Four solutions of the protein were made with a dilute salt solution (0.15 *M* sodium chloride) and were dialyzed against the same solution for two weeks at 4°. The *p*H of the protein solution at the end of the dialysis was 7.5. The solutions were then centrifuged for twenty minutes in a field 32,000 times that of gravity to remove any suspended dust, placed in a scattering cell, and the intensity of the scattered light compared with that scattered from carbon disulfide for a wave length of 5461 Å. Depolarization measurements were made. The refractive index increment was computed from the difference between the refractive indices of the solution and the solvent. Two of the solutions were slightly colored. Optical density measurements were made on these solutions with a spectrophotometer at the wave length used so that the magnitude of the scattering could be corrected for the true absorption. Concentrations were determined as described in the previous section.

The molecular weight of the dissolved protein is given by

$$M = \frac{\lambda^4 N_0}{2\pi^2 n^2 \left(\frac{\partial n}{\partial c}\right)^2 (c/i/ics_2)_{c\to 0}(I_0/ics_2)} \qquad (3)$$

which follows from (1) and (2)

$c/i/ics_2$ is the concentration of the solution divided by the ratio of the intensity of the light scattered from the solution to that scattered from carbon disulfide. This quantity is corrected for the depolarization of the scattered light and is extrapolated to zero concentration.

A plot of $c/i/ics_2$ *vs.* c is given in Fig. 1. The refractive index increment of this protein is 0.171. The depolarization of the solution is 0.032 and apparently is independent of concentration.

The value of the molecular weight which is calculated from light scattering measurements, 158,000 ± 10,000 compares favorably with previously published data from sedimentation and osmotic pressure studies.

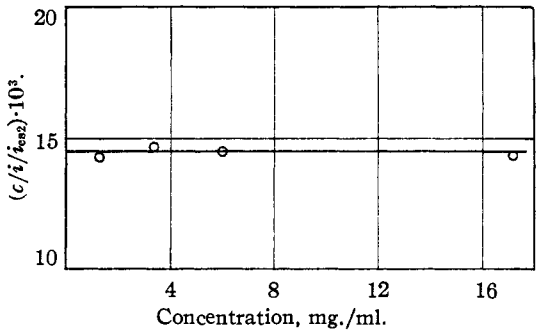

Fig. 1.—Light scattering data for purified rabbit antibody.

There is evidence, however, that the turbidity, depolarization, and refractive index of a protein solution change somewhat with *p*H and perhaps with salt content.[15,16] Not enough work has yet been done to understand how these changes should be taken into account when a value of the molecular weight is to be calculated.

We wish to express our thanks to Professor R. M. Badger and Dr. Stanley Swingle for their suggestions and assistance.

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Summary

Methods are described for the isolation and purification of rabbit antibody against *p*-azophenylarsonic acid. The purified preparations were electrophoretically homogeneous and similar to gamma globulin.

Molecular weight studies from osmotic pressure and light scattering data gave values of approximately 140,000 and 158,000, respectively.

(15) Unpublished work on solutions of human serum albumin, human serum globulin, and blood group A-Specific substance.

(16) S. Armstrong and others, THIS JOURNAL, **69**, 1747 (1947).

PASADENA, CALIF. RECEIVED FEBRUARY 6, 1948

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

The Inhibition of Microbiological Growth by Allylglycine, Methallylglycine and Crotylglycine[1,2]

BY KARL DITTMER, HARLAN L. GOERING,[3] IRVING GOODMAN AND STANLEY J. CRISTOL

The interchange of an aromatic sulfide for a vinylene group in thiamin,[4] nicotinic acid[5] and phenylalanine[6] has led to the formation of specific metabolite antagonists. Because of these effects and because of the theoretical basis for the similarity of the vinylene group (—CH=CH—) and a divalent sulfur atom (—S—),[7] it has been possible to assume that substituting a sulfur for a vinylene group or *vice versa* may be the basis for the preparation of one type of specific metabolite antagonist.[8,9,10]

Since all of these examples are of aromatic com-

(1) This work was supported in part by a research contract with the Office of Naval Research.

(2) This paper, which is Number 1 of the Unsaturated Amino Acid Series, was presented in part at the 111th meeting of the American Chemical Society at Atlantic City, April, 1947.

(3) American Cyanamid Company Fellow.

(4) Woolley and White, *J. Exp. Med.*, **78**, 489 (1943).

(5) Erlenmeyer, Block and Kiefer, *Helv. Chim. Acta*, **25**, 1066 (1942).

(6) Dittmer, Ellis, McKennis and du Vigneaud, *J. Biol. Chem.*, **164**, 761 (1946).

