

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY, UNIVERSITY OF COLORADO, AND FLORIDA STATE UNIVERSITY]

The Syntheses and Microbiological Properties of Acetylenic Amino Acids. Propargylglycine and 2-Amino-3-methyl-4-pentynoic Acid¹

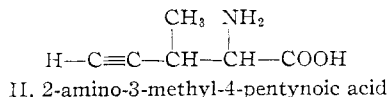
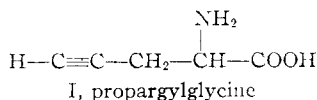
BY HERMAN GERSHON, JACOB SHAPIRA, JOHN S. MEEK AND KARL DITTMER²

RECEIVED JANUARY 29, 1954

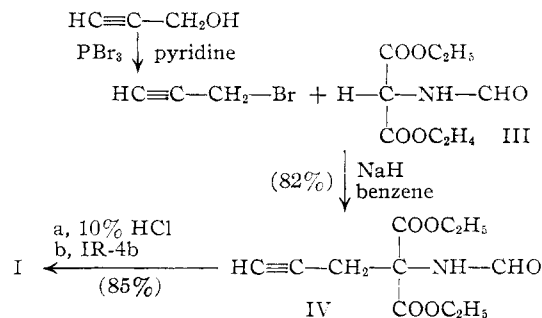
Since ethylenic unsaturated amino acids, allylglycine, methallylglycine and others were found to be powerful inhibitors of microbial growth, it seemed of importance to synthesize acetylenic amino acid analogs. The syntheses of propargylglycine and 2-amino-3-methyl-4-pentynoic acid and derivatives are described. These two acetylenic amino acids were potent inhibitors of the growth of *S. cerevisiae* and less potent, but effective, inhibitors of *E. coli*.

Early studies of metabolite antagonists indicated that replacement of a $-\text{CH}=\text{CH}-$ group by a $-\text{S}-$ atom or *vice versa* in an aromatic metabolite may result in the production of an antagonist.³ Dittmer and co-workers demonstrated that this relationship existed among aliphatic compounds when they prepared and studied the vinylene analogs of cysteine and methionine: allylglycine and 2-amino-5-heptenoic acid.⁴ They also studied the related unsaturated amino acids, methallylglycine and crotylglycine. These unsaturated amino acids, most of which were first prepared by Albertson and co-workers⁵ were found to be potent inhibitors of microorganisms^{4a} but not specific inhibitors of the related sulfur-containing metabolite. Since introduction of olefinic unsaturates into an amino acid produced powerful inhibitors of microorganisms, two amino acids containing the acetylenic linkage were prepared. These compounds are of further interest because the antibiotic, mycomycin, has been shown to be an aliphatic acetylenic acid.⁶

In this paper we wish to report the details of our investigations in the synthesis of the acetylenic amino acids, propargylglycine (I)⁷ and 2-amino-3-methyl-4-pentynoic acid (II) and their preliminary microbiological properties.



The procedure for the synthesis of propargylglycine (I) finally evolved is that shown by the reaction



Propargylglycine was also prepared *via* the acetamidomalonate derivative, but the acid hydrolysis of this intermediate was more difficult and resulted in the formation of by-products which were difficult to remove. Hydrolysis with dilute sulfuric acid had a tendency to hydrate the triple bond, while hydrobromic acid hydrolysis resulted in the addition of hydrogen bromide. The hydrolysis of IV proceeds readily in 10% hydrochloric acid solution, which avoids these side reactions. In the condensation of propargyl bromide with III, good yields were obtained consistently by the use of sodium hydride in benzene.⁸ Sodium ethoxide in ethanol effected the condensation quite well, but occasional contamination with a trace of water resulted in considerably lower yields. I was also prepared by the use of ethyl acetamidocyanacetate and ethyl acetamidomalonate, but inferior over-all yields were obtained.

