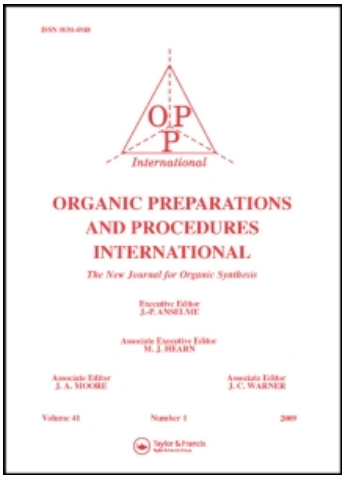

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PREPARATION OF *DL -threo -* AND *DL- erythro -* 3,5 - DIAMINOHEXANOIC ACID

V. Krasnobajew[ab]; E. A. Rimerman[a]; H. A. Barker[a]
[a] Department of Biochemistry, University of California, Berkeley, California [b] Givandan Forschungsgesellschaft, Dübendorf, Switzerland

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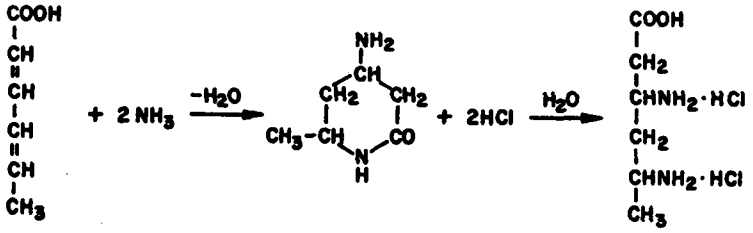
PREPARATION OF DL-*threo*- AND DL-*erythro*-3,5-DIAMINOHEXANOIC ACID

V. Krasnobajew[1], E. A. Rimerman and H. A. Barker
Department of Biochemistry
University of California, Berkeley, California 94720

3,5-Diaminohexanoic acid (III), probably the L-*erythro* isomer, was first reported as an intermediate in the enzymic degradation of lysine by clostridia and was isolated in mg amounts and characterized.[2,3] To obtain larger quantities of this compound and its isomers for biochemical investigations, a convenient synthetic method was needed. Fischer and Schlotterbeck[4] had reported the synthesis from sorbic acid (I) and ammonia of a diaminohexanoic acid which was isolated as a picrate but not adequately characterized. Recently the major product of this synthesis was crystallized as the dihydrochloride, identified as 3,5-diaminohexanoic acid, and partially resolved by fractional crystallization under unspecified conditions into two racemates, one of which contained the biologically active isomer.[2,5] We now describe a well-tested method of synthesizing III from sorbic acid and separating the *threo* and *erythro* racemates on a 200 g scale in a purity of 80 to 98%.



I II III

In alkaline, neutral or weakly acidic (pH 3) solution, 3,5-diaminohexanoic acid is converted to the lactam (II) slowly at 40°, more rapidly

at higher temperatures; consequently heating under these conditions should be minimized. The diamino acid can be estimated by reaction with ninhydrin[6] to form a yellow-orange product that has an absorption maximum at 413 nm and a shoulder in the 540-570 nm region. On a molar basis the diamino acid (*threo* or *erythro*) gives ninhydrin color values 0.55, 3.02 and 0.25 of those of leucine at 413, 440 and 570 nm, respectively.[7] The amounts of the *threo* and *erythro* forms were determined[8] by converting the acids to their lactams, reacting the lactams with L-glutamyl-N-carboxyanhydride to form the L-glutamyl peptides[9], and estimating the latter by means of an amino acid analyzer.

EXPERIMENTAL

To 250 g (2.23 moles) of sorbic acid in a 1 l. screw capped flask provided with a magnetic stirring bar was cautiously added 700 ml (11.5 moles) of conc. aqueous ammonia and the mixture was stirred until the sorbic acid was mostly dissolved. The solution and remaining solid was then transferred to a stainless steel pressure reaction vessel[10] fitted with a pressure gauge and thermometer. The reaction vessel was kept at 150 ± 3° (internal pressure, 260 psi) for 20 hr and then allowed to cool to room temperature. The clear, pale yellow solution was concentrated to a thick syrup under reduced pressure (bath temperature, 40-45°). The syrup was diluted with 200 ml of water and again concentrated under reduced pressure to remove residual ammonia.

