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The Preparation of N-Ethylglycocyamine. Double Salt Formation Between Guanidines and Amino Acids[1]

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N-Ethylglycocyamine and N-ethylglycocyamidine, homologs of creatine and creatinine, respectively, have been prepared. N-Ethylglycine and N-methyl-DL-alanine have been found to form stable double salts with guanidine hydrochloride and hydrobromide; none of several other amino acids and N-substituted amino acids form analogous double salts. N-Ethylglycocyamine and N-ethylglycine have been found to form a stable complex. It was not possible to form similar complexes by substituting creatine or sarcosine, respectively, for their homologs.

In a study of the relation between the pathological changes occurring in muscle tissue in muscular dystrophy and the accompanying abnormality in creatine metabolism, it became desirable to attempt the preparation of some compounds which might function *in vivo* as antagonists for creatine. One of the most attractive compounds for this purpose appeared to be N-ethylglycocyamine (N-ethylguanidinoacetic acid), a homolog of creatine. The preparation of the ethyl homolog was prompted by the fact that in other cases substitution of an ethyl group for the methyl group of a natural metabolite has resulted in the formation of an effective antagonist for the natural compound; *e.g.*, triethylcholine for choline[3] and ethionine for methionine.[4]

Unexpected difficulties were encountered when the preparation of N-ethylglycocyamine from N-ethylglycine was undertaken with procedures which give good results for the preparation of creatine from sarcosine. The preparation of creatine by the reaction of sarcosine with S-ethylisothiourea[5] or with cyanamide[6] in ammoniacal solution is facilitated by the crystallization of creatine hydrate from the reaction mixtures. When the reactions were carried out in the same manner with N-ethylglycine, no product crystallized. When the reaction mixtures were concentrated and examined, no product having the expected properties could be obtained in either case.

The product obtained by the reaction of N-ethylglycine with cyanamide did not have the properties expected for N-ethylglycocyamine. The elementary analysis and other properties showed this compound to be a stable complex of N-ethylglycocyamine and N-ethylglycine. N-Ethylglycocyamine finally was prepared from the complex by dissolving it in a small amount of conc'd ammonium hydroxide and cooling; N-ethylglycocyamine·H_2O crystallized in large dodecahedra. The hydrate is much less stable than creatine hydrate and quickly loses water when it is exposed to the atmosphere. Anhydrous N-ethylglycocyamine was purified by recrystallization from aqueous ethanol. N-Ethylglycocyamine was readily converted to N-ethylglycocyamidine by heating it with mineral acid according to the procedure used for the formation of creatinine from creatine. N-Ethylglycocyamidine gives the same color, on a molar basis, as does creatinine in the Jaffé determination for creatinine.

The product which was obtained in good yield by the addition of S-ethylisothiourea hydrobromide to a strongly ammoniacal solution of N-ethylglycine had salt-like properties, and was shown to be a stable salt having the composition: N-ethylglycine·guanidine·HBr. It could also be formed by the addition of an equivalent of guanidine hydrobromide to an alcoholic solution of N-ethylglycine. The corresponding hydrochloride double salt also is stable and can be recrystallized repeatedly without signs of separation of N-ethylglycine and the guanidine salt.

A brief survey of the generality of the formation of complex salts between a few guanidine compounds and amino acids was undertaken in an attempt to gain a better understanding of the nature of the compounds encountered in the synthesis. Long ago, Baumann[7] reported that a stable compound, which could be recrystallized from ethanol, was obtained when sarcosine and guanidine hydrochloride were heated together. The hydrochloride salt corresponding to the hydrobromide isolated in the attempted formation of N-ethylglycocyamine from N-ethylglycine and S-ethylisothiourea was prepared from N-ethylglycine and guanidine hydrochloride and was proved to be stable upon recrystallization from ethanol. An attempt to prepare similar salts from sarcosine and guanidine hydrobromide or hydrochloride gave some indications

(1) This research was supported by a grant from the National Institutes of Health, U. S. Public Health Service. A preliminary abstract of part of the work has appeared previously.[2]

(2) Armstrong, *Federation Proc.*, **12**, 170 (1953).

