methylaminoethyl chloride[10] [prepared from 21.6 g. (0.15 mole) of the hydrochloride and 20% sodium hydroxide solution] was added to the sodium salt suspension. The mixture was heated at reflux temperature for six hours, and the turbid light brown solution cooled and filtered to remove sodium chloride. Benzene was evaporated from the filtrate, and 75 ml. of absolute alcohol was added. Dry hydrogen chloride gas was passed in, and upon evaporation of solvent to one-third of the original volume, 14.5 g. (40% yield) of the dihydrochloride of α-(2-dimethylaminoethylamino)-pyridine (III) was unexpectedly obtained; m.p. 223–225°.

The identity of the product was confirmed by a mixed melting point with an authentic sample[2] and by analysis.

Anal. Calcd. for $C_9H_{15}N_3 \cdot 2HCl$: C, 45.39; H, 7.20; Cl, 29.77. Found: C, 45.02; H, 7.18; Cl, 29.85.

The filtrate from which III dihydrochloride was isolated was evaporated to a viscous black liquid, which was extracted twice with hot benzene. The insoluble layer was distilled at 4 mm. to give 5.5 g. of *sym*-tetraphenylethane; m.p. 209–210.5°.[11] The benzene layer was evaporated and the residue distilled at 135–145° (2–4 mm.) to give 5 g. of benzohydryl chloride.[12] Heated gently, in accord with the literature,[5] the chloride was converted to *sym*-tetraphenylethane, m.p. 204–209°.

(10) Burckhalter, Stephens and Hall, *J. Am. Pharm. Assoc.*, **39**, 271 (1950).

(11) Anschütz found 207°.

(12) Huntress lists 135–145° (4 mm.).

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
UNIVERSITY OF KANSAS SCHOOL OF PHARMACY
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Preparation of Nitroaminoguanidine

BY RONALD A. HENRY, ROBERT C. MAKOSKY AND G. B. L. SMITH

The synthesis of nitroaminoguanidine reported by Phillips and Williams[1] involves the reaction of nitroguanidine with an equivalent amount of hydrazine sulfate and two equivalents of one normal ammonium hydroxide solution at 55–60°. Reduced to its simplest terms, this hydrazinolysis is represented by the equation

$$NH_2C(NH)NHNO_2 + N_2H_4 \longrightarrow NH_2NHC(NH)NHNO_2 + NH_3$$

Although these authors claimed yields of 50%, repeated duplication of their procedure, coupled with more precise methods for the analysis of nitroaminoguanidine, indicated that their yields were actually 30–35% and the purity of their product 70–80%.

In an attempt to improve the yield of nitroaminoguanidine, certain variables in the reaction of hydrazine with nitroguanidine in aqueous systems were investigated in this Laboratory.

As a result, a modified procedure has been developed which consistently yields nitroaminoguanidine of improved purity in 40–50% yield. The by-products formed in this reaction were described previously.[2]

Experimental

Typical Procedure for the Preparation of Nitroaminoguanidine.—In a two-liter, three-necked flask, equipped with a stirrer, a dropping funnel and a thermometer was placed 52 g. (0.5 mole) of nitroguanidine and one liter of distilled water at 60–65°. To the well-agitated slurry was added, dropwise, 31.9 g. (0.55 mole) of 87% hydrazine hydrate in 500 ml. of water. The temperature was maintained at 55–60°. The addition of the hydrazine required 55–60 minutes after which the solution was stirred for an additional 15 minutes. Ammonia was copiously evolved throughout the entire reaction period. When the reaction was completed, the orange-colored solution was rapidly cooled to below 45° and neutralized with concentrated hydrochloric acid to stop further reaction. The solution obtained from the reaction was chilled at 0° for several hours, after which the impure crystalline product was removed by filtration, washed with several small volumes of ice-cold water, and air-dried. The yield of impure product, containing about 96% nitroaminoguanidine, was 25–30 g. (41–49%); m. p. 186–187° (dec.). Two recrystallizations from water (30 ml. per gram) gave a white powder, decomposing at 190°. The melting point is an unsatisfactory criterion for ascertaining the purity of this compound.

Anal. Calcd. for $CH_5O_2N_5$: hydrazino nitrogen, 23.53. Found: hydrazino nitrogen, 23.51, 23.56, 23.53, 23.49.

Rapid evaporation of the original mother liquor over an open flame to one-third the original volume and cooling for 24 hours at 0° gave a second crop of crude material (12–20 g.), containing about 30% nitroaminoguanidine. Recovery of the pure nitroaminoguanidine, as such, from this crop was very difficult.