In view of other types of reactions of propargyl halides leading to allenic compounds,⁹ the possibility of allene formation had to be considered in this study. In order to exclude this possibility, the infrared spectra were determined for propargyl bromide, the condensate IV, the amino acid I and the N-benzoyl amino acid V. In no case was evidence obtained for the sharp band at about 1900 cm^{-1} characteristic of the allenic group, whereas strong primary acetylenic bands were observed around 3320 cm^{-1} for all of these compounds. However, the acetylenic band at 2135 cm^{-1} was very weak, but always detectable, in the condensates and benzoyl derivatives of the amino acids and of medium strength in the amino acids. In contrast Wotiz and Miller^{9a} found this band was always strong in compounds containing a terminal triple bond. A titration of the acid liberated when IV was treated with silver nitrate gave the results ex-

(1) Taken from the thesis submitted by Herman Gershon in partial fulfillment of the requirements for the Degree Doctor of Philosophy, University of Colorado. This work was supported in part under a contract with the Office of Naval Research and Research Grant 2714 from U. S. Public Health Services.

(2) Florida State University, Tallahassee, Florida.

(3) D. W. Wooley and A. G. C. White, *J. Biol. Chem.*, **149**, 285 (1943); V. du Vigneaud, H. McKennis, Jr., S. Simmonds, K. Dittmer and G. B. Brown, *ibid.*, **159**, 385 (1945).

(4) K. Dittmer, H. L. Goering, I. Goodman and S. J. Cristol, *THIS JOURNAL*, **70**, 2499 (1948); (b) H. L. Goering, S. J. Cristol and K. Dittmer, *ibid.*, **70**, 3310 (1948); (c) H. L. Goering, S. J. Cristol and K. Dittmer, *ibid.*, **70**, 3314 (1948).

(5) N. F. Albertson, *ibid.*, **68**, 450 (1946); N. F. Albertson and B. F. Tullar, *ibid.*, **67**, 502 (1945).

(6) W. D. Celmer and I. A. Solomons, *ibid.*, **75**, 1372 (1953).

(7) H. Gershon, J. S. Meek and K. Dittmer, *ibid.*, **71**, 3573 (1949).

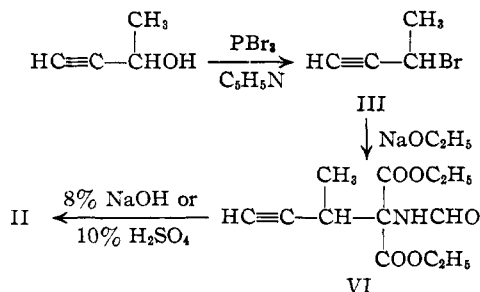
(8) J. Shapira, R. Shapira and K. Dittmer, *ibid.*, **75**, 3655 (1953).

(9) J. H. Wotiz, J. S. Matthews and J. A. Lieb, *ibid.*, **73**, 5503 (1951).

(9a) J. H. Wotiz and F. A. Miller, *ibid.*, **71**, 3441 (1949).

pected for IV.¹⁰ Paper chromatography of the amino acid in a variety of solvents gave no indication that more than one compound was present. Determinations of the relative reactivities of propargyl bromide and bromopropadiene by Jacobs and Brill¹¹ toward sodium iodide in acetone strongly imply that even if our propargyl bromide did contain a small amount of bromopropadiene, it would not condense with III.

2-Amino-3-methyl-4-pentynoic acid (II) was prepared by the same general reactions



2-Bromobutyne-3 was condensed with III in 20–35% yields. Basic hydrolysis of VI gave II in 12% yield. When VI was hydrolyzed with 10% sulfuric acid, II was obtained in 71% yield.

Experimental¹²

Diethyl Propargylformamidomalonate (IV).—In a 500-ml. three-necked flask equipped with a mechanical stirrer, dropping funnel and reflux condenser (all dried at 110° overnight) was placed 9.5 g. (0.40 mole) of sodium hydride covered with about 10 ml. of dry benzene. There was then added a hot solution of 74.2 g. (0.36 mole) of III, m.p. 50–52°, in 150 ml. of dry benzene. This solution had been boiled down to about three-fourths of its original volume to expel traces of moisture. The resulting mixture evolved hydrogen slowly for a few minutes as determined by a mercury bubble-trap connected to the top of the reflux condenser. There was then added 43.3 g. (0.35 mole) of dry propargyl bromide,¹³ and the mixture was stirred and refluxed gently for five hours. Liberation of hydrogen had virtually ceased after the third hour.

