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α-Methyl-α-amino Acids. I. Homologs of Glutamic Acid, Methionine and Diaminopimelic Acid

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α-Methyl homologs of glutamic acid, methionine and α,α′-diaminopimelic acid have been synthesized from methyl ketones *via* the hydantoins; various derivatives of the first two acids were prepared. α-Phenylglutamic acid was also made but cyclizes readily to the corresponding pyroglutamic acid.

A number of α-methylamino acids have been synthesized in this Laboratory. The present paper discusses preparation of methyl homologs of glutamic acid, methionine and α,α′-diaminopimelic acid.

There has long been an interest, in connection with enzymatic, microbiological and chemotherapeutic studies, in antagonists of the naturally-occurring amino acids. Generally antagonists of amino acids of the type formula $R\underset{\displaystyle NH_2}{\underset{|}{C}}HCOOH$ have been sought by variation of the group R. Notably successful examples of such variations are ethionine, thienylalanine and 6-methyltryptophan. Another interesting possibility is replacement of the α-hydrogen by methyl to give compounds of formula $R-\underset{\displaystyle NH_2}{\underset{|}{\overset{\displaystyle CH_3}{\overset{|}{C}}}}-COOH$, α-methylamino acids. The provocative biochemical data on α-aminoisobutyric acid or α-methylalanine[1] suggested that investigation of other α-methylamino acids may be worthwhile.

α-Methyl homologs of valine,[2] leucine,[3] serine,[4] aspartic acid[5,6] and asparagine[5] are known and a mixture believed to contain α-methylthreonine has also been described.[7] Dextrorotatory α-methylserine was recently reported to be an hydrolysis product of the antibiotic Amicetin.[8] This seems to be the only well-established case for natural occurrence of an α-methylamino acid. There has just come to our attention a synthesis of α-methylglutamic acid by a Schmidt reaction on diethyl α-acetyl-α-methylglutarate or the corresponding half-nitrile, then hydrolysis.[9]

Homologs of α-amino acids which do not occur in nature have also been reported. The most interesting are the α-methyl relatives of α-aminobutyric and valeric acids. These are metabolized by yeast and dogs.[10] α-Methyl-α-aminobutyric acid, the so-called "isovaline," has been resolved for reaction mechanism studies.[11]

Higher α-alkyl derivatives of valine and leucine have been made[12] as well as α-ethylserine.[4b] There appears to be no record, however, of an enzymatic reaction being effected or blocked by an α-amino acid in which none of the substituents on the α-position is either hydrogen or methyl. Hog kidney acylase I hydrolyzes asymmetrically N-chloroacetyl-DL-α-amino-α-methyl-*n*-butyric acid, but not the corresponding α-ethyl compound.[12b]

The α-methylamino acids of the present report were obtained *via* the appropriate methyl ketones. From these the hydantoins were prepared and hydrolyzed to the amino acids. Thus hydrolysis of the hydantoin secured from levulinic acid gave α-methylglutamic acid. α-Methylmethionine was prepared from 5-methyl-5-(methylmercapto)-ethylhydantoin. The synthesis of α,α′-diamino-α,α′-dimethylpimelic acid required formation of a bishydantoin in two stages. 5-Methyl-5-(δ-hydroxypentyl)-hydantoin was oxidized to the ketone and the bishydantoin formed from this. Only one diastereoisomeric form of the hydantoin and, in consequence, one form of the acid was obtained.

A number of derivatives of α-methylmethionine and α-methylglutamic acid were made. α-Methylmethionine was converted to the sulfonium iodide and to α,α′-dimethylhomocystine. Several esters and N-acyl derivatives were obtained from α-methylglutamic acid, as well as the carbamyl compound and α-methylpyroglutamic acid.

A synthesis of α-phenylglutamic acid was successful in only a limited sense. Hydrolysis of α-acetamido-α-phenylglutaronitrile, prepared by cyanoethylation of α-acetamidobenzyl cyanide, gave the desired acid, but this spontaneously cyclized to α-phenylpyroglutamic acid.