(3) Keston and Wortis, *Proc. Soc. Exptl. Biol. Med.*, **61**, 439 (1946).

(4) Dyer, *J. Biol. Chem.*, **124**, 519 (1938).

(5) *Org. Syntheses*, Coll. Vol. **3**, 440 (1955).

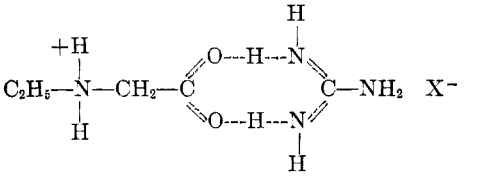
(6) Block, Schoenheimer, and Rittenberg, *J. Biol. Chem.*, **138**, 155 (1941).

(7) Baumann, *Ber.*, **7**, 1151 (1874).

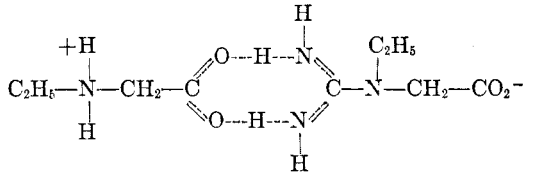
of the possible formation of double salts. Analyses of successive crops of the products, however, showed them to be inhomogeneous, and it was possible to separate sarcosine in a pure form by repeated recrystallizations of the crude products. Thus, the double salts, if formed at all, are not nearly as stable as the compounds prepared from N-ethylglycine.

An attempt then was made to prepare double salts from other amino acid derivatives and guanidine salts. Of these derivatives, only N-methyl-DL-alanine yielded isolable stable double salts similar to those obtained with N-ethylglycine. It is possible, however, that some of the N-substituted amino acids formed salts which could not be obtained crystalline because of their solubility behavior. Attempts to form other salts corresponding to the N-ethylglycocyamine·N-ethylglycine complex were all unsuccessful.

The stable salts obtained may possibly have structures such as



N-Ethylglycine·Guanidine Hydrohalide



N-Ethylglycocyamine·N-Ethylglycine Complex

where a particular and unusual set of circumstances insofar as basicity of guanidine group and alkylamino group, and possibly the degree of dipolar ion formation of the dipolar compounds, leads to an unusually stable, unionized guanidine-carboxylate combination. It was beyond the scope of this investigation to explore this type of compound further. It is of interest, however, to note the possibility that stable combinations of guanidine and carboxyl groups such as were observed in these compounds could be of importance in connection with the forces which maintain protein molecules in their native state. The well-known action of guanidine salts as a denaturing agent for proteins might be related to a dissociation of bonds of this type.

EXPERIMENTAL

N-Ethylglycocyamine. N-Ethylglycine (89.4 g.) was dissolved in 90 ml. of water, 35.0 g. of cyanamide and 1.0 ml. of conc'd NH_4OH were added, and the solution was allowed to stand at room temperature for 10 days. The solution then was cooled, saturated with ammonia gas, and cooled overnight in the refrigerator. The precipitate of dense granular crystals was collected, washed two times with abs. ethanol, and dried. The hydrate lost water when air-dried and yielded 55.5 g. of N-ethylglycocyamine. The mother liquor was conc'd to a small volume, saturated with ammonia, and cooled to yield an additional 7.3 g. of product. Total yield, 63.8 g. (a quantitative yield on the basis that only 50 per cent of the N-ethylglycine gave rise to N-ethylglycocyamine).

The crude product (63.8 g.) was dissolved in 65 ml. of hot water (crystals of hydrate, which appeared to be more insol. in water than was the anhydrous material, formed at this time) and the aqueous solution was diluted with 650 ml. of hot abs. ethanol. The solution was cooled overnight in the refrigerator and the elongated hexagonal plates of anhydrous N-ethylglycocyamine that separated were collected, washed with abs. EtOH, and dried. Yield, 52.0 g.; m.p., 264–270°d.,[8] after subl. to rectangular plates beginning at 195°.

Anal. Calc'd for $C_5H_{11}N_3O_2$: C, 41.37; H, 7.63; N, 28.95. Found: C, 41.86; H, 7.70; N, 29.20.

