

of material boiling at 55–59° distilled in 2.5 hr. The residue was transferred to a Claisen flask and fractionally distilled at reduced pressure. A total of 39 g. (99%) of product boiling at 170–172°/0.05 mm. was collected, m.p. 129–135°. Two recrystallizations from cyclohexane gave 35 g., m.p. 138.5–139.5°.

Anal. Calcd. for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10. Found: C, 81.71; H, 6.19.

4-Phenyl-5-oxohexanamide. 1-Phenyl-2-propanone (40.3 g., 0.3 mole) was dissolved in *tert*-butyl alcohol (150 ml.) and 40% aqueous benzyltrimethylammonium hydroxide (3 ml.) were placed in a one-liter flask equipped with a mechanical stirrer and dropping funnel. A solution of acrylamide (21.3 g., 0.3 mole) dissolved in *tert*-butyl alcohol (300 ml.) was added, with stirring, over a period of 80 min. The temperature was maintained at 20–25° during the addition by external cooling.

The cooling bath was removed and more benzyltrimethylammonium hydroxide solution (12 ml.) was added. During the next 30 min. the temperature rose to 32°. Addition of a few crystals of product, obtained by evaporation of several drops of reaction mixture, initiated the separation of solid product. After stirring for another 3 $\frac{3}{4}$ hr. the mixture was neutralized with dilute sulfuric acid and the solid removed by filtration.

The product was washed with a little *tert*-butyl alcohol and dried. The yield was 23.7 g. The solvent was removed from the combined mother liquors and washings by reduced pressure distillation. The residue was dissolved in ethyl acetate (200 ml.) and the solution dried over sodium sulfate. Evaporation of the solvent left another 12.5 g. of product, bringing the total to 36.2 g. (59%). Recrystallization first from ethanol and then from water gave material melting at 145.5–147°.

Anal. Calcd. for $C_{12}H_{15}N_2O$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.30; H, 7.45; N, 6.79.

N-(3-Dimethylaminopropyl)-4,4-diphenyl-5-oxohexanamide. 4,4-Diphenyl-5-hydroxy-5-hexenoic acid lactone (13.2 g., 0.05 mole) was placed in a small flask and treated with dry 3-dimethylaminopropylamine (5.6 g., 0.055 mole). The solid partially dissolved with the evolution of heat.

The flask was fitted with a reflux condenser whose open end was protected from moisture by means of a drying tube.

The mixture was heated for 15 min. on a steam bath. The resultant solution was treated with petroleum ether (b.p. 30–60°) which caused the product to solidify. The solid was removed by filtration, washed with more petroleum ether, and dried. The yield was 17.7 g. (97%), m.p. 62–65°. One recrystallization from hexane, and then two from petroleum ether gave a product melting at 64–66°.

Anal. Calcd. for $C_{23}H_{30}N_2O_2$: C, 75.37; H, 8.25; N, 7.64. Found: C, 75.30; H, 8.15; N, 7.63.

4,4-Diphenyl-5-hydroxyhexanoic acid lactone. 4,4-Diphenyl-5-oxohexanoic acid (100 g., 0.356 mole) was dissolved in a solution of sodium hydroxide (320 g., 8 mole) and water (2500 ml.). The solution was placed in a 5-liter, 3-necked flask equipped with a mechanical stirrer, reflux condenser, thermometer, and electric heating mantle. The solution was vigorously stirred and, while maintaining the temperature at 88–92°, Raney nickel–aluminum alloy (300 g.) was added, portionwise, over 14 hr.

The mixture was filtered and the filtrate diluted with water (1 liter) and then added slowly to concd. hydrochloric acid (2 liters). The mixture was warmed for a short time and then cooled and the oil removed by decantation. The mother liquor was extracted with ethyl acetate (four 400-ml. portions). The combined extracts and oily product were dried over sodium sulfate and then the solvent removed by reduced pressure distillation.

The residual oil was treated with saturated sodium bicarbonate solution (250 ml.) and the mixture stirred and warmed. After the evolution of gas had ceased the solid that separated was removed by filtration, washed with water, and dried. [Acidification of the filtrate gave 49 g. (49%) of unreacted starting material.] The yield of crude lactone was 42.1 g. (45%), m.p. 117.5–118.5°. Recrystallization from heptane gave 41.9 g., m.p. 118–119°.

