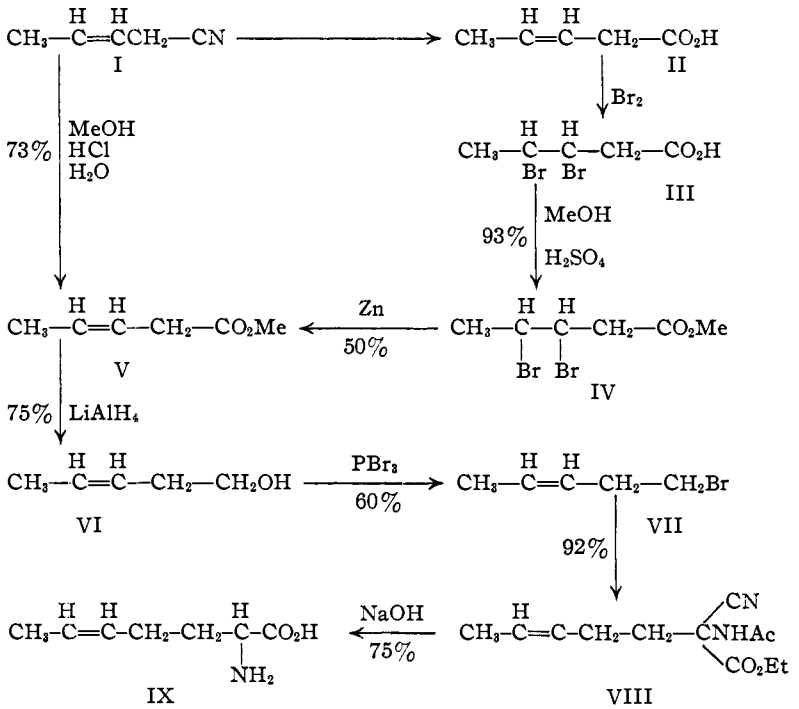
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

Unsaturated Amino Acids. III. 2-Amino-5-heptenoic Acid, the Vinylene Analog of Methionine[1]

BY HARLAN L. GOERING,[2] STANLEY J. CRISTOL AND KARL DITTMER

In the course of an extended study on the permissible variances in structure resulting in the development of metabolite–antagonist relationships, we have shown that replacement of the sulfur atom in the amino acid cysteine by a vinylene group results in an antagonist of the growth of certain microörganisms. Since this compound, allylglycine,[1] was so interesting biologically,[3] it seemed important to us to prepare the corresponding analog of the other biologically important sulfur-containing amino acid, methionine. The synthesis and properties of this analog, 2-amino-5-heptenoic acid (VII), are the subjects of this paper.

The method of synthesis which we found successful is outlined in the following chart.



The most promising method of amino acid synthesis is that recently developed by Snyder[4] and by Albertson[5] and their co-workers involving alkylation of ethyl acetamidomalonate or ethyl acetamidocyanoacetate followed by hydrolysis of the alkylation products. To apply this method to the synthesis of IX there was required a five-carbon alkylating agent such as 5-bromo-2-pentene (VII). Fortunately this compound is now easily available through application of the reducing agent lithium aluminum hydride, recently developed by Nystrom and Brown,[6] in a three-step process starting with the known 3-pentenonitrile (I).[7] The nitrile was converted to the corresponding methyl ester V directly in 73% yield by solvolysis catalyzed by hydrogen chloride. This substance had properties identical with those of the same material prepared by debromination with zinc dust of the ester IV prepared from 3,4-dibromopentanoic acid (III).[8] The ester V was converted to 3-penten-1-ol (VI) in 75% yield with lithium aluminum hydride, and the alcohol upon treatment with phosphorus tribromide and pyridine at −40° gave the required 5-bromo-2-pentene (VII) in 60% yield, according to the procedure of Young and Lane[9] for similar treatment of butenyl alcohols.

The bromide was condensed with ethyl acetamidocyanoacetate in 92% yield, and the alkylation product was hydrolyzed in base to the required amino acid IX in 75% yield.

The structure of IX was proved by reduction to the known 2-aminoheptanoic acid (X)[5a] and by ozonolysis to acetaldehyde.

The 2-amino-5-heptenoic acid inhibited the growth of one strain of *Escherichia coli*, but had no effect on the growth of *Saccharomyces cerevisiae* and two other strains of *E. coli*. Relatively large amounts (20 mg. per 7.5 ml.) were required for complete inhibition of the growth of *E. coli*, and very small amounts of methionine almost completely reversed the bacteriostatic action.

