dl-2,6-Diamino-4-hexynoic Acid, a Growth Inhibitor

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***dl*-2,6-Diamino-4-hexynoic acid, a lysine analog, was synthesized by the interaction of *N*-(4-chloro-2-butynyl)phthalimide and the sodium salt of ethyl acetamidomalonate. A two-step hydrolysis yielded the desired acetylenic diamino acid. *dl*-Lysine, identical with the known amino acid, was obtained by the reduction of the triple bond in I. This acetylenic analog of lysine inhibited the growth of DNA, RNA, and protein synthesis in resting cell suspensions of pneumococci at a concentration of 100 mcg./ml.**

THE UNSATURATED amino acids, methallylglycine and crotylglycine, were first prepared by Albertson and co-workers, (1) and were found to be potent inhibitors of microorganisms. On the basis that olefinic unsaturation produces powerful inhibition of microorganisms, Gershon and co-workers (2) prepared two amino acids containing the acetylenic linkage, propargylglycine and 2-amino-3-methyl-4-pentynoic acid, and found that these acetylenic analogs were more potent growth inhibitors than their ethylenic analogs.

Similarly, several cyclopentenyl, cyclopentyl, and cyclohexenyl amino acids have been demonstrated (3, 4) to be competitive inhibitors of certain naturally occurring amino acids in various microorganisms. Unsaturation within the cycloalkyl moiety in all cases resulted in compounds acting as amino acid antagonists. The importance of steric requirements was further exemplified by Shive and co-workers (4) who synthesized *cis* and *trans* dehydrolysine and found the *trans* compounds to be the only isomer active as a competitive lysine antagonist.

Davis *et al.* (5) have recently reported that the introduction of a carbon-carbon triple bond into the carbon chain of lysine (I) forces the lysine into a fixed conformation and yields a competitive growth inhibitor of *Leuconostoc dextranicum* 8086. The authors were thus prompted to report their findings on the synthesis and biological properties of *dl*-2,6-diamino-4-hexynoic acid (I), their investigations having been carried out independently.

$$NH_2CH_2C{\equiv}C{-}CH_2CHNH_2COOH$$

"Acetylenic" Lysine

I

The authors' biological results (Table I) show that at a concentration of 100 mcg./ml., "acetylenic" lysine (I) is an effective inhibitor of DNA, RNA, and protein synthesis. Higher concentrations than 100 mcg./ml. do not increase the inhibition, while lower concentrations than 100 mcg./ml. are less effective. The control levels are depressed equally for all three components (about 80% inhibition). Since these results are obtained with "resting" cells (with respect to multiplication), it cannot be stated definitely that similar results would be obtained in growing (multiplying) cultures. However, since the synthesis of three essential components (DNA, RNA, and protein) required for *any* multiplication are inhibited by the modified lysine, it is likely that multiplication in growing cultures would also be inhibited. As for the mechanism of action of "acetylenic" lysine, at least two possibilities exist. One is that modified lysine may be a general growth inhibitor rather than a specific antagonist of DNA, RNA, and protein synthesis, since it is unlikely that the drug would affect reactions involved in the synthesis of all three components. Rather, it is possible that "acetylenic" lysine acts on some basic cell process that indirectly affects DNA, RNA, and protein synthesis, such as respiration. Two is that since the modified lysine was reported (5) to be a competitive antagonist of lysine, it is possible that the drug inhibits protein synthesis specifically, which then results in an inhibition of DNA and RNA synthesis.

TABLE I.—EFFECTS OF *dl*-2,6-DIAMINO-4-HEXYNOIC ACID HYDROCHLORIDE ON NUCLEIC ACID AND PROTEIN SYNTHESES OF PNEUMOCOCCI

dl-2,6-Diamino-4-hexynoic Acid Hydrochloride (mcg./ml.)	% Increase Over 0 Time after 70 min.		
	DNA	RNA	Protein
None	20	18	30
25	18	18	25
50	10	9	14
100	4	3	6
200	4	None	3

EXPERIMENTAL[1]

N-(4-Chloro-2-butynyl)phthalimide.—To a solution of 74 Gm. (0.6 mole) of 1,4-dichloro-2-butyne (General Aniline and Film Corp.) in 1000 ml. of dimethylformamide at 90° in a 2-L. 3-necked flask equipped with a stirrer, thermometer, and a funnel was slowly added with constant stirring 55 Gm. (0.3 mole) of potassium phthalimide. Stirring with heating (90°) was continued for 4 hr. after which the solution was poured into an ice-water mixture.

The precipitate which formed was filtered (suction) and washed with 200 ml. of 5% sodium hydroxide followed by two washings with 200 ml. of water. The white crystals were dried (vacuum) and recrystallized from ethanol to yield 28 Gm. (40%), m.p. 121–122°. [Lit. (5) m.p. 120–121°.]

Anal.—Calcd. for $C_{12}H_8ClNO_2$: C, 61.75; H, 3.41; Cl, 15.21. Found: C, 61.69; H, 3.61; Cl, 15.15.

Ethyl 2-Acetamido-2-carbethoxy-6-phthalimido-4-hexynoate.—To a 500-ml. 3-necked flask fitted with a condenser and mechanical stirrer was added a solution of 21.8 Gm. (0.1 mole) of ethyl acetamidomalonate (Winthrop Laboratories) in 200 ml. of absolute ethanol. After the malonate had completely dissolved in the ethanol, 2.3 Gm. of sodium was added and the mixture was rapidly taken up to reflux temperature. At this point 30.4 Gm. of

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[1] All melting points are uncorrected and were obtained in a Thomas-Hoover silicone-filled capillary melting point apparatus.