Anal. Calcd. for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.16; H, 6.74.

WEST POINT, PA.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, MEDICAL COLLEGE OF VIRGINIA]

meso and *dl*-2,3-Diaminosuccinic Acids¹

HERBERT McKENNIS, JR.,² AND ALLAN S. YARD

Received February 6, 1958

meso and *dl*-2,3-Diaminosuccinic acids have been synthesized by hydrogenolysis of the corresponding bisbenzylamino compounds at room temperature in the presence of Pd-C and hydrogen at atmospheric pressure. The synthesis of *meso*-*N,N'*-dimethyl-2,3-diaminosuccinic acid was carried out in an analogous manner with platinum oxide or Pd-C (preferably the latter).

As a result of the increased use of hydrazine and its derivatives, biological attention has been focused on a number of its metabolic products.^{3,4} Of these, 2,3-diaminosuccinic acid has been impli-

cated in the metabolism of hydrazine through enzymatic reactions which are at present not completely understood. Jacobsohn and Soares⁵ considered diaminosuccinic acid to result from addition of hydrazine across the double bond of fumaric acid, but did not state which diastereo or optical isomer was involved. Suzuki, Suzuki, and Egami⁶ reported that *E. coli* were capable of carrying out the

(1) Presented in part at the Southwide Chemical Conference of the American Chemical Society, Memphis, Tenn., Dec. 7, 1956.

(2) Appreciation is expressed for financial aid under National Institutes of Health Grant RG 5337.

(3) A. S. Yard and H. McKennis, Jr., *Federation Proc.*, **14**, 309 (1955).

(4) A. S. Yard and H. McKennis, Jr., *J. Pharmacol. Exptl. Therap.*, **114**, 391 (1955).

(5) K. P. Jacobsohn and M. Soares, *Enzymologia*, **1**, 183 (1936–1937).

(6) S. Suzuki, N. Suzuki, and F. Egami, *J. Biochem. (Tokyo)*, **39**, 305 (1952).

reverse reaction, *i.e.*, converting *meso*-2,3-diaminosuccinic acid to fumaric acid and hydrazine. Garcia-Hernandez and Kun⁷ found that *meso*-2,3-diaminosuccinic acid inhibited the transamination of aspartate and α -ketoglutarate. Shive and Macow⁸ reported an anti-aspartic acid action on *E. coli*. Convenient methods for the synthesis of the diaminosuccinic acids are, therefore, desirable.

dl-2,3-Diaminosuccinic acid has been prepared⁹⁻¹³ by reduction of sodium dihydroxytartrate osazone with sodium amalgam. This laborious reduction affords a mixture of *meso* and *dl* forms. The latter was obtained only in approximately 18% yield.

Wenner^{14,15} prepared *meso*-2,3-diaminosuccinic acid by reduction of *meso*-2,3-bis(benzylamino)succinic acid in acid and obtained yields of 90% when the reduction was carried out at 800 pounds pressure and temperatures up to 60°. He also reported the reduction of *dl*-2,3-bis(benzylamino)succinic acid under similar conditions to give the *meso*-diamino acid in unspecified yield. In the latter case the final product was identified as the dibenzoyl derivative.

The fact that *N*-debenzylations¹⁶ can be conducted under relatively mild conditions suggested that the high pressures employed by Wenner could be avoided. Successful hydrogenolysis of *meso*-2,3-bis(benzylamino)succinic acid to *meso*-2,3-diaminosuccinic acid at room temperature and atmospheric pressure in the presence of Pd-C was then accomplished in high yield. *dl*-2,3-Bis(benzylamino)succinic acid was reduced to *dl*-2,3-diaminosuccinic acid with comparably good yields. *meso*-*N,N'*-Dibenzyl-*N,N'*-dimethyl-2,3-diaminosuccinic acid was debenzylated to yield *meso*-*N,N'*-dimethyl-2,3-diaminosuccinic acid under similar conditions. Thus, benzylamino acids in general appear to offer a wide range of possibilities as intermediates in the preparation of α -amino and α -*N*-methylamino acids.