Experimental

Methyl 3,4-Dibromopentanoate (IV).—3-Pentenonitrile (I) was prepared from a butenyl chloride mixture accord-

(1) Previous paper in series: Goering, Cristol and Dittmer, THIS JOURNAL, **70**, 3310 (1948).

(2) American Cyanamid Fellow.

(3) Dittmer, Goering, Goodman and Cristol, THIS JOURNAL, **70**, 2499 (1948).

(4) (a) Snyder, Shekleton and Lewis, *ibid.*, **67**, 310 (1945); (b) Howe, Zambito, Snyder and Tishler, *ibid.*, **67**, 38 (1945).

(5) (a) Albertson, *ibid.*, **68**, 450 (1946); (b) Albertson, Archer and Suter, *ibid.*, **67**, 36 (1945); (c) Albertson and Archer, *ibid.*, **67**, 308 (1945); (d) Albertson and Archer, *ibid.*, **67**, 2043 (1945).

(6) Nystrom and Brown, *ibid.*, **69**, 1198 (1947).

(7) Lane, Fentress and Sherwood, *ibid.*, **66**, 545 (1944).

(8) Mackenzie and Fittig, *Ann.*, **283**, 82 (1894).

(9) Young and Lane, THIS JOURNAL, **59**, 2051 (1937).

ing to the method of Lane and co-workers.[7] 3-Pentenoic acid (II) was prepared from I by the previously described method.[7] The unsaturated acid was converted to the known 3,4-dibromopentanoic acid according to the method of Mackenzie and Fittig,[8] using carbon tetrachloride as solvent instead of carbon disulfide. The esterification of III was carried out in the usual manner using sulfuric acid as a catalyst. Methyl 3,4-dibromopentanoate, obtained in 93.5% yield, boiled at 122.5° (17 mm.) and had $n^{20}D$ 1.5105, d^{20}_4 1.7416, M_D (calcd.) 47.19, M_D (found) 47.08.

Anal. Calcd. for $C_6H_{10}O_2Br_2$: C, 26.30; H, 3.68. Found: C, 26.22; H, 3.46.

Methyl 3-Pentenoate (V).—(A) 3-Pentenonitrile (I) was converted to V by a modification of the general method of Pfeiffer.[10]

A solution of 45.4 g. (0.56 mole) of 3-pentenonitrile and 10.1 g. (0.56 mole) of water in 650 ml. of absolute methanol[11] was prepared in a two-neck round-bottom flask provided with a reflux condenser and an inlet tube for gaseous hydrogen chloride. Dry hydrogen chloride was passed through the refluxing solution for a period of two hours, after which the solution was allowed to stand for two more hours. Ammonium chloride began to separate from the solution after the first half hour of refluxing. An equal amount of ice-cold saturated brine was added to the solution causing the ester layer to separate. The aqueous layer was extracted with several portions of ether. The ester and ether extracts were combined and extracted with a small amount of saturated potassium carbonate solution and then with water and finally with brine. The ether solution was dried over anhydrous sodium sulfate. Fractionation of the ether solution through a one-foot column packed with helices yielded 46.5 g. (73%) of methyl 3-pentenoate, b. p. 128.1–128.3° (625 mm.), and had $n^{20}D$ 1.4217, d^{20}_4 0.9284, M_D (calcd.) 31.10, M_D (found) 31.23.

(B). To a solution of 73.6 g. of IV in 700 ml. of absolute methanol, in a one-liter three-neck flask equipped with an efficient stirrer, thermometer and condenser, was added 132 g. of zinc dust. The zinc was added slowly with continuous stirring and the exothermic reaction was controlled by placing the reaction flask in an ice-bath intermittently. The reaction subsided after the addition of the zinc was complete and the stirred solution was held at a temperature of 60° for an additional hour. The solution was filtered and chilled, and an equal volume of saturated brine was added. The solution was extracted with five 100-ml. portions of ether, and the combined ether extracts were washed with saturated brine and dried over Drierite. Fractionation of the ether solution yielded 15 g. (50%) of product having the same physical properties as the product described in part A.

3-Penten-1-ol (VI).—The ester V was converted to VI in 75% yield by reduction with lithium aluminum hydride according to the general directions of Nystrom and Brown.[6] This compound boiled at b. p. 129.9° (628 mm.), and had $n^{20}D$ 1.4327, d^{20}_4 0.8492, M_D (calcd.) 26.35, M_D (found) 26.34.

Anal. Calcd. for $C_5H_{10}O$: C, 69.72; H, 11.70. Found: C, 69.86; H, 11.68.