Two batches of syrup (550 ml) were combined and conc. HCl (950 ml) was slowly added with stirring to give a final concentration of about 5 N. The resulting solution was refluxed for 6 hr to hydrolyze the lactam (II). Upon standing overnight, the solution deposited a thick layer of crystals on the walls of the flask and a sticky brown solid on the surface of the solution. The latter was skimmed off and discarded. The solution was

then decanted from the crystalline material and the latter was washed with 100 ml of 1:1 (v/v) 2-propanol-water. The wash solution was decanted and combined with the mother liquor. The crystalline residue (Fraction 1; 300-500 g moist wt) contained 80-85% *threo* and 15-20% *erythro* racemates of 3,5-diaminohexanoic acid·2HCl.

The combined mother liquor and wash from Fraction 1 was concentrated three times[11] under reduced pressure (water aspirator; bath temperature, 45°) to remove excess HCl. The resulting partially crystalline syrup was diluted with about 350 ml of water, warmed briefly to 45° on a steam bath to dissolve crystalline material, treated with 40 g of moist charcoal, and filtered while warm through a layer of filter paper and filter aid.[12] The filtrate was concentrated to a thick syrup (about 350 ml) and, while still warm[13], slowly diluted with 2 volumes of 2-propanol with continuous stirring. Crystallization of the product began almost immediately. The solution was left at 4° overnight; then the precipitate was collected and the filtrate discarded. The crystalline product (Fraction 2) was washed with 2-propanol (400 ml) and ethyl ether and dried at 37°. The yield of the colorless product (mainly *erythro* racemate) was 200 g.

Purification of the *threo* racemate. The crude product (Fraction 1; 400 g) was dissolved in a minimum volume of water (about 350 ml) at 45° with stirring. The solution (about 650 ml) was then allowed to cool and the brown oily material on the surface was removed with a spatula. Moist charcoal (25 g) was then added, the solution was heated to 45° and filtered through a layer of filter aid. The filtrate was concentrated twice under reduced pressure to remove excess HCl. The syrup was then diluted with about 450 ml of water, warmed to 40° to dissolve solid material, and slowly mixed while stirring with 2 volumes (about 1.5 l.) of 2-propanol. Crystallization began immediately. Stirring was continued for an additional hour in ice and then the solution was left overnight at 4°. The

colorless product (Fraction 1a) was collected, washed with 80% 2-propanol-water, 2-propanol and ethyl ether, and dried at 37°. The yield of product (94-98% *threo* racemate) was about 250 g (24.4 moles %), m.p. ranging from 230° to 249°.

Anal. Calcd. for $C_6H_{16}N_2O_2Cl_2$: C, 32.9; N, 7.34; N, 12.8

Found: C, 32.2; H, 7.49; N, 12.9

The combined mother liquor and wash from Fraction 1a was concentrated to syrup and then diluted to about 200 ml. Two volumes of 2-propanol were added to give a second crop of about 40 g (Fraction 1b) consisting mainly of *erythro* racemate.

Purification of the *erythro* racemate. The crude product (Fraction 2; 200 g) was dissolved in a minimal amount of water at 25° (final volume, 480 ml) and 960 ml of 2-propanol was added. Crystallization began within 30 min. After standing for another hour at room temperature, the solution was cooled in ice for several hours. The suspension was then filtered cold and the colorless product (Fraction 2a) was washed with 2-propanol and ether and dried *in vacuo* over NaOH at room temperature. The yield was about 125 g. An additional 45 g was obtained by work up of the concentrated mother liquor in the same manner. Another 30 g of mostly *erythro* racemate was obtained by recrystallization of Fraction 1b. The total yield of *erythro* racemate amounted to 200 g (18.8 moles %). The m.p. ranged from 180° to 205° with moderately slow heating (<8°/min); with more rapid heating melting occurs at 118-125° (loss of H_2O) followed by crystallization and remelting at 180-194°.

Anal. Calcd. for $C_6H_{18}N_2O_3Cl_2$ (monohydrate): C, 30.2; H, 7.60; N, 11.8

Found: C, 30.5; H, 7.41; N, 11.8

Preparations having a m.p. below 190° generally contained 80-90% *erythro* racemate, the remainder being *threo*; with a m.p. above 190° the *erythro* content was 90-97%.

Solubility of 3,5-Diaminohexanoic Acid[a]

	Water	2-Propanol-Water (v/v)		
		1:1	2:1	3:1
Threo	325(370)[b]	88	44	30
Erythro	500(580)[b]	168	100	68

[a] In mg/ml of solution at 4°. [b] At 25°.

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11. Add about 125 ml of water before concentrating the second and third times.
12. The filtrate should be clear and light yellow-brown in color. If it is not clear, another 10 g of charcoal is added and the solution is filtered again.
13. If allowed to cool, the solution becomes very viscous.

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