The convenient intermediates, as noted by Wenner, for *meso*- and *dl*-2,3-bis(benzylamino)succinic acids are the corresponding *meso*- and *dl*-dibromosuccinic acids. *meso*-2,3-Dibromosuccinic acid is commercially available, and *dl*-2,3-dibromosuccinic acid has been previously prepared.¹⁷ Conditions for the bromination of maleic acid to yield *dl*-dibromosuccinic acid were reinvestigated, and a

procedure was developed which readily gave dibromosuccinic acid of high purity directly in much less time and without the purifications necessary in the original procedure.

After conversion of the dibromo acid to *dl*-2,3-bis(benzylamino)succinic acid, hydrogenolysis was carried out. This reduction was effected with both palladium-charcoal and platinum¹⁵ oxide catalysts. (Use of the latter was discontinued on account of apparently variable activity of the oxide. More active samples of Adams' catalyst appreciably reduced liberated toluene to methylcyclohexane as well as effecting debenzylation.) When the reaction was carried out at room temperature, no evidence for the formation of the *meso* compound was observed. The apparently complete conversion to the *meso* acid as reported by Wenner¹⁴ which results when higher temperatures are employed could result from epimerization of either the bisbenzylamino compound or the amino acid. Earlier workers observed the epimerization¹⁸ of the amino acid in the presence of acid and heat. In consequence, it was considered¹⁴ that epimerization occurred after formation of the amino acid.

Although both *meso*-2,3-diaminosuccinic acid and the *dl* compound form the usual amino acid derivatives, neither gives a positive ninhydrin reaction under conditions which are commonly employed.¹⁹ Kuhn and Zumstein¹³ reported that both compounds gave positive tests, but did not state the conditions. Garcia-Hernandez and Kun⁷ reported that the *meso* compound reacted with ninhydrin, but only after strong heating.

EXPERIMENTAL²⁰

meso-2,3-Diaminosuccinic acid. *meso*-2,3-Bis(benzylamino)succinic acid (10.0 g.), prepared in alcohol by the procedure of Wenner,¹⁴ was dissolved in a mixture of 50 ml. of glacial acetic acid and 42 ml. of concentrated hydrochloric acid. Hydrogenolysis was effected at room temperature and atmospheric pressure in the presence of 1.0 g. of 10% palladium on charcoal. An equal volume of water was added, and the catalyst was removed by filtration. The filtrate was concentrated to a sirupy mass at the water pump. The residue was dissolved in dilute sodium hydroxide. Upon treatment with glacial acetic acid to pH 5-6, the solution deposited colorless crystals of *meso*-2,3-diaminosuccinic acid, m.p. 304° (dec.). The yield was 4.1 g. (91%). After extraction with hot water, the compound was dissolved in ammonia water. Upon boiling, the ammonia was liberated and the amino acid precipitated. For analysis the compound was dried at room temperature *in vacuo*.

Anal. Calcd. for C₄H₈N₂O₄: C, 32.43; H, 5.44; N, 18.91. Found: C, 32.47; H, 5.49; N, 18.95.

The melting point of the *meso* diamino acid varied considerably with the rate of heating and was not sufficiently different from the *dl* compound to be used for differentiation. The *meso* compound by the Schotten-Baumann proce-

(18) R. Kuhn and F. Zumstein, *Ber.*, **59**, 479 (1926).

(19) P. B. Hawk, B. L. Oser, and W. H. Summerson, *Practical Physiological Chemistry*, 12th Ed., The Blakiston Company, N. Y., N. Y., 1947, p. 157.

(20) Microanalyses by the Clark Microanalytical Laboratory and Spang Microanalytical Laboratory.

(7) M. Garcia-Hernandez and E. Kun, *Biochem. et Biophys. Acta*, **24**, 78 (1957).

(8) W. Shive and J. Macow, *J. Biol. Chem.*, **162**, 451 (1946).

(9) J. M. Farchy and J. Tafel, *Ber.*, **26**, 1980 (1893).