5-Bromo-2-pentene (VII).—A solution of 37 g. (0.43 mole) of VI and 11.5 g. of dry pyridine was placed in a 200-ml. distilling flask closed with a two-hole stopper carrying a dropping funnel and a low temperature thermometer. The side arm of the distilling flask and the opening of the dropping funnel were protected with calcium chloride drying tubes. The solution was chilled to −40° and 16.5 ml. (0.174 mole) of phosphorus tribromide was added dropwise from the dropping funnel at such a rate that the temperature was maintained between −40 and −30°. The flask was shaken during this addition which required about one and one-half hours. After the addition of the phosphorus tribromide the thermometer was replaced by a capillary, and the crude product was distilled at about 15 mm. from the solid residue. The crude bromopentene was washed with ice-cold sodium bicarbonate solution and then with ice water. After drying over Drierite the bromopentene was fractionated through a 12-plate glass spiral column to give 38.1 g. (60%) of 5-bromo-2-pentene, b. p. 121.7° (621 mm.) and had d^{20}_4 1.2715, $n^{20}D$ 1.4695, M_D (calcd.) 32.59, M_D (found) 32.68.

Anal. Calcd. for C_5H_9Br: Br, 53.62. Found: Br, 53.44.

Ethyl 2-Acetamido-2-cyano-5-heptenoate (VIII).—5-Bromo-2-pentene was condensed with ethyl acetamidocyanoacetate in 92% yield according to the general directions of Albertson.[5a] The crude product melted at 70–75° and was recrystallized with but little loss from an acetone–water mixture. An analytical sample melted at 81°.

Anal. Calcd. for $C_{12}H_{18}O_2N_2$: N, 11.76. Found: N, 11.50.

2-Amino-5-heptenoic Acid (IX).—Ten and one-half grams of VIII yielded 4.7 g. (75% yield) of IX when hydrolyzed according to the general method of basic hydrolysis for substituted ethyl acetamidocyanoacetates.[5a] The amino acid was purified by recrystallization from water, about 40 ml. of water for each gram of amino acid being required. An analytical sample had a decomposition point of over 260° when heated rapidly in a capillary.

Anal. Calcd. for $C_7H_{13}O_2N$: C, 58.49; H, 9.12; N, 9.75. Found: C, 58.23; H, 9.44; N, 9.65.

2-Benzamido-5-heptenoic Acid.—Two hundred and eighty-four milligrams (0.002 mole) of IX was benzoylated in the usual manner.[12] An analytical sample melted at 149.5–150°.

Anal. Calcd. for $C_{14}H_{17}O_3N$: N, 5.66. Found: N, 5.72.

Reduction of 2-Amino-5-heptenoic Acid (IX).—To a solution of 0.197 g. (0.00139 mole) of IX in 10 ml. of water and 1.38 ml. of *N* sodium hydroxide was added 11 mg. of platinum oxide catalyst. The quantitative reduction required slightly over one hour at room temperature and atmospheric pressure and 1.02 equivalents of hydrogen were taken up. The solution was filtered, and the reduced amino acid was benzoylated in the usual manner[12] without isolation.

The benzoyl derivative of the reduced IX was recrystallized from aqueous ethanol and melted at 133–134°. When this compound was mixed with α-benzamidoheptanoic acid[5a] (m. p. 133–134°), the melting point of the mixture was not depressed. This proved the skeleton structure of IX.

Ozonolysis of 2-Amino-5-heptenoic Acid (IX).—The position of the double bond in IX was established in the following manner: A solution of 0.213 g. of IX in 2 ml. of 0.5 *N* sulfuric acid was ozonized at 0°. The ozonized solution was steam distilled and the volatile aldehyde trapped in 0.5 ml. of ethanol at −10°. The ethanolic solution of the aldehyde was converted to the methone derivative according to the general method of Horning.[13]

The methone derivative obtained was recrystallized from aqueous ethanol and melted at 139.5–141°. When this compound was mixed with an authentic sample of the methone derivative of acetaldehyde (m. p. 139.5–141°), the melting point of the mixture was not depressed.

Microbiological Tests.—2-Amino-5-heptenoic acid was tested on the growth of three strains of *Escherichia coli* and strain 139 of *Saccharomyces cerevisiae*.[14] When this vinylene analog of methionine was added up to a concen-

(10) Pfeiffer, Engelhardt and Alfuss, *Ann.*, **467**, 158 (1928).

(11) Absolute methanol was used so that an exact equivalent of water could be provided for the reaction.

(12) Steiger, *J. Org. Chem.*, **9**, 396 (1944).

(13) Horning and Horning, *ibid.*, **11**, 95 (1946).