Analysis of Nitroaminoguanidine.—The purity of nitroaminoguanidine samples was determined by the following modification of the Jamieson method[3] for estimating hydrazine nitrogen: A 90- to 110-mg. sample of material is accurately weighed into a 125-ml. iodine flask; 20 ml. of water is added and the sample dissolved by heating and swirling. The solution is then cooled to 20–25°, 25 ml. of concentrated hydrochloric acid is added and the solution recooled to 25°, after which 15 ml. of chloroform is added and the solution titrated with standardized 0.1 *N* potassium iodate solution (theoretically 5.3505 g./liter) until the iodine color is completely discharged from the chloroform layer. Initially the iodate is added in increments of 5 to 6 ml., followed by shaking. As the end-point is approached (gradual fading of the iodine color), the size of the increment is progressively decreased so that ultimately the iodate solution is being added dropwise. The shaking between each addition must be maintained and must be very vigorous. If the approximate titer of the sample is known, 90 to 95% of the iodate solution can be added all at once, followed by the more careful addition. The usual precautions must be taken to prevent spurting when the stopper from the iodine flask is being removed.

$$\% \text{ nitroaminoguanidine} = \frac{(\text{ml. of } KIO_3)(\text{normality of } KIO_3)(119.09/4)}{(\text{sample weight})(10)}$$

(3) Jamieson, "Volumetric Iodate Methods," Chemical Catalog Co., Inc., New York, N. Y., 1926, p. 36.

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The Rates of Absorption of and the Formation of Liver Glycogen by L-Proline and L-Hydroxyproline[1]

BY W. C. HESS AND I. P. SHAFFRAN

L-Proline fed to chickens, as the half sodium salt, was found by Kratzer[2] to be adsorbed at the rate of 49.8 mg. per 100 g. body weight per hour. No previous reports upon the rate of absorption of L-hydroxyproline were found in the literature. Dakin[3] fed L-proline to phlorhizinized dogs and noted the production of extra urinary glucose. Stöhr[4] reported that feeding L-proline and L-

(1) Presented in part before the Division of Biological Chemistry of the American Chemical Society, Phila., April, 1950.

(2) Kratzer, *J. Biol. Chem.*, **153**, 237 (1944).

(3) Dakin, *ibid.*, **14**, 321 (1913).

(4) Stöhr, *Biochem. Z.*, **299**, 242 (1938).

(1) Phillips and Williams, THIS JOURNAL, **50**, 2469 (1928).

(2) Henry, Lewis and Smith, *ibid.*, **72**, 2015 (1950).

hydroxyproline to fasting rats produced extra liver glycogen but the results were variable. Butts, Blunden and Dunn[5] fed the anhydride of glutamic acid, pyrrolidone carboxylic acid, a compound closely related to both proline and hydroxyproline, to fasting rats and found an increase in liver glycogen. McFarlane and Guest[6] and Guest[7] have described quantitative colorimetric methods for the determination of hydroxyproline and proline, and we have applied these methods in an investigation of the rates of absorption of these two amino acids. The amino nitrogen method of Pope and Stevens,[8] which estimates quantitatively the nitrogen in these two amino acids, has been used to confirm the results obtained by the colorimetric methods. The formation of extra liver glycogen was followed simultaneously with the determination of the rates of absorption

Experimental

White rats, fasted for 48 hours, were fed the amino acids by stomach tube. The amino acids, dissolved in water, were administered at levels of 100, 200 and 300 mg. per 100 g. of body weight for the 1-, 2- and 3-hour absorption periods, respectively.[9]

The animals were sacrificed at the end of the period and the entire gastrointestinal tract and the liver removed. Glycogen was determined immediately in a portion of the liver by the method of Good, Kramer and Somogyi.[10] The gastrointestinal tract was ground in a Waring blendor with 50 ml. of a 10% aqueous solution of trichloroacetic acid, the extract filtered through a Celite pad and aliquots of the filtrate taken for analysis. A series of experiments with each of the amino acids was conducted in which the amino acid was neutralized by the addition of the calculated amount of 5 *N* NaOH solution prior to administration. The results of the experiments are given in Tables I and II. All the results are corrected for the loss occurring in the procedure; for L-proline the loss was 4.3% and for L-hydroxyproline 3.8%. The loss was determined by adding the amino acid to the isolated gastrointestinal tract of a control rat and carrying out the procedure described above.