(10) J. Tafel, *Ber.*, **20**, 244 (1887).

(11) J. Tafel and H. Stern, *Ber.*, **38**, 1589 (1905).

(12) T. Tamura, *J. Biochem.*, **27**, 335 (1938).

(13) R. Kuhn and F. Zumstein, *Ber.*, **58**, 1429 (1925).

(14) W. Wenner, *J. Org. Chem.*, **13**, 26 (1948).

(15) W. Wenner, U. S. Patent **2,389,099**, November 13, 1945.

(16) W. H. Hartung and R. Simonoff, *Org. Reactions*, **7**, 263 (1955).

(17) A. McKenzie, *J. Chem. Soc.*, **101**, 1196 (1912).

dure afforded a dibenzoyl derivative, m.p. 210° (dec.). This melting point, which was also dependent upon rate of heating, agrees with that reported by Wenner¹⁴ (208–210°) and Kuhn and Zumstein¹³ (212–213°). The derivative was more readily purified by solution in dilute sodium hydroxide and reprecipitation with acid than by the recrystallization from acetic acid and water employed by the previous workers.

Di-N-benzylmethylammonium meso-N,N'-dibenzyl-N,N'-dimethyl-2,3-diaminosuccinate. To a solution of 10.36 g. of *meso*-2,3-dibromosuccinic acid in 80 ml. of 95% ethanol, 40 g. of *N*-benzylmethylamine was added. The solution was refluxed for 8 hr. Upon cooling, 37.15 g. of colorless crystals of the crude dibenzylmethylammonium salt precipitated. These were collected and washed with alcohol. For analysis, the compound was recrystallized from hot ethanol, m.p. 191° (dec.), and dried at 60° and 3 mm. Hg over potassium hydroxide.

Anal. Calcd. for C₃₆H₄₆O₄N₄: C, 72.21; H, 7.74; N, 9.35. Found: C, 72.53; H, 7.71; N, 8.98.

meso-N,N'-Dibenzyl-N,N'-dimethyl-2,3-diaminosuccinic acid. The warm solution from the reaction (above) of benzylmethylamine and *meso*-2,3-dibromosuccinic acid was acidified to pH 1–2 by addition of concentrated hydrochloric acid. The solution was adjusted to pH 5 (hydron paper) by addition of 10*N* sodium acetate. The cooled solution deposited a colorless product which was washed with water (7.2 g., 54%, m.p. 171°). For analysis the acid was recrystallized from warm 95% ethanol and then from warm water, m.p. 176°, and dried over potassium hydroxide at 1 mm. Hg.

Anal. Calcd. for C₂₀H₂₄N₂O₄: C, 67.43; H, 6.73; N, 7.86. Found: C, 67.20; H, 7.03; N, 7.58.

meso-N,N'-Dimethyl-2,3-diaminosuccinic acid. *N,N'*-Dibenzyl-*N,N'*-dimethyl-2,3-diaminosuccinic acid (5.15 g.) was dissolved in a mixture of 20 ml. of glacial acetic acid and 1.5 ml. of concentrated hydrochloric acid. Hydrogenolysis was conducted at atmospheric pressure and room temperature in the presence of 0.5 g. of 5% palladium on charcoal. An equal volume of water was added to dissolve the solid which separated during the reaction, and the catalyst was removed by filtration. The filtrate was adjusted to pH 6 by addition of sodium acetate. The methylamino acid deposited in colorless crystals (0.75 g., 29.4%, m.p. 250° dec.). For analysis the compound was recrystallized from water, m.p. 276–277° (dec.), and dried over potassium hydroxide at 3 mm. Hg. The compound is very insoluble in alcohol.

Anal. Calcd. for C₆H₁₂O₄N₂: C, 40.93; H, 6.82; N, 15.91. Found: C, 41.26; H, 6.85; N, 15.95.