(14) The methods employed and the test organisms were the same as those previously described.[3]

tration of 20 mg. per 7.5 ml. of medium the growth of one strain of *E. coli* (unidentified strain T) was completely inhibited. The growth of this microörganism was inhibited to 50% of normal by 13 mg. per 7.5 ml. and completely inhibited by 20 mg. The addition of 0.4 microgram of methionine to 20 mg. of this new inhibitor nullified the inhibitory action and permitted growth to 50% of normal; larger amounts of methionine almost completely reversed the toxicity. Typical experimental results are listed in the table.

TABLE I

THE EFFECT OF 2-AMINO-5-HEPTENOIC ACID ON THE GROWTH OF *E. coli*, STRAIN T

Amount of 2-amino-5-heptenoic acid added per 7.5 ml., mg.	Growth of *E. coli* as turbidity, colorimeter units
0	100 (normal growth)
1	100
5	95
10	87
15	25
20	0
25	0
20 plus 0.01γ methionine	0
20 plus 0.1γ methionine	15
20 plus 0.5γ methionine	58
20 plus 1.0γ methionine	82
20 plus 5.0γ methionine	94
20 plus 10.0γ methionine	95

Preliminary results indicate that several other amino acids will also reverse the toxicity of 2-amino-5-heptenoic acid, but methionine seems the most active.

The growth of two other strains of *E. coli* (Unidentified strain N and the strain listed by the American Type Culture Collection as number 9723) and the yeast were either not at all or only slightly affected by 20 mg. per 7.5 ml. of medium.

These results indicate a metabolite antagonistic relationship between methionine and its vinylene analog, but the potency of the antagonist is very low.

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Summary

2-Amino-5-heptenoic acid, the vinylene analog of methionine, has been prepared by the acetamidocyanoacetate method. The structure of the compound has been established.

The new unsaturated amino acid in high concentrations inhibited the growth of one strain of *E. coli* but did not affect the growth of two other strains of *E. coli* nor one strain of yeast. Where the 2-amino-5-heptenoic acid was bacteriostatic, methionine counteracted the toxicity.

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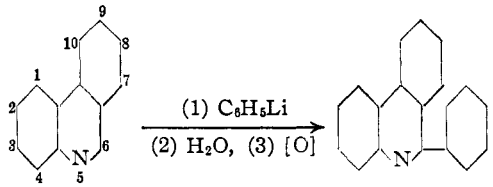
The Reaction of Aryllithium Compounds with 6-Arylphenanthridines

BY HENRY GILMAN AND R. DAVID NELSON

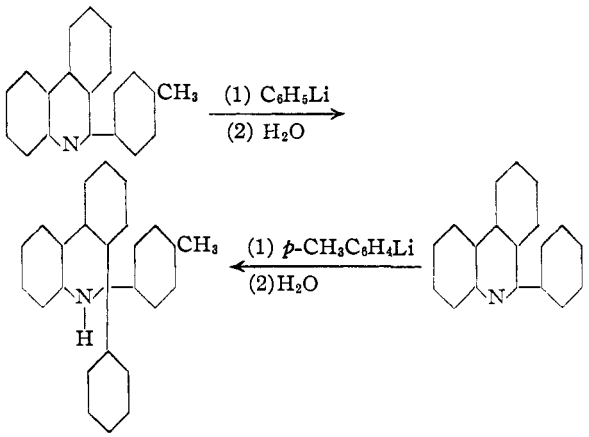
In the study of the addition of organolithium compounds to 2-arylquinolines,[1] 2-aryl-3,4-benzoquinolines (6-arylphenanthridines) were used. These compounds were chosen since 1,4-nuclear addition to the azomethine linkage is hindered because of the blocked 4-position of quinoline. This left only the possibility of 1,2-nuclear or 1,4-binuclear addition to the 6-arylphenanthridines.

Initially, to prove that phenyllithium added 1,2 to phenanthridine, 6-phenylphenanthridine was prepared by the cyclization of *o*-benzamidobiphenyl by means of $POCl_3$[2] in the presence of nitrobenzene, and this authentic specimen was compared with the compound isolated from the reaction of phenyllithium with phenanthridine. The compounds were found to be identical by means of the mixed melting points. Since phenanthridine is obtained in quite poor yields from *o*-formamidobiphenyl,[3] the preferred method for preparing 6-phenylphenanthridine is by the cyclization of *o*-benzamidobiphenyl.

On the basis of work[1] carried out previously in these Laboratories, an aryllithium compound



should add 1,2 to the azomethine linkage of a 6-arylphenanthridine giving, after hydrolysis, a



(1) Gilman and Gainer, THIS JOURNAL, **69**, 877 (1947).
(2) Morgan and Walls, *J. Chem. Soc.*, 294 (1945).
(3) Pictet and Hubert, *Ber.*, **29**, 1182 (1896).