N-Ethylglycocyamidine. N-Ethylglycocyamine (6.0 g.) was dissolved in 10 ml. of conc'd HCl, the solution was heated under reflux for 2 hours, and the mixture was concentrated to dryness *in vacuo*. Then 10 ml. of abs. EtOH was added and was removed *in vacuo*. The residue was dissolved in 10 ml. of hot water, the solution was made slightly alkaline by the addition of an excess of conc'd NH_4OH, and 2 volumes of hot methanol were added. The solution was cooled overnight and the product was collected, washed with abs. EtOH, and dried. Yield, 3.1 g. (59%), m.p. 274–284°d., after subliming to long rectangular plates at 180°. The mother liquors were reworked to yield an additional 1.9 g. of N-ethylglycocyamidine; total yield, 5.0 g. (96%). For analysis, the N-ethylglycoamidine was recrystallized from hot water; m.p. 270–275°d., after subliming to rectangular plates, beginning at 195°.

Anal. Calc'd for $C_5H_9N_3O$: C, 47.23; H, 7.13; N, 33.05. Found: C, 47.37; H, 7.29; N, 32.7.

N-Ethylglycocyamine·N-Ethylglycine complex. A solution of 4.4 g. of cyanamide in 4.0 ml. of water was added to 10.3 g. of N-ethylglycine in 10.0 ml. of water, 2 drops of conc'd NH_4OH were added, and the resulting solution was allowed to stand 10 days at room temperature. Then it was conc'd to dryness *in vacuo*, keeping the temperature of the bath below 40°, the residue was taken up in 20 ml. of warm abs. EtOH, and the solution was cooled in the refrigerator overnight. The product was collected and washed with cold abs. EtOH; first crop., 5.3 g. The mother liquor was again conc'd to dryness *in vacuo*, the residue was taken up in 30 ml. of warm abs. EtOH, and a second crop of 6.8 g. was collected. Total yield, 12.1 g. (98%). The combined crops were recrystallized from 120 ml. of hot abs. EtOH plus enough water to cause complete solution of the solid (about 8 ml.); the product crystallized as long silky needles. Yield, 9.8 g.; m.p. 175–176°, then resolidified into sheafs of rectangular plates, m.p. 231–232°d.

Anal. Calc'd for $C_9H_{20}N_4O_4$: C, 43.57; H, 8.12; N, 22.56. Found: C, 43.64; H, 8.00; N, 22.5.

Ethylglycocyamine. Calc'd (Jaffé): 57.5. Found: 59.0.

N-Ethylglycocyamine from N-ethylglycine complex. The N-ethylglycocyamine·N-ethylglycine complex (5.0 g.) was suspended in 5 ml. of conc'd NH_4OH, the suspension was warmed to 40°, and then was cooled overnight in the refrigerator. The dense granular precipitate of N-ethylglycocyamine hydrate was collected, washed with abs. EtOH, and dried. Yield, 2.3 g. (80%) of anhydrous product; m.p. 255–265°d. after subliming to rectangular plates at 170–185°.

N-Ethylglycine·Guanidine hydrobromide. A. A solution of 5.2 g. of N-ethylglycine in 5 ml. of conc'd NH_4OH was added to 10 g. of S-ethylisothiourea hydrobromide in 20 ml. of conc'd NH_4OH and the resulting solution was allowed to stand overnight. A reaction proceeded readily, as evidenced by the rapid formation of ethyl mercaptan. The reaction

(8) All melting points were made on the micro hot stage and are corrected.

mixture then was conc'd to dryness *in vacuo*, with the bath temperature below 50°. The residue was taken up in 30 ml. of hot abs. EtOH, the hot solution was filtered, and then was cooled in an ice bath. The product that crystallized in long flat blades was collected, washed with a small amount of cold abs. EtOH, and dried. Yield, 9.2 g.; m.p. 182–185°. The mother liquor was reworked to yield an additional 1.3 g. Total yield 10.5 g. (86%). The crude product was recrystallized from 80 ml. of abs. EtOH containing 0.5 ml. of water to yield 9.5 g.; m.p., 186–187°, which was not changed on further recrystallization.