The micromelting points (dec.) for analytical samples of the methylamino acid were 267 and 272° (rate of heating approximately one degree per minute). The decomposition of the compound, in common with other amino acids in this study, was influenced by rate of heating and particle size. The capillary melting points were similarly influenced.

dl-2,3-Dibromosuccinic acid. Bromine (19 ml.) was added to 400 ml. of dry ether¹⁷ with cooling to keep the temperature below 25°. Powdered technical maleic acid (40 g.) was added in small portions with stirring. The temperature of the reaction mixture was kept between 18 and 25°. After washing with water and sulfurous acid, the ether solution was dried with anhydrous magnesium sulfate. Upon evaporation at room temperature in an atmosphere of dry nitrogen the solu-

tion directly afforded almost colorless crystals of *dl*-2,3-dibromosuccinic acid, m.p. 167–168°, (50–58 g.). The product was sufficiently pure for reaction with benzylamine.

dl-2,3-Bis(benzylamino)succinic acid. To a solution of 35.0 g. of *dl*-2,3-dibromosuccinic acid in 150 ml. of 95% ethanol was added cautiously, in portions, 107.5 g. of benzylamine. The mixture was heated under reflux for 5 hr. Approximately 30 ml. of ethanol was removed by vacuum distillation. The mixture solidified when cooled to room temperature. The solid mixture of benzylammonium salts was collected by suction filtration, washed with cold ethanol, and dried at room temperature. The yield was 19.6 g. of benzylammonium salts. Wenner's procedure¹⁴ yields similar material but in our hands only after some solvent has been removed. The crystals were dissolved in a minimum volume of 2*N* potassium hydroxide. After extraction thrice with ether (125 ml. portions), the acid was precipitated by acidifying the solution with glacial acetic acid. The bisbenzylamino acid (11.1 g.) was obtained, m.p. 197–205° (dec.). For analysis the compound was recrystallized from water, dried at 60° over potassium hydroxide and 3 mm. Hg. The analytical sample melted at 188° (dec.). Although the compound was difficult to characterize by melting point, higher melting samples were generally found to be contaminated with benzylamine.

Anal. Calcd. for C₁₈H₂₀N₂O₄: C, 65.86; H, 6.14; N, 8.53. Found: C, 65.91; H, 6.21; N, 8.52.

dl-2,3-Diaminosuccinic acid. The *dl*-2,3-bis(benzylamino)succinic acid (10.0 g.) was dissolved in 80 ml. of warm glacial acetic acid. Palladium on charcoal (200 mg.) was added, and the hydrogenolysis effected at room temperature and atmospheric pressure. During the course of the debenzylation the *dl*-2,3-diaminosuccinic acid precipitates. The reaction mixture was filtered with suction, and the *dl*-2,3-diaminosuccinic acid was extracted from the catalyst with 2*N* potassium hydroxide. The potassium hydroxide solution was treated with glacial acetic acid (to pH 5–6). The air-dried precipitate weighed 3.52 g. (75% yield, m.p. 295° dec.). For analysis the *dl* acid was purified by solution in potassium hydroxide and precipitated by acidification with glacial acetic acid, m.p. 295° (dec.). The analytical sample was washed with 95% ethanol and dried at room temperature.

Anal. Calcd. for C₄H₈N₂O₄ · H₂O: C, 28.92; H, 6.05; N, 16.86; H₂O, 10.83. Found: C, 28.88; H, 5.87; N, 16.71; H₂O (by loss at 100° and 1 mm.) 10.43.

Dibenzoyl derivative of dl-2,3-diaminosuccinic acid. The acid yielded a dibenzoyl derivative obtained as the monohydrate.¹³ The colorless crystals of the dibenzoyl derivative (113 mg.) melted at 163–176°. For analysis the compound was dissolved in a minimum quantity of 2*N* sodium hydroxide. The solution was acidified with 5*N* sulfuric acid. The precipitate was washed with water. After three reprecipitations, the product was dried over potassium hydroxide at 1 mm., m.p. 175–176°, with frothing and decomposition. Kuhn and Zumstein¹³ reported the melting point at 164°. Tamura¹² reported that the same compound melted at 152°. Heating rate and particle size are possible variables.

Anal. Calcd. for C₁₈H₁₆O₆N₂ · H₂O: C, 57.74; H, 4.85; N, 7.49. Found: C, 57.95; H, 4.84; N, 7.62.

RICHMOND 19, VA.