Enzyme studies on α-methylglutamic acid and related compounds have been reported by several groups.[13] Umbreit[14] has recently reviewed these

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(11) P. A. Levene and R. E. Steiger, *ibid.*, **76**, 299 (1928).

(12) (a) Y. Huang, K. Lin and L. Li, *J. Chinese Chem. Soc.*, **15**, 31, 46 (1947); *Science and Technol. China*, **1**, 5 (1948); *C. A.*, **42**, 523, 4941 (1948); **43**, 2166 (1949); (b) S.-C. Fu and S. M. Birnbaum, THIS JOURNAL, **75**, 918 (1953).

(13) E. Roberts, *Proc. Soc. Exp. Biol. Med.*, **83**, 720 (1953); *J. Biol. Chem.*, **202**, 359 (1953); J. A. Jacquez and F. Mottram, *Cancer Res.*, **13**, 605 (1953); N. Lichtenstein, H. E. Ross and P. P. Cohen, *J. Biol. Chem.*, **201**, 117 (1953); *Nature*, **171**, 45 (1953); S. Grisolia and P. P. Cohen, *J. Biol. Chem.*, **204**, 753 (1953); B. N. Braganca, J. H. Quastel and M. Schucher, *Arch. Biochem. Biophys.*, **41**, 478 (1952).

(14) W. W. Umbreit, "Symposium on Amino Acid Metabolism," McCollum-Pratt Institute, Baltimore, Md., 1954.

investigations in which the strong inhibition of mammalian glutamic decarboxylase is particularly noteworthy. α-Methylmethionine appears to be a potent methionine antagonist; also it blocks very markedly the action of D-amino acid oxidase on phenylalanine.[15] α,α'-Diamino-α,α'-dimethylpimelic acid failed to interfere with utilization of diaminopimelic acid by a lysine-deficient *E. coli* strain.[16]

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Experimental[17]

β-[5-(5-Methylhydantoin)]-propionic Acid.[18]—Five hundred grams of levulinic acid was dissolved in 500 ml. of water in a 4-l. erlenmeyer flask. Four pounds of lump ammonium carbonate was added and, when foaming ceased, a solution of 320 g. of sodium cyanide in 600 ml. of water. An air condenser was attached and the flask immersed in a constant temperature bath at 58–60°. After 18 hours the contents were transferred to a 4-l. beaker and heated on the steam-bath for 3–5 hours to drive off excess ammonium carbonate. The solution was then acidified to congo red with concentrated hydrochloric acid, boiled for 20 minutes over a free flame to remove hydrogen cyanide, charcoaled and filtered while hot. The residue was washed with a little hot water and the washings combined with the filtrate; the volume at this point was about 3 liters. The solution was chilled for several hours to crystallize the hydantoin which was then collected, washed with 200 ml. of ice-water and air dried. About 700 g. of crude hydantoin was obtained which was suitable for hydrolysis. A sample which was recrystallized from alcohol melted at 159–160° (lit. 157.5–158.5°).

Anal. Calcd. for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05. Found: C, 45.52; H, 5.29; N, 15.06.

α-Methylglutamic Acid.—The crude hydantoin (700 g.) was dissolved in 1500 ml. of water and 800 g. of sodium hydroxide was slowly added. The solution was refluxed for 20 hours, then cooled, acidified to litmus with concentrated hydrochloric acid and filtered through Super-cel to remove silica. After washing the residue with 400 ml. of hot water, the filtrate was acidified to *p*H 3 and allowed to stand at room temperature. When crystallization was complete, the crystals were collected, washed with 300 ml. of cold water, then with 300 ml. of alcohol and air-dried, yielding a first crop of 330 g.

The filtrate and washings were combined and evaporated to a slurry of salt crystals, then 200 ml. of concentrated hydrochloric acid was added and the mixture filtered through a sintered glass funnel. The salt cake was washed with a little concentrated hydrochloric acid and the combined filtrates concentrated to a sirup. Two hundred ml. of water was added and the solution evaporated under vacuum to remove excess hydrochloric acid. The residual sirup was dissolved in 200 ml. of water, 800 ml. of alcohol was added and the solution neutralized to congo red with aniline. After chilling overnight, the α-methylglutamic acid was collected, washed with alcohol and air-dried. A second crop of 100 g. was then obtained. The combined yields were dissolved in 3 l. of hot water, charcoaled and filtered. Concentration of the filtrate under vacuum to about 1 l. caused crystallization of the α-methylglutamic acid in the form of large hydrated prisms. The slurry was then chilled and filtered yielding 400 g., m.p. 168–170°.[19] A sample was dried at 100° for analysis.