Anal. Calc'd for $C_5H_{15}BrN_4O_2$: C, 24.70; H, 6.22; N, 23.05; Br, 32.87. Found: C, 25.28; H, 6.25; N, 22.50; Br, 32.73.

B. N-Ethylglycine (0.52 g.) and 0.70 g. of guanidine hydrobromide were dissolved in 15 ml. of absolute ethanol, the solution was cooled overnight in the refrigerator, and the product was collected, washed, and dried. Yield, 1.06 g. (87%) of a product that melted at 186–187° and gave no depression in melting point when mixed with the substance obtained in *A*.

Anal. Calc'd for $C_5H_{15}BrN_4O_2$: N, 23.05; Br, 32.87. Found: N, 23.07; Br, 32.98.

Formation of guanidine picrate from N-ethylglycine·guanidine hydrobromide. The N-ethylglycine·guanidine hydrobromide salt (1.0 g.) was dissolved in 10 ml. of warm water and was added to a solution of 2 g. of picric acid in 190 ml. of hot water. When the solution was cooled, a copious precipitate of compact dendritic yellow crystals of guanidine picrate was obtained. Yield, 1.15 g. (98%). After recrystallization from hot water containing a small amount of picric acid, this material melted at 350–355°d.

Anal. Calc'd for $C_{11}H_{17}N_7O_9$: C, 29.26; H, 3.19; N, 29.93. Found: C, 29.17; H, 2.80; N, 29.16.

Authentic guanidine picrate which was prepared from guanidine hydrochloride and picric acid had identical properties.

Attempted preparation of N-ethylglycocyamine from the N-ethylglycine·guanidine hydrobromide salt. The above salt (2.43 g.) was dissolved in 3 ml. of conc'd NH_4OH, a solution of 1.90 g. of S-ethylisothiourea hydrobromide in 4 ml. of conc'd NH_4OH was added, and the reaction mixture was allowed to stand overnight. It was worked up as above and yielded 1.70 g. (70% recovery) of starting salt; m.p. 184–186°, no depression when mixed with starting material.

Attempted formation of creatine·ethylglycine salt. Creatine hydrate (0.75 g.) and 0.52 g. of N-ethylglycine were dissolved in 6 ml. of hot water. When the solution was cooled, characteristic crystals of creatine hydrate separated and were recovered. Yield, 0.70 g.

N-Ethylglycine·Guanidine hydrochloride salt. Procedure *B* for the preparation of the hydrobromide salt was repeated using 1.03 g. of N-ethylglycine and 0.96 g. of guanidine hydrochloride in 20 ml. of hot abs. EtOH. Yield, 1.68 g. (85%); m.p. 173–174°. After two recrystallizations from abs. EtOH, m.p. 174–176°.

Anal. Calc'd for $C_5H_{15}ClN_4O_2$: N, 28.20; Cl, 17.85. Found: N, 28.22; Cl, 18.03.

N-Methylalanine·Guanidine hydrobromide salt. N-Methyl-DL-alanine·$^1/_2H_2O$ (0.55 g.) and 0.70 g. of guanidine hydrobromide were dissolved in 10 ml. of hot abs. EtOH. A crystalline product (0.85 g., 65%) was obtained; m.p. 132–135°. After two recrystallizations from abs. EtOH, there remained 0.67 g., m.p. 133–135°.

Anal. Calc'd for $C_5H_{15}BrN_4O_2$: N, 23.05; Br, 32.87. Found: N, 22.88; Br, 32.84.

N-Methylalanine·Guanidine hydrochloride salt. The above procedure was repeated with 0.55 g. of N-methyl-DL-alanine·$^1/_2H_2O$ and 0.44 g. of guanidine hydrochloride in 5 ml. of abs. EtOH. Yield, 0.70 g. (73%); m.p. 127–130°. After two recrystallizations from abs. ethanol the m.p. was 122–124°.