After cooling, a small amount of ethanol was added to decompose the excess sodium hydride and after 30 minutes of stirring, 150 ml. of water was added. The product was separated from the reaction mixture by extraction with ether. The extract was taken to dryness on the steam-bath to yield an oil which rapidly solidified on cooling to give 79 g. of a sticky yellow solid. This was recrystallized from ether to give 50 g. of light tan product, m.p. 73.5–74°. The mother liquors yielded an additional 20.6 g. of product, m.p. 70–72°. The total yield of recrystallized product amounted to 81.5%. Several recrystallizations from aqueous ethanol gave pure white material melting at 75.5–76.0° (cor.) and showed no depression in a mixed m.p. made with a sample prepared by sodium ethoxide condensation.⁷

Ethyl Acetamidocyanopropargylacetate.—To a solution containing 1.15 g. (0.05 g. atom) of sodium dissolved in 45 ml. of absolute alcohol was added 8.5 g. (0.05 mole) of ethyl acetamidocyanacetate. Six and two-tenths grams (0.062 mole) of propargyl bromide in 20 ml. of absolute ethanol was added and the mixture refluxed for 18 hours. After con-

centrating to dryness, the residue was taken up in a mixture of chloroform and water. The product obtained by evaporating the chloroform was decolorized with "Darco C-60" and recrystallized from water. The ethyl acetamidocyanopropargylacetate melted at 83°, and the yield was 87%. An analytical sample was obtained from isopropyl ether, m.p. 88–89°.

Anal. Calcd. for $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_4$: N, 13.46. Found: N, 13.14.

Ethyl Acetamidopropargylmalonate.—By a procedure similar to the one described above, propargyl bromide was condensed with ethyl acetamidomalonate in 0.2 molar quantities. Ethyl acetamidopropargylmalonate was produced in 33% yield and melted at 90–94°. An analytical sample, m.p. 95–96°, was obtained from isopropyl ether.

Anal. Calcd. for $\text{C}_{12}\text{H}_{17}\text{NO}_5$: N, 5.49. Found: N, 5.44.

Ethyl Formamido- α -methylpropargylmalonate (VI).—2-Bromobutyne-3 as prepared by a modification of the method of Kirmann¹³ was condensed with ethyl formamidomalonate in 0.05–0.2 molar quantities. VI was obtained in 20–35% yields, m.p. 79–80°. An analytical sample was prepared by recrystallizing from water and then from *n*-butyl ether and melted at 97–98°.

Anal. Calcd. for $\text{C}_{12}\text{H}_{17}\text{NO}_5$: C, 56.47; H, 6.67; N, 5.49. Found: C, 56.98; H, 6.78; N, 5.53.

Propargylglycine.—A mixture of 19.3 g. (0.08 mole) of IV and 100 ml. of 10% hydrochloric acid was refluxed gently for four hours. After about two hours evolution of carbon dioxide ceased, as determined by a mercury bubble-trap attached to the condenser and identified with a barium hydroxide solution. The resulting mixture was concentrated on a steam-bath under reduced pressure to a solid, 50 ml. of water was added and it was evaporated again. The solid was dissolved in 100 ml. of water, decolorized with "Darco S-51" and the filtrate added to 100 g. of the basic form of "Duolite A-2" resin. After ten minutes standing, the neutral supernatant liquid was decanted and the resin was washed with warm water until free of amino acid. The combined filtered aqueous solutions were concentrated to a small volume under reduced pressure, diluted to about 50 ml. with water and an equal volume of acetone was added.

After standing at –15° for 12 hours, the product was filtered and white crystals weighing 7.7 g. (85% yield), m.p. 236.5–238° dec., were obtained. This product was halogen free and a purified sample had a m.p. of 238–240° dec. (cor.) (lit. 243° dec.).

Benzoylpropargylglycine (V).—0.02 mole of propargylglycine was benzoylated by the method described by Steiger.¹⁴ After three recrystallizations from alcohol and water, a yield of 46% of benzoylpropargylglycine was obtained, m.p. 134–135°.

Anal. Calcd. for $\text{C}_{12}\text{H}_{11}\text{NO}_3$: N, 6.45. Found: N, 6.49.

Reduction of V to N-Benzoylnorvaline.—A solution of 200 mg. of pure V in 5 ml. of anhydrous ethanol was hydrogenated at room temperature and 45 p.s.i. in the presence of a trace of platinum oxide. After one hour, the solution was filtered and an equal volume of water was added.