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.86; H, 6.85; N, 8.40.

This acid has pK_a values of 2.2, 4.4 and 9.7.

Carbamyl-α-methylglutamic Acid.—α-Methylglutamic acid (16.1 g.) was dissolved in 25 ml. of 4 *N* potassium hydroxide and potassium cyanate (9.0 g.) was dissolved therein. The reaction mixture was allowed to stand at room temperature for one hour; it was then cooled in an ice-bath and 17.5 ml. of concentrated hydrochloric acid was slowly added with vigorous stirring. After standing in an ice-bath for one hour the crystals were collected, washed with a small volume of ice-water and air-dried. The product weighed 6.3 g., melted at 155–157° and contained 2.2% ash.

An aqueous solution of the crude product was passed through an IR-210 resin column (hydrogen cycle, washed to *p*H 4 to *p*H 5). The eluate was freeze-dried to give a white powdery solid, m.p. 152–153° dec.

Anal. Calcd. for $C_7H_{12}N_2O_5$: C, 41.17; H, 5.93. Found: C, 41.25; H, 6.10.

***p*-Nitrobenzoyl-α-methylglutamic Acid.**—An ice-cold stirred solution of 48.3 g. of α-methylglutamic acid in 600 ml. of *N* sodium hydroxide was simultaneously treated with a solution of 56.0 g. of *p*-nitrobenzoyl chloride in dioxane and 150 ml. of 2 *N* sodium hydroxide. The cooled reaction mixture was stirred for one hour after the addition was completed and then acidified to congo red with 6 *N* hydrochloric acid. The solid was collected and washed with 100 ml. of water. The cake was dissolved in 500 ml. of boiling water and the solution charcoaled and filtered. The filtrate was cooled in ice, the crystalline solid collected and air-dried. A crude yield of 46.0 g., m.p. 175–180°, was obtained. Recrystallization from 200 ml. of water yielded 42.4 g. of white crystals, m.p. 183–185°.

Anal. Calcd. for $C_{13}H_{14}N_2O_7$: N, 9.03. Found: N, 8.99.

Benzoyl-α-methylglutamic Acid.—This was prepared by a method similar to the above, m.p. 200–201°.

Anal. Calcd. for $C_{13}H_{15}NO_5$: C, 59.00; H, 5.65; N, 5.28. Found: C, 59.19; H, 5.60; N, 5.17.

α-Methylpyroglutamic Acid.—α-Methylglutamic acid (35.0 g.) was heated slowly with stirring to 185°. At 130° water evolution started. Heating at 185° was continued until no further water was evolved. The melt was cooled to room temperature and triturated with ethyl acetate to give 29.7 g. (90%) of a white solid, m.p. 145–146°. The product gave a negative ninhydrin test. A small sample was recrystallized from acetone–petroleum ether, m.p. 145–146°.

Anal. Calcd. for $C_6H_9NO_3$: C, 50.34; H, 6.32; N, 9.79. Found: C, 50.08; H, 6.20; N, 9.40.

α-Methylglutamic Acid, γ-Methyl Ester Hydrochloride.—Acetyl chloride (8.5 ml.) was dissolved in 100 ml. of ice-cold anhydrous methanol. To the solution was added 16.1 g. of α-methylglutamic acid. The mixture was shaken until solution was complete and then allowed to stand at room temperature for 24 hours. The solution was filtered and concentrated *in vacuo* at 35° to a sirup. The sirup was dissolved in 25 ml. of chloroform and an equal volume of anhydrous ether slowly added. After seeding and cooling 150 ml. of anhydrous ether was added and the mixture allowed to stand overnight in a refrigerator.

The solid was collected, washed well with anhydrous ether and dried *in vacuo*. The white crystalline product weighed 18.0 g., m.p. 165–167° dec. and it gave a strong ninhydrin test.