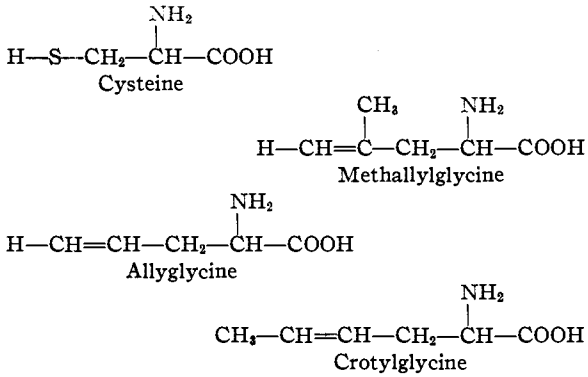
(7) Neuhaus, *Die Chemie*, **57**, 33 (1944).

(8) Wagner-Jauregg, *Naturwissenschaften*, **31**, 335 (1943).

(9) Woolley, *Physiol. Rev.*, **27**, 308 (1947).

(10) Roblin, *Chem. Rev.*, **38**, 255 (1946).

pounds, it seemed desirable to determine whether similar changes in aliphatic compounds containing sulfur or vinylene groups would likewise result in the formation of antagonists. To test this we chose to make the vinylene analogs of the sulfur-containing amino acids. In this paper we wish to report on the growth-inhibitory properties of the vinylene analogs of cysteine, allylglycine and the two closely related unsaturated amino acids methallylglycine and crotylglycine. The structural relationships of these unsaturated amino acids to cysteine are illustrated by the following chemical formulas. The position of the double bond in these compounds is inferred from their syntheses.



We have determined the amounts of these unsaturated amino acids required to inhibit the growth of three strains of *Escherichia coli* and strain 139 of *Saccharomyces cerevisiae*. The effects of various amino acids and vitamins on the toxicity of these unsaturated amino acids are now being investigated.

Experimental

Preparation of Unsaturated Amino Acids.—The syntheses of the unsaturated amino acids by procedures similar to those reported by Albertson[11] will be described in a separate paper.[12] The starting materials were allyl chloride, methallyl chloride and crotyl chloride from which were obtained *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine, respectively.

Inhibition of Growth of *Escherichia coli*.—Three strains of *E. coli* were used in this study; one was an unidentified strain[13] which will be referred to as strain N; the second is listed by the American Type Culture Collection as number 9723; and the third was kindly supplied by Dr. William Shive; it will be referred to as strain T. For these tests the organisms were grown for sixteen hours in the synthetic medium described by MacLeod.[14] Best results were obtained when the medium was prepared daily and the *p*H carefully adjusted to 7.3. Six and five-tenths milliliters of medium was added to the various addenda dissolved in a volume of 1.0 ml. in the assay tubes (20 × 150-mm.). The tubes were capped by aluminum caps and autoclaved for five minutes at 15 pounds pressure and then inoculated with 1 drop of a pure *E. coli* suspension. The inoculum was prepared and handled as described previously,[6] except that during these experiments each culture was washed once with sterile saline before it was diluted for the inoculum.

The effects of increasing amounts of *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine on the growth of three strains of *E. coli* are illustrated by the curves plotted in Figs. 1, 2 and 3, respectively. The amounts of each

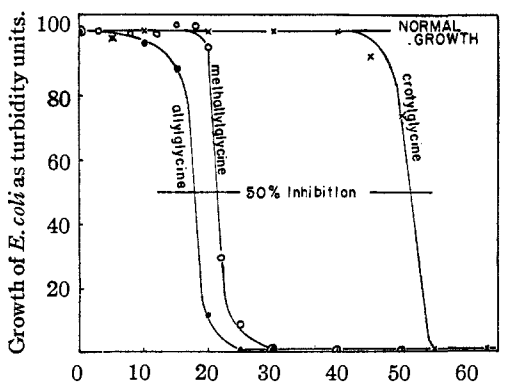


Fig. 1.—The inhibition of the growth of *E. coli*, unidentified strain N, by *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine.

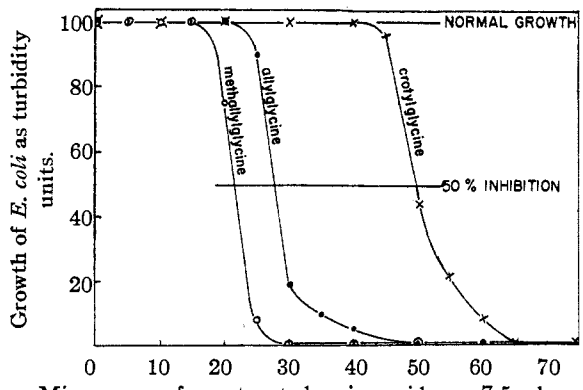


Fig. 2.—The inhibition of the growth of *E. coli*, ATCC 9723, by *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine.

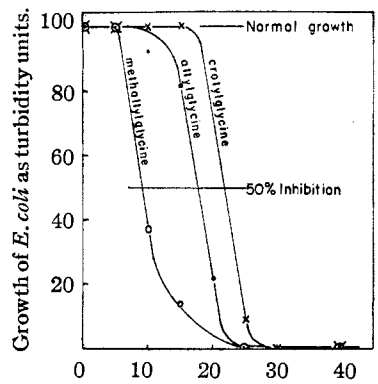


Fig. 3.—The inhibition of the growth of *E. coli*, unidentified strain T, by *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine.