The crystals which appeared after standing in the cold were recrystallized from ethanol-water to give N-benzoylnorvaline, m.p. 151.5–153° (cor.). This same melting point was observed for an authentic sample prepared in the conventional way from DL-norvaline. A mixed melting point of these samples showed no depression.

Determination of Acetylenic Hydrogen of IV.—Samples of 100 mg. of IV were treated with silver nitrate according to the method described by Siggia.¹¹ The nitric acid produced was found to be 102% of the theoretical.

2-Amino-3-methyl-4-pentynoic Acid (II).—When 0.005 mole of condensation product VI was hydrolyzed with 10% sulfuric acid for 18 hours and the acid removed by "Duolite A-2," a yield of 71% of II was obtained, m.p. 194° dec.

An analytical sample was obtained from aqueous alcohol and melted at 213–214° dec. Although this compound can exist in two racemic modifications, only one isomer appeared to be present.

Anal. Calcd. for $\text{C}_6\text{H}_9\text{NO}_2$: C, 56.69; H, 7.09. Found: C, 56.40; H, 7.10.

Isoleucine.—Hydrogenation of 64 mg. (0.0005 mole) of 2-amino-3-methyl-4-pentynoic acid in the presence of 10

(10) S. Siggia, "Quantitative Organic Analysis vis Functional Groups," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 54.

(11) T. L. Jacobs and Wm. F. Brill, *THIS JOURNAL*, **75**, 1314 (1953).

(12) All melting points are uncorrected unless specifically noted as corrected (cor.). The infrared spectra of all new compounds reported and compounds used in the structure determinations are available at Florida State University, and will be published in the Ph.D. thesis of Jacob Shapira.

(13) A. Kirmann, *Bull. soc. chim.*, (IV) **39**, 698 (1926). Later preparations of I were made with a generous sample of propargyl bromide provided by Commercial Developments Department of General Aniline and Film Corp.

(14) R. E. Steiger, *J. Org. Chem.*, **9**, 396 (1944).

mg. of Adams catalyst, at 23.3° and 627 mm. required 0.00103 mole (103%) of hydrogen and produced 60 mg. of alloisoleucine. The formation of alloisoleucine on hydrogenation was established by a comparison of the product with an authentic sample of alloisoleucine by paper chromatography and by a comparison of its infrared spectrum with those of isoleucine and alloisoleucine.

2-Benzamido-3-methyl-4-pentynoic Acid.—2-Amino-3-methylpentynoic acid was benzoylated according to the method of Steiger¹⁴ to give the benzoyl derivative, m.p. 127°.

Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.53; H, 5.63. Found: C, 67.50; H, 5.63.

Microbiological Tests.—Propargylglycine and 2-amino-3-methyl-4-pentynoic acid were tested for their ability to inhibit the growth of *E. coli*, ATCC 9723, and *S. cerevisiae*, strain 139, according to the method previously described.⁶

By these tests 2–3 μ g. of propargylglycine per 7.5 ml. of medium inhibited the growth of yeast to 50% of normal;

14 μ g. of 2-amino-3-methyl-4-pentynoic acid was required to give 50% inhibition of yeast growth. The growth of *E. coli* was inhibited to 50% of normal by 130 mg./7.2 ml. of propargylglycine and 50 μ g./7.2 ml. of 2-amino-3-methyl-4-pentynoic acid. The acetylenic analogs of amino acids were more potent yeast growth inhibitors than the ethylenic analogs, allylglycine and methallylglycine. In the inhibition of the growth of *E. coli*, the ethylenic analogs were more potent than the acetylenic amino acids. Propargylglycine is the most potent yeast growth inhibitor so far prepared in our laboratory.

The relationship of the acetylenic amino acids to amino acid metabolism in these microorganisms will be reported elsewhere.

