2-(1-pyrrolidyl)-4,6-diamino-s-triazine. In contrast, the reaction of several heterocyclic primary amines such as 2-aminopyridine, 2-aminopyrimidine and 2-aminothiazole produced products which were infusible and insoluble in the common organic reagents and water. After considering the physical properties and the data obtained from complete analyses of the products obtained from this reaction, we have reached the conclusion that if the desired products were initially formed (which is doubtful) they hydrolyzed to produce a mixture of what may possibly be ammeline and ammelide.

The most satisfactory method of producing N,N-disubstituted melamines was found to be through the interaction of 2-chloro-4,6-diamino-s-triazine with an excess of the appropriate cycloaliphatic amine; 2-(1-pyrrolidyl)-, 2-(1-piperidyl)- and 2-(4-morpholinyl)-4,6-diamino-s-triazine were produced in yields ranging between 75 and 79%. After our work had been completed, the latter two s-triazines were reported[4] to have been prepared in yields of 22 and 48%, respectively. The present superior yields and the vigor with which our reactions took place seem to indicate that this type of reaction might best be run in such an excess of the amine that it can act as its own solvent.

An excess of piperazine reacted vigorously with 2-chloro-4,6-diamino-s-triazine to give a mixture of products from which 2-(1,4-piperazinyl)-bis-(4,6-diamino-s-triazine) was isolated in 48% yield.

The fusion of a large excess of 2-aminopyridine with 2-chloro-4,6-diamino-s-triazine produced what appeared to be a quantitative yield of 2-(2-pyridylamino)-4,6-diamino-s-triazine hydrochloride. It seemed to us unlikely that this apparent hydrochloride would be capable of isolation from the reaction mixture which contained a large excess of 2-aminopyridine.[5] A dry pyridine extraction of this substance removed 11% of the total weight; from this pyridine extract a water-insoluble free base was isolated. This substance upon analysis compared rather closely to 2-(2-pyridylamino)-4,6-diamino-s-triazine or an isomer of it. The pyridine-insoluble portion of the reaction product gave, after further purification, a precipitate with acidified silver nitrate solution. This water-soluble halogen containing compound upon analysis yielded results rather close to the theoretical values required for 2-(2-pyridylamino)-4,6-diamino-s-triazine hydrochloride or an isomer of it. These data make it appear as though the initial product of this reaction was possibly the pyridinium salt (A) which partially rearranged under the influence of heat to produce 2-



A B

(1) Taken from a thesis presented by W. K. Detweiler to the Graduate Faculty of Lehigh University in partial fulfillment of the requirements for the Ph.D. degree, June, 1951.

(2) The fusion of 2-aminopyrimidine hydrochloride with dicyandiamide at 178° produced a pitch which could not be purified; the same reaction in refluxing butanol did not yield any product which could be identified. The fusion of 2-aminothiazole hydrochloride with dicyandiamide resulted in the evolution of ammonia and hydrogen sulfide.

(3) D. E. Nagy, U. S. Patent 2,392,608 (1946); *C. A.*, **40**, 3480 (1946),

(4) D. F. Walker, Y. J. L'Italien, W. M. Pearlman and C. K. Banks, *J. Am. Pharm. Assoc.*, **39**, 393 (1950).

(5) It was suggested by the referee that the pyridylmelamine may actually be a stronger base than melamine. While this is true it would not explain the difficulty experience in removing the hydrochloric acid from the salt.