Anal. Calcd. for $C_7H_{14}ClNO_4$: C, 39.72; H, 6.67. Found: C, 39.77; H, 6.44.

Carbobenzoxy-α-methylglutamic Acid.—Carbobenzoxy chloride (10.2 g.) dissolved in 70 ml. of toluene was slowly added with shaking to an ice-cold suspension of α-methylglutamic acid (9.7 g.) and magnesium oxide (7.4 g.) in 100 ml. of water. The reaction mixture was shaken for 16 hours at room temperature. After filtration the layers were separated and the aqueous layer extracted with two 100-ml. portions of ether, acidified with 6 *N* hydrochloric acid and extracted with five 100-ml. portions of ethyl acetate. The

(15) C. O. Gitterman, T. L. Sourkes, W. W. Umbreit, personal communications.

(16) B. D. Davis, *Nature*, **169**, 534 (1952); unpublished observations.

(17) All compounds described are the racemic modifications.

(18) H. R. Henze and R. J. Speer, THIS JOURNAL, **64**, 522 (1942), reported this hydantoin in an article on the identification of carbonyl compounds; however, specific isolation procedures and yields were not given.

(19) Šmrt and Šorm[9] record m.p. 169°. The paper of A. E. Cal- S. Arakian and G. J. Martin, THIS JOURNAL, **76**, 4181 (1954), which appeared after this contribution had been submitted, gives 168–170° dec.

combined ethyl acetate layers were washed with 25 ml. of *N* hydrochloric acid, 25 ml. of water and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residual sirup dissolved in 25 ml. of ethyl acetate. Addition of petroleum ether yielded a precipitate which after trituration solidified and was collected. The white solid, m.p. 140–141°, weighed 14.1 g. Recrystallization of a small portion from water yielded white crystals, m.p. 148.5–149°.

Anal. Calcd. for $C_{14}H_{17}NO_6$: C, 56.94; H, 5.81. Found: C, 57.23; H, 5.78.

Dimethyl α-Methylglutamate Hydrochloride.—A suspension of 15.0 g. of α-methylglutamic acid in 100 ml. of absolute methanol was cooled in an ice-bath and saturated with dry hydrogen chloride. All of the solid dissolved. The resultant solution was refluxed for three hours, the solvent removed *in vacuo* and the sirup redissolved twice in 75 ml. of methanol and concentrated *in vacuo* at which time the residue was a crystalline solid. The solid was dissolved in chloroform, filtered and ether added. The crystalline product was collected after cooling, was washed with ether and dried *in vacuo* over calcium chloride. A yield of 17.2 g. (81%) of white crystals, m.p. 142–143° dec., which gave a negative ninhydrin test was obtained.

Anal. Calcd. for $C_8H_{16}ClNO_4$: C, 42.57; H, 7.15. Found: C, 42.60; H, 6.99.

α-Acetamido-α-phenylglutaronitrile.—A solution of 10 g. of acrylonitrile in 10 ml. of dioxane was added during one-half hour to a stirred solution of 29 g. of α-acetamidobenzyl cyanide[20] and 5 ml. of 40% benzyltrimethylammonium hydroxide (Triton B) in 60 ml. of dioxane. The reaction mixture was maintained at 37–40° during addition and for two hours afterwards. It was then diluted with water and extracted with chloroform. The chloroform extractive yielded, after two crystallizations from ethyl acetate, 13.4 g. of α-acetamido-α-phenylglutaronitrile, m.p. 154–155°.

Anal. Calcd. for $C_{13}H_{13}N_3O$: N, 18.49. Found: N, 18.46.

α-Phenylglutamic Acid, α-Phenylpyroglutamic Acid.—Attempted alkaline hydrolysis of α-acetamido-α-phenylglutaronitrile caused extensive decomposition, but acid hydrolysis proceeded satisfactorily. Ten grams of nitrile were refluxed overnight in 200 ml. of 6 *N* hydrochloric acid. The solution was evaporated to dryness *in vacuo*, taken up in 30 ml. of water and cooled; 2.2 g. of α-phenylpyroglutamic acid, m.p. 204–205°, crystallized out. The filtrate was neutralized to congo red with aniline, causing precipitation of 6.5 g. of α-phenylglutamic acid, m.p. 170°. This compound gave a positive ninhydrin reaction. On overnight drying *in vacuo* at room temperature cyclization occurred. The pyroglutamic acid formed was recrystallized from boiling water, m.p. 207–208°.