(11) Albertson, THIS JOURNAL, **68**, 450 (1946).

(12) Goering, Cristol and Dittmer, *ibid.*, in press.

(13) This culture of *E. coli* was originally obtained from Professor James Neill of the Department of Bacteriology, Cornell University Medical College, and is the same as that used in the work previously reported.[6]

(14) MacLeod, *J. Exp. Med.*, **72**, 217 (1940).

inhibitor required to reduce the growth of each strain to 50% of normal and to complete inhibition are tabulated in Table I. The data of Table I represent the averages obtained from a large number of determinations. From these data and the curves of Figs. 1, 2 and 3, it can be seen that allylglycine and methallylglycine have very similar inhibitory activity, but crotylglycine is less active for all three strains of *E. coli*.

TABLE I

DATA ILLUSTRATING THE RELATIVE EFFECTIVENESS OF THREE UNSATURATED AMINO ACIDS AS MICROBIAL GROWTH INHIBITORS

Microörganism	Amounts of unsaturated amino acid required[a] per 7.5 ml. of medium: *dl*-Allylglycine γ	*dl*-Methallylglycine γ	*dl*-Crotylglycine γ
E. coli, strain N			
for 50% Inhibition[a]	16[b]	22	50
for 100% Inhibition[a]	20–40	25–40	50–80
E. coli, strain 9723			
for 50% Inhibition	27	20	50
for 100% Inhibition	30–50	25–40	50–70
E. coli, strain T			
for 50% Inhibition	17	10	23
for 100% Inhibition	20–30	15–25	30–40
S. cerevisiae, strain 139			
for 50% Inhibition	6	55–100	700–1000
for 100% Inhibition	50	>1 mg.	>4 mg.

[a] The amounts required for complete inhibition vary much more than the amounts required for 50% inhibition. The values for 50% inhibition are averages while the range of amounts required for 100% inhibition are given. [b] For a short period of time during these tests, between 30 and 50γ were required for 50% inhibition of normal growth.

Allyl chloride, allyl alcohol and allylurea were tested at a concentration of 1 mg. per 7.5 ml. of medium and were found to be either completely inactive or only slightly inhibitory.

Inhibition of the Growth of *Saccharomyces cerevisiae*.—The technique followed in the yeast growth experiments was similar to that described previously.[6] The medium employed was that used by Snell, Eakin and Williams'[15] except for the level of aspartic acid, which was increased to 2 g. per 10 liters of medium.

The effects of increasing amounts of *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine on the growth of *S. cerevisiae* are shown in Fig. 4. The amounts of the inhibitors required to produce 50 and 100% inhibition of normal growth are also listed in Table I. From the curves of Fig. 4 and the data of Table I it is evident that of the three unsaturated amino acids, allylglycine is by far the best yeast growth inhibitor and crotylglycine is the poorest.

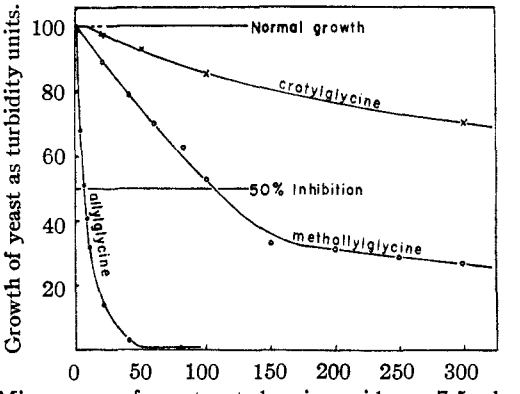


Fig. 4.—The inhibition of the growth of *S. cerevisiae*, strain 139, by *dl*-allylglycine, *dl*-methallylglycine and *dl*-crotylglycine.

Acknowledgment.—The authors are greatly indebted for the very excellent assistance received from Mrs. Charmion McMillan, Mrs. Hester P. McNulty and Mrs. Virginia Janda. The authors also wish to thank Mr. V. C. Irvine and Mr. W. S. Thornhill of the Shell Chemical Corporation for generous supplies of allyl chloride and methallyl chloride.

Summary

As possible antagonists for cysteine three unsaturated alpha amino acids, allylglycine, methallylglycine and crotylglycine, were tested for their growth inhibition of three strains of *Escherichia coli* and strain 139 of *Saccharomyces cerevisiae*. Allylglycine and methallylglycine were almost equally effective inhibitors of *E. coli*, producing complete inhibition of growth for sixteen hours in very small concentrations. Crotylglycine was less active on the growth of all three strains of *E. coli*. For *S. cerevisiae*, allylglycine was the most effective inhibitor of the three unsaturated amino acids and crotylglycine showed only low inhibitory action.

(15) Snell, Eakin and Williams, THIS JOURNAL, **62**, 175 (1940).

BOULDER, COLORADO RECEIVED FEBRUARY 18, 1948