N-(4-chloro-2-butynyl)phthalimide was added in 1 part. The reaction mixture was then heated at reflux for 8 hr., allowed to cool, and the solid precipitate was filtered off. The precipitate was then extracted with hot absolute ethanol and the extract was allowed to cool. White crystals of the hexynoate separated. These crystals were again recrystallized from absolute ethanol to yield 37% of product, m.p. 149–150°. [Lit. (5) m.p. 139–142°.]

Anal.—Calcd. for $C_{21}H_{22}N_2O_7$: C, 60.86; H, 5.35; N, 6.76. Found: C, 61.24; H, 5.62; N, 7.08.

Ethyl 2-Acetamido-6-phthalimido-4-hexynoate.—A small amount could be isolated from the above reaction by evaporation of the filtrate from the reaction mixture. The resulting red residue was triturated with water, the water decanted off, and the remaining solid recrystallized from absolute ethanol, m.p. 224–226°.

Anal.—Calcd. for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30. Found: C, 63.09; H, 5.55.

dl-2,6-Diamino-4-hexynoic Acid Hydrochloride.—Ethyl 2-acetamido-2-carbethoxy-6-phthalimido-4-hexynoate (41.5 Gm., 0.1 mole) was hydrolyzed with 5.5 Gm. of 85% aqueous hydrazine in 200 ml. of ethanol by the method of Sheehan (6). As the solution was taken up to reflux temperature, the hexynoate slowly went into solution and the reaction became clear, but shortly thereafter, phthalhydrazide began to precipitate as a white solid. After 1 hr. of stirring at reflux, the reaction mixture was cooled to room temperature, and the phthalhydrazide was filtered off. The reaction liquor was then reduced under vacuum to yield a viscous semi-clear yellow residue. Without further isolation the crude hydrazine hydrolysate was dissolved in a small amount of water and then was added to an excess of concentrated hydrochloric acid. This mixture was then stirred at reflux for 1.5 hr. The reaction mixture was cooled to room temperature and the solvents partially removed under vacuum, cooled in an ice bath, and filtered. This filtration was carried out in order to remove any residual phthalhydrazide.

The filtrate, containing the product, was then completely evaporated under vacuum, the residue triturated with acetone to yield a crystalline product. The product thus formed consisted of a mixture of the monohydrochloride and the dihydrochloride of "acetylenic" lysine (I). Pure monohydrochloride (11.1 Gm., 62% yield) was obtained by recrystallization of the mixture of the mono- and dihydrochlorides from an ethanol–water–pyridine solution. This compound melted at 236° when a sample was placed in the oil bath at 220° and heated at approximately 2°/min.[2]

Anal.—Calcd. for $C_6H_{10}N_2O_2 \cdot HCl$: C, 40.35; H, 6.16; Cl, 19.85; N, 15.69. Found: C, 40.56; H, 6.21; Cl, 19.3; N, 15.71.

Catalytic Hydrogenation of 2,6-Diamino-4-hexynoic Acid Monohydrochloride.—A solution of the monohydrochloride (0.1816 Gm.) of the acetylenic amino-acid (I) in water (10 ml.) was catalytically hydrogenated over platinum oxide (0.053 Gm.) at room temperature and atmospheric pressure. When 2 *M* equivalents of hydrogen had been absorbed, the catalyst was removed, the filtrate was reduced to dryness under reduced pressure, and the residue was crystallized from 5 ml. of 95% ethanol containing 2 drops of pyridine. The crystals which formed were filtered off and dried, m.p. 260–262°. [Lit. (7) m.p. 262–264°.] The melting point of a mixture of an authentic sample of *dl*-lysine monohydrochloride with a sample of the product isolated above was not depressed (m.p. 261–262°). The infrared spectra of this product was identical with that of an authentic sample of *dl*-lysine monohydrochloride.

Biological Test Methods.—Virulent pneumococci derived from type III (strain A66) were grown in 3 L. of a casitone, tryptone (both Difco), albumin (fraction V from bovine serum albumin, Armour) medium supplemented with glucose and K_2HPO_4 (8) for 24 hr. at 37°. After this time, additional glucose and phosphate were added and the cells were incubated for 3 hr. The viable cell count after this 3-hr. period was approximately $4–6 \times 10^9$ cells/ml. The culture was centrifuged in a refrigerated centrifuge (International) at 30,000 × g for 20 min., washed 3 times with cold Na-K-phosphate buffer (0.02 *M*, pH 7.5), and inoculated into 3 ml. of a suspending medium consisting of this same buffer, glucose, casitone (both 1.0%), and catalase (0.005%, Nutritional Biochemicals). The suspending medium was prepared double strength so that additions could be made without affecting the over-all concentration of the basic constituents. *dl*-2,6-Diamino-4-hexynoic acid hydrochloride was added in 4 concentrations: 25, 50, 100, and 200 mcg./ml. (in the suspending medium). All samples were prepared in duplicate and the inoculated suspensions contained $1–2 \times 10^9$ viable cells/ml. The suspensions were incubated at 37° for 70 min. in a gyrotory water bath shaker (New Brunswick Scientific) at 200 r.p.m. DNA, RNA, and protein were then extracted as described previously (9) and measured by the procedures of Brody (10), Drury (11), and Lowry *et al.* (12), respectively.

[2] Davis *et al.* (5) reported their 2,6-diamino-4-hexynoic acid dihydrochloride as having a m.p. 178–183° dec.

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