Anal. Calcd. for $C_{11}H_{11}NO_3$: C, 64.09; H, 5.40; N, 6.82. Found: C, 64.42; H, 5.49; N, 6.47.

Methylthiobutane-3-one.—This ketone was prepared by Cardwell[21] in 30% yield by the reaction of methyl mercaptan with methyl 2-chloroethyl ketone. The present method is analogous to that used by Pierson, *et al.*,[22] in the synthesis of β-methylmercaptopropionaldehyde.

A mixture of 26.2 g. of dry methyl vinyl ketone, 0.2 g. of cupric acetate and 0.2 g. of hydroquinone was placed in a 3-neck round-bottomed flask equipped with inlet tube, stirrer, condenser and thermometer. An exit line was arranged to pass through a solution of lead acetate. Liquid methyl mercaptan (40 ml.) was placed in a small round-bottomed flask connected to the inlet tube. The methyl mercaptan was heated with warm water, vaporizing it, and forcing it through the inlet tube, which extended below the surface of the reaction mixture. The temperature of the reaction was maintained at 35–40° with cooling. After complete addition stirring was continued overnight at room temperature. The reaction mixture was then filtered and distilled *in vacuo*. The product boiled at 75° (14 mm.), $n^{25}D$ 1.4772. The yield was 33.8 g. (77%).

5-(β-Methylmercapto)-ethyl-5-methylhydantoin.—A mixture of 33.8 g. of methylthiobutan-3-one and 28.6 g. of potassium cyanide was dissolved in an alcohol–water mixture to form a homogeneous solution, then 132.2 g. of ammonium carbonate was added. The reaction mixture was heated 18 hours at 58–60°, then placed in an open dish and heated four hours longer on a steam-bath. The solution was acidified to congo red and evaporated to a small volume, charcoaled, filtered and cooled. The crystalline precipitate was filtered and extracted with ethanol. The ethanol extract was evaporated to yield a sirup, which was dissolved in ether, filtered, treated with petroleum ether, and cooled. The crystalline product obtained weighed 40.8 g. (82%), m.p. 105–107°. A sample for analysis was recrystallized from ether–petroleum ether and melted at 109–110°.

Anal. Calcd. for $C_7H_{12}N_2O_2S$: N, 14.89. Found: N, 14.92.

α-Methylmethionine.—Forty and three-tenths grams of 5-(β-methylmercapto)-ethyl-5-methylhydantoin, 217.4 g. of barium hydroxide octahydrate and 150 ml. of water were heated under reflux until evolution of ammonia ceased. Carbon dioxide was then passed into the solution until no further precipitation occurred. After acidifying, the solution was filtered and concentrated to dryness. The residue was redissolved in hot water, a small amount of insoluble material being rejected. On cooling, the product crystallized, m.p. 280° dec. The filtrate yielded additional material, m.p. 279° dec. The combined yield was 15.8 g. (45%). A sample for analysis was obtained by recrystallization from aqueous alcohol, m.p. 283–284° dec.

Anal. Calcd. for $C_6H_{13}NO_2S$: C, 44.15; H, 8.03; N, 8.58. Found: C, 44.58; H, 7.89; N, 8.60.

α,α′-Dimethylhomocystine.—This was obtained by a method similar to the preparation of homocystine by Butz and du Vigneaud.[23]

α-Methylmethionine (8.5 g.) was dissolved in 70 ml. of 18 *N* sulfuric acid and the solution heated under reflux in an oil-bath at 125–135° for eight hours. The cooled solution was poured into 1200 ml. of water and the sulfate precipitated with barium hydroxide. The mixture was heated to boiling, filtered and the residue washed twice by suspension in 1500 ml. of boiling water. The combined filtrates were concentrated *in vacuo* to about 300 ml. while the product gradually precipitated. The product was collected, dissolved in dilute hydrochloric acid and reprecipitated by neutralization to litmus. The precipitate was collected and washed with water until free of chloride; yield 1.1 g., m.p. 278° dec. A sample was dried at 80° and 3 mm. for analysis.