Acknowledgment.—The authors wish to thank Mrs. Ann E. Johnson and Miss Virginia Peck for the microbiological determinations.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TORONTO]

Preparation and Properties of the Epimeric 2,3-Dimethylbutane-1,4-diols and Some Derivatives

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RECEIVED FEBRUARY 12, 1954

DL- and *meso*-2,3-dimethylbutane-1,4-diol were prepared by the lithium aluminum hydride reduction of the corresponding dimethylsuccinic acid diethyl esters. The diols are colorless, viscous oils and were characterized by conversion to their crystalline di-*p*-nitrobenzoates and ditrityl ethers. The diols were also converted to the corresponding dibromides. The colorless, liquid dibromides were characterized by conversion to their di-*p*-nitrophenyl ethers, and also by reaction with pyrrolidine to give the dimethylspirobipyrrrolidinium bromides, picrates and chloroaurates.

To facilitate the solution of a more fundamental problem, still in progress, we have prepared *meso*- and DL-2,3-dimethylbutane-1,4-diol and the corresponding dibromides. Each diol and dibromide has been characterized by the preparation of a number of solid derivatives.

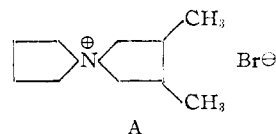
Synthetic Route.—The key intermediate needed for preparation of the *meso*-diol was *meso*-2,3-dimethylsuccinic acid.³ Since direct reduction of the *meso*-diacid with lithium aluminum hydride⁴ gave us a poor yield of diol, the *meso* diethyl ester⁵ was prepared; on reduction it gave a 56% yield of *meso*-diol. The diol obtained was a colorless viscous liquid, readily soluble in water and miscible with ether, whose molecular refraction was in agreement with the theoretical value.

The *meso*-diol was characterized by conversion to its di-*p*-nitrobenzoate and ditrityl ether,⁶ both sharp-melting crystalline derivatives.

On treatment with anhydrous hydrogen bromide,

the *meso*-diol gave the colorless, liquid *meso*-dibromide. The dibromide differs from the diol by its much higher density (1.6 vs. 1.0), by its lower viscosity and boiling point, and by its much lower solubility in water. The molecular refraction of the dibromide was in agreement with theory.

The *meso*-dibromide was first characterized by reaction with sodium nitrophenoxide to give the crystalline *meso*-di-*p*-nitrophenyl ether.⁷ Since the yield of this reaction was only 25%, and the conditions somewhat drastic, the dibromide was characterized also by reaction with pyrrolidine, to give the *meso*-spiroquaternaryammonium bromide, A. This reaction which proceeds under mild conditions gave



a 60% yield. This spirane bromide was further characterized by converting it to its picrate and chloroaurate.⁸

The work on the racemic diol and dibromide and their derivatives (see Experimental Section) was closely parallel to that on the *meso* epimers.

(7) Similar preparations of diaryl ethers from dibromides have been described by (a) A. Müller, *Monatsh.*, **49**, 27 (1928); (b) A. Wedige, *J. prakt. Chem.*, [2] **24**, 246 (1881); (c) E. Wagner, *ibid.*, [2] **27**, 201 (1883).

(8) In order to prove that our synthetic route did not cause any structural rearrangement, the spirane derivatives (both *meso* and DL) also were prepared by an independent route, namely: the substituted succinamic acid obtained by reaction of 2,3-dimethylsuccinic anhydride with pyrrolidine was reduced and cyclized. Details of the spirane work will be given in a subsequent communication.

(1) Nadine Phillips Fellow, 1953–1954.

(2) From a Ph.D. Thesis to be submitted by Stephen Proskow to the Graduate School, University of Toronto.

(3) W. A. Bone and C. H. G. Sprankling, *J. Chem. Soc.*, **75**, 839 (1899). In this reference the *meso*-diacid (m.p. 209°) is incorrectly called "trans."

(4) (a) "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, Chap. 10; (b) R. F. Nystrom and W. G. Brown, *THIS JOURNAL*, **69**, 2548 (1947).

(5) N. Zelinsky and S. Krapivin, *Ber.*, **22**, 646 (1889). In this reference the DL-diacid (m.p. 129°) is incorrectly called "maleinoid." These authors claim that the esterification of either pure isomer of dimethylsuccinic acid with ethanol and mineral acid causes partial isomerization and that the silver salt method does not.

(6) (a) "Advances in Carbohydrate Chemistry," Vol. 3, Academic Press, Inc., New York, N. Y., 1948, Chap. IV; (b) F. Valentin, *Chem. Zentr.*, **103**, I, 2160 (1932); (c) B. Helferich, *et al.*, *Ber.*, **56**, 766 (1923).