Anal. Calc'd for $C_5H_{15}ClN_4O_2$: N, 28.20; Cl, 17.85. Found: N, 28.76; Cl, 17.75

Sarcosine and guanidine hydrobromide. The above procedure was repeated with 0.45 g. of sarcosine and 0.70 g. of guanidine hydrobromide in 15 ml. of hot abs. EtOH and 10 drops of water. Yield, 0.75 g.; m.p. 118–120°. One recrystallization of this material from 10 ml. of abs. EtOH and 6 drops of water yielded 0.30 g. of material, m.p. 200–213°d., which had a sweet taste and gave a negative test for the bromide ion. One more recrystallization from 5 ml. of 95% EtOH yielded 0.25 g., m.p. 212–214°d. A mixture melting point with authentic sarcosine (m.p. 214–215°d.) showed no depression. Thus a stable complex salt was not formed between sarcosine and guanidine hydrobromide.

Sarcosine and guanidine hydrochloride. The preceding experiment was repeated with 0.89 g. of sarcosine and 0.96 g. of guanidine hydrochloride in 20 ml. of hot abs. EtOH and 6 drops of water. Thus 1.50 g. of crystals were obtained, m.p. 149–151°. Titration of samples with standard $AgNO_3$ solution gave varying results with different samples, indicating that the product was inhomogeneous, and probably not a definite compound. Several wasteful recrystallizations led to the recovery of 0.19 g. of material, m.p. 213–215°d., which gave a neg. test for the chloride ion, had a sweet taste, and showed no depression of melting point when mixed with authentic sarcosine.

Arginine hydrochloride and N-ethylglycine. L-Arginine monohydrochloride (2.11 g.) and 1.03 g. of N-ethylglycine were dissolved in 30 ml. of hot 85% EtOH. The crystals that separated after the solution had cooled were collected, washed with abs. EtOH, and dried. Yield, 1.35 g.; m.p. 219–221°d. (Lit., m.p. 222°d. for L-arginine monohydrochloride).[9]

Anal. Calc'd for arginine monohydrochloride: Cl, 16.83. Found: Cl, 17.01.

Guanidinium glutamate. L-Glutamic acid (1.47 g.) and 0.90 g. of guanidine carbonate were suspended in 10 ml. of water. When the evolution of CO_2 had ceased the solution was diluted with 20 ml. of ethanol, treated with charcoal, and evaporated to a clear sirup (steam-bath). The sirup was dissolved in 30 ml. of abs. ethanol plus sufficient water to yield a clear solution, and was allowed to stand overnight at room temperature. Then it was placed in the refrigerator for another day, and the crystals were collected and dried; yield, 1.7 g. (72%), m.p. 185–192°. After one more recrystallization from aqueous ethanol the air-dried material melted at 192–195°d.

Anal. Calc'd for $C_6H_{14}N_4O_4 \cdot H_2O$: N, 24.95. Found: N, 25.59. The material was dried over P_2O_5 at 1 mm. of Hg and 95° and reanalyzed.

Anal. Calc'd for $C_6H_{14}N_4O_4$: N, 27.18. Found: N, 27.82.

Treatment of individual amino acids with guanidine hydrochloride. Glycine. Glycine (1.27 g.) and 1.62 g. of guanidine hydrochloride were dissolved in 20 ml. of aqueous ethanol. The solution was conc'd to dryness *in vacuo*, and the residue was digested with abs. ethanol, and filtered; 1.15 g. of glycine was recovered.

Alanine. DL-Alanine (0.89 g.) and 0.96 g. of guanidine hydrochloride were dissolved in 30 ml. of 60% ethanol. After the solution had cooled overnight, 0.50 g. of alanine was recovered as a crystalline precipitate.

Alanine (0.89 g.) and 1.90 g. of guanidine hydrochloride were dissolved in 2 ml. of hot water, and the solution was diluted with 25 ml. of abs. ethanol. There separated 0.83 g. of alanine which was collected.

N-Phenylglycine. N-Phenylglycine (1.5 g.) and 0.96 g. of guanidine hydrochloride were dissolved in 10 ml. of hot absolute ethanol. No crystalline material could be obtained from this mixture.

N,N-Dimethylglycine, N-propylglycine, N-ethyl-DL-alanine. These amino acids were treated with guanidine hydrochloride in the same manner as above. No crystalline products could be obtained.

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(9) Cox, King, and Berg, *J. Biol. Chem.*, **81**, 755 (1929).