Anal. Calcd. for $C_{10}H_{20}N_2O_4S_2 \cdot H_2O$: C, 38.20; H, 7.05; N, 8.91. Found: C, 38.32; H, 6.40; N, 8.72; wt. loss at 100°, 6.6%.

α-Methylmethioninemethylsulfonium Iodide.—Following the procedure of Toennies[24] for the preparation of methioninemethylsulfonium iodide, a mixture of 4.9 g. of α-methylmethionine, 49 ml. of 88% formic acid, 15 ml. of glacial acetic acid and 7.5 ml. of methyl iodide was allowed to stand in the dark for six days. After removal of solvent *in vacuo* the sirup was dissolved in 40 ml. of methanol and 100 ml. of ether was added. The oily product gradually solidified and was collected. The precipitation from a methanol solution with ether was repeated giving 8.1 g. of a cream colored solid. This was dissolved in 8 ml. of 50% alcohol and crystallized by the addition of 100 ml. of absolute alcohol and cooling. The yield was 5.6 g. of white crystals, m.p. 95° dec.

Anal. Calcd. for $C_7H_{16}INO_2S$: C, 27.54; H, 5.28. Found: C, 26.96; H, 6.15.

5-Methyl-5-(δ-hydroxypentyl)-hydantoin.—Forty-seven grams of 6-hydroxyheptanone-2[25] was heated with 50 g. of sodium cyanide and 200 g. of ammonium carbonate in 500 ml. of 30% aqueous alcohol at 58–60° for 18 hours. The solution was further heated in an open beaker on the steam-bath for four hours then acidified with concentrated hydrochloric acid and boiled for one-half hour to remove unreacted hydrogen cyanide. It was then concentrated to a sirup and absolute alcohol added to precipitate inorganic salts. These were removed by filtration and the filtrate concentrated and redissolved in a small amount of ethyl

(20) H. Reihlen, G. V. Hessling, W. Hühn and E. Weinbrenner, *Ann.*, **493**, 20 (1932).

(21) H. M. E. Cardwell, *J. Chem. Soc.*, 715 (1949).

(22) E. H. Pierson, M. Giella and M. Tishler, THIS JOURNAL, **70**, 1450 (1948).

(23) L. W. Butz and V. du Vigneaud, *J. Biol. Chem.*, **99**, 136 (1933).

(24) G. Toennies and J. J. Kalb, THIS JOURNAL, **67**, 848 (1945).

(25) W. H. Perkin, *J. Chem. Soc.*, **105**, 1361 (1914).

acetate and charcoaled. The product was precipitated by the addition of ether and cooling. It was then collected and washed with ether; yield 60 g. (77%), m.p. 115–120°. An analytical sample recrystallized from ethyl acetate melted at 131°.

Anal. Calcd. for $C_9H_{16}N_2O_3$: N, 14.00. Found: N, 13.64.

The crude product is probably a mixture of diastereomers. However, both forms would give the same ketone on oxidation.

5-Methyl-5-(δ-ketopentyl)-hydantoin.—Forty grams of the above crude hydantoin was heated with a solution of 24 g. of sodium dichromate and 28 g. of sulfuric acid in 500 ml. of water at 60° for 18 hours. The solution was allowed to stand at 5° overnight and the precipitated ketohydantoin collected and washed with ice-water until the filtrate was colorless; 1st crop 27 g., m.p. 155°. A second crop of 8 g. was obtained on concentration of the mother liquors; total yield 35 g. (88%). A sample was recrystallized from ethyl acetate, m.p. 155–157°.

Anal. Calcd. for $C_9H_{14}N_2O_3$: C, 54.54; H, 7.12. Found: C, 54.50; H, 7.14.