TABLE I

RATE OF ABSORPTION OF AND GLYCOGEN FORMATION BY L-PROLINE

No. of animals	Time, hours	Rate, mg. per 100 g. per hour Colorimet. method	Amino N method	Glycogen, %
4	1	68.7	66.3	0.45[a]
6	2	71.0	70.0	0.69
6	3	68.8	67.2	1.41
Av. 16		69.6 ± 5.6	68.0 ± 5.8	
4[b]	1	75.0	73.1	0.23
4[b]	2	73.7	73.0	.49
4[b]	3	75.5	74.5	.81
Av. 12		74.7 ± 4.4	73.3 ± 4.9	

[a] The glycogen content of the livers of 12 control rats averaged 0.04%. [b] Fed as the sodium salt.

Discussion and Summary.—When fed to white rats previously fasted for 48 hours, L-proline and L-hydroxyproline are absorbed at substantially the same rate, 69.6 and 68.6 mg. per 100 g. of body weight per hour, respectively. When fed as their sodium salts, the rate of absorption of L-hydroxyproline, but not L-proline, is increased at a rate that is statistically significant ($t = 6.30$) a finding that has been observed with other amino acids.[11] The rates determined from the estimation of the amino nitrogen content of the extracts of the gastrointestinal tracts are in excellent agreement with those calculated from the colorimetric estimations. No significant differences in the rates were found after absorption periods of 1, 2 or 3 hours.

Both L-proline and L-hydroxyproline produced extra liver glycogen within 1 hour following their administration, 0.45 and 0.64% compared with 0.04% in the livers of 12 control rats fasted for 48 hours.

TABLE II

RATE OF ABSORPTION OF AND GLYCOGEN FORMATION BY L-HYDROXYPROLINE

No. of animals	Time, hours	Rate, mg. per 100 g. per hour Colorimet. method	Amino N method	Glycogen, %
6	1	70.3	59.9	0.64
6	2	65.5	67.8	.80
5	3	70.6	68.3	.86
Av. 17		68.6 ± 6.7	65.5 ± 7.2	
5[a]	1	77.5	74.1	.50
5[a]	2	84.0	84.1	.72
5[a]	3	81.8	84.2	.80
Av. 15		81.0 ± 4.6	79.1 ± 6.9	

[a] Fed as the sodium salt.

(5) Butts, Blunden and Dunn, *J. Biol. Chem.*, **119**, 247 (1937).

(6) McFarlane and Guest, *Can. J. Research*, **B17**, 139 (1939).

(7) Guest, *ibid.*, **17**, 143 (1939).

(8) Pope and Stevens, *Biochem. J.*, **33**, 170 (1939).

(9) The L-proline and L-hydroxyproline were obtained from Mann Fine Chemicals, Inc. The purity was determined by amino nitrogen (Method of Pope and Stevens[8]) and total nitrogen estimation; both amino acids were at least 98.4% pure. The L-proline had a specific rotation, in water, of $[-84.2°]^{20}D$ and the L-hydroxyproline, also in water, of $[-73.8°]^{20}D$.

(10) Good, Kramer and Somogyi, *J. Biol. Chem.*, **100**, 485 (1933).

(11) Hess, THIS JOURNAL, **72**, 1407 (1950).

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The Enzymatic Resolution of DL-Phenylalanine

BY H. T. HUANG AND CARL NIEMANN[1]

In principle any one of a number of enzymatic methods may be used for the resolution of a particular α-amino acid.[2] However in practice it has been our experience that no one method is ideal for all amino acids and that in general the most suitable procedure will be determined by the nature of the amino acid to be resolved. For DL-phenylalanine it has been found that from the standpoint of reliability, convenience, cost and yield, the method of choice appears to be that based upon the papain-catalyzed synthesis of acetyl-L-phenylalanin-*p*-toluidide from acetyl-DL-phenylalanine and *p*-toluidine[3] followed by hydrolysis of the L-toluidide and the residual D-acid to the corresponding amino acids. The yields obtained with this procedure are superior to those previously reported for the resolution of DL-phenylalanine by another enzymatic process,[4] and are comparable with those achieved in the resolution of the three isomeric nuclear substituted monofluoro-

(1) To whom inquiries regarding this article should be sent.

(2) For a résumé of available methods *cf.* P. J. Fodor, V. E. Price and J. P. Greenstein, *J. Biol. Chem.*, **178**, 503 (1949); and L. Levinton, V. E. Price and J. P. Greenstein, *ibid.*, **184**, 55 (1950).

(3) *Cf.* C. Niemann and P. L. Nichols, Jr., *ibid.*, **143**, 191 (1942).

(4) J. B. Gilbert, V. E. Price and J. P. Greenstein, *ibid.*, **180**, 473 (1949).