5,5'-Dimethyl-5,5'-trimethylenebishydantoin.—The bishydantoin was obtained by a method similar to the hydroxyhydantoin. Forty-three grams of the ketohydantoin was heated with 25 g. of sodium cyanide and 250 g. of ammonium carbonate. The product precipitated immediately upon acidification and was collected and washed with water; yield 50 g., m.p. 290°. Recrystallization from 1200 ml. of boiling water gave 45 g., m.p. 293°.

Anal. Calcd. for $C_{11}H_{16}N_4O_4$: C, 49.25; H, 6.01; N, 20.89. Found: C, 49.44; H, 5.85; N, 21.19.

α,α'-Diamino-α,α'-dimethylpimelic Acid.—A solution of 30 g. of the bishydantoin and 40 g. of sodium hydroxide in 300 ml. of water was refluxed in a copper flask for 22 hours. The solution was acidified to *p*H 6 with concentrated hydrochloric acid and the resulting flocculent precipitate digested over an open flame for one hour. The product was collected and purified by solution in sodium hydroxide and reprecipitation with acid, yielded 6.6 g. (28%) of amino acid which decomposes above 350°. The mother liquors and filtrates were combined, evaporated to a small volume and the sodium chloride precipitated by the addition of concentrated hydrochloric acid. The salt was removed by filtration through a sintered glass funnel and the filtrate evaporated to dryness. The residue was dissolved in water and neutralized to congo red giving an additional 3.5 g. of crude amino acid.

Anal. Calcd. for $C_9H_{18}N_2O_4$: C, 49.53; H, 8.31; N, 12.84. Found: C, 49.60; H, 8.24; N, 12.36; Van Slyke N, 12.69.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES, CHEMICAL DIVISION, MERCK & CO., INC.]

α-Methyl α-Amino Acids. II.[1] Derivatives of DL-Phenylalanine

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A variety of α-methylphenylalanine derivatives was prepared for biological study.

As part of a program to investigate chemotherapeutic possibilities among amino acid analogs, our attention was turned to derivatives of phenylalanine and particularly of the biologically important 3,4-dihydroxyphenylalanine (DOPA). This compound may well figure in the biosynthesis of the pressor amines noradrenaline and epinephrine.[2] Furthermore decarboxylation of DOPA by mammalian enzyme preparations had been especially thoroughly studied[3,4] and appeared in excellent point for interference in the biosynthetic sequence by metabolite analogs.[5] Also earlier success with α-methylglutamic acid as inhibitor of mammalian decarboxylase[1,6] suggested a new approach to the inhibition of DOPA decarboxylation.

Synthesis of α-methyl DOPA (VI) was from 3,4-dimethoxyphenylacetonitrile (I) *via* 3,4-dimethoxyphenylacetone, the corresponding hydantoin IV and the dimethoxy acid V. This route proved superior to the alternate method *via* VIIa. It is of interest that acid hydrolysis of VIIa at 100° gave only partial cleavage of methoxyl. The product was assigned the structure α-methyl-3-hydroxy-4-methoxyphenylalanine (VIIIa) on the basis of a positive response in the indophenol test for phenols having an unsubstituted *p*-position.[7] A negative result was obtained with V and with 3-methoxy-4-hydroxyphenylalanine. Acid treatment at 150° was effective in cleaving the remaining methoxyl of VIIIa and yielding α-methyl DOPA (VI).

A similar sequence of reactions provided α-methyl-3-hydroxyphenylalanine (VIa) which was considered also a possible DOPA decarboxylase inhibitor since 3-hydroxyphenylalanine serves as a substrate for this enzyme.

The parent compound, α-methylphenylalanine (VIIIb) corresponded in properties to the preparation of Herbst and Johnson.[8] Its conversion to α-methyltyrosine (XII), desired for studies on bacterial tyrosine decarboxylase, was made the occasion for synthesis of the chloramphenicol-resembling substance X. The formulas given are for those related compounds which were synthesized.

The α-methylphenylalanine was also converted *via* its N-acetyl methyl ester to 2-amino-2-methyl-3-phenylpropanol.

A comparison of the physical properties of the natural amino acids (racemic modifications) and their α-methyl homologs is of interest. Thus while α-methyl DOPA is nearly ten times as soluble in water as DOPA itself, the differences in pK_a values are small. The α-methyl compound showed

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