1:1 mixture of adducts A and B. The less strained compound **9**-(*Z*) shows greater selectivity and gives a single adduct with **10**.

It is remarkable that **3**-(*E*) does not rapidly isomerize to its geometric isomer **3**-(*Z*). Thus, **3**-(*E*) must lie in an energy well of sufficient depth to prevent isomerization at a rate competitive with its reactions with **10** and *tert*-butyl alcohol. Other examples of intermediates with trans double bonds in six-membered rings are bicyclo-[2.2.1]hept-1-ene,[10] adamantene,[11] and the *trans*-cyclohexenones[12] formed in photochemical reactions.

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Supplementary Material Available. A listing of atomic parameters and calculated structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-6121.

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(11) (a) D. Lenoir, *Tetrahedron Lett.*, 4049 (1972); (b) D. Grant, M. A. McKervey, J. J. Rooney, N. G. Samman, and G. Step, *J. Chem. Soc., Chem. Commun.*, 1186 (1972); (c) J. E. Gano and L. Eisenberg, *J. Amer. Chem. Soc.*, **95**, 972 (1973).
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(13) NDEA Graduate Fellow, 1969–1970; National Institutes of Health Graduate Fellow, 1970–1973.

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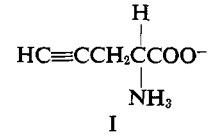
Acetylenic Enzyme Inactivators. Inactivation of γ-Cystathionase, *in Vitro* and *in Vivo*, by Propargylglycine

Sir:

It has previously been shown that the enzyme β-hydroxydecanoylthiolester dehydrase is inactivated by a 3-decynoylthiolester;[1] the basis for inactivation derives from enzymatic abstraction of a C_2 hydrogen as a proton, followed by rearrangement to a conjugated allene which is attacked by a nucleophilic active site histidine.[2]

It occurred to us that acetylenic substrate analogs may be generally useful in the inactivation of enzymes which abstract carbon-bound hydrogens as protons at a position adjacent to the acetylenic linkage. Such inactivations are particularly attractive since their specificity is not based merely on structural similarity to substrates but also upon the mechanism of the catalytic process; the acetylenic linkage is made chemically reactive as a result of specific enzymatic action on the inhibitor molecule. These inactivators are therefore not only useful as active site directed reagents for purified enzymes but also as agents for *in vivo* inactivation of the target enzymes.

We have synthesized and tested a number of acetylenic enzyme substrates[3,4] and now report the irreversible inactivation of the pyridoxal phosphate dependent rat liver enzyme, γ-cystathionase, both *in vitro* and *in vivo* by D,L-2-amino-4-pentynoic acid (propargylglycine) (I).[5] γ-Cystathionase catalyzes the elimination of a



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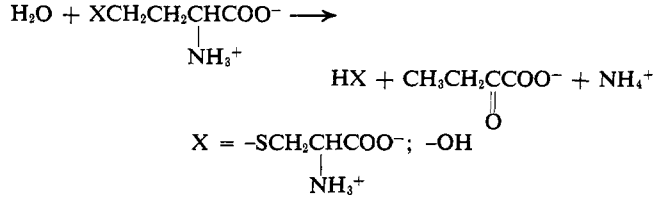
number or substituents from the γ carbon of susceptible amino acid substrates.[6]



Incubation of highly purified γ-cystathionase[7] with D,L-propargylglycine produces a time-dependent pseudo-first-order irreversible loss of catalytic activity. At 6.6×10^{-5} *M* inactivator,[8] the loss of enzymatic activity has a $t_{1/2}$ of 2 min at 25°. Enzymatic activity could not be recovered by dialysis for 12 hr or by gel filtration of the enzyme through Sephadex or by addition of pyridoxal phosphate, suggesting a covalent modification of the enzyme. In support of this idea, incubation of [2-^{14}C]propargylglycine with the enzyme followed by gel filtration on a Sephadex G-25 column resulted in incorporation of radioactivity into the protein peak. One mole of ^{14}C inactivator is incorporated per 80,000 g of protein.[9] (A control experiment in which the bound pyridoxal coenzyme had been reduced by borohydride treatment prior to addition of [^{14}C]-propargylglycine resulted in no radioactivity associated with the protein after gel filtration.) Radioactivity was not removed from the ^{14}C-labeled protein by subsequent dialysis against 6 *M* urea or 10^{-3} *M* HCl. These experiments suggest that propargylglycine inactivates γ-cystathionase by covalent modification of

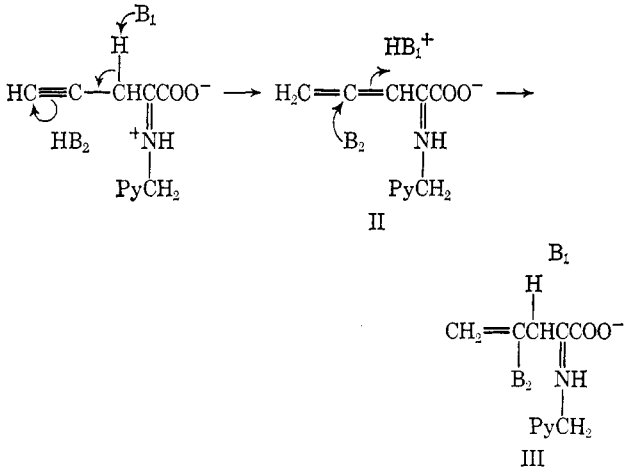
(1) G. M. Helmkamp, R. R. Rando, D. J. H. Brock, and K. Bloch, *J. Biol. Chem.*, **243**, 3229 (1968).
(2) K. Bloch, *Accounts Chem. Res.*, 2, 193 (1969); K. Bloch, *Enzymes, 3rd Ed.*, **5**, 441 (1971).
(3) C. T. Walsh, A. Schonburnn, O. Lockridge, V. Massey, and R. H. Abeles, *J. Biol. Chem.*, **247**, 6004 (1972).
(4) R. Hevey and R. H. Abeles, unpublished experiments; W. Washtien and R. H. Abeles, unpublished experiments.
(5) DL-Propargylglycine was synthesized by the method of A. C. A. Jansen, R. J. M. Weustink, K. E. T. Kerling, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **88**, 819 (1969) and gave a satisfactory melting point (185–187°), nmr spectra, and chromatographic mobility compared with a sample of authentic L-propargylglycine kindly provided by Dr. H. R. Kaback, The Roche Institute for Molecular Biology, Nutley, N. J.
(6) L. Davis and D. E. Metzler, *Enzymes, 3rd Ed.*, **7**, 33 (1972).
(7) γ-Cystathionase, purified and assayed according to D. Greenberg, *Methods Enzymol.*, **5**, 936 (1962).
(8) Inactivation experiments utilized 3–10 units of γ-cystathionase, 4×10^{-5} *M* 2-mercaptoethanol, 6×10^{-3} *M* EDTA, and 8×10^{-7} *M* potassium phosphate (pH 7.5). To this was added varying amounts of propargylglycine or other inhibitors at 25°. At intervals, aliquots were withdrawn and used to assay the remaining enzymatic activity.
(9) A minimum molecular weight of 85,227 has been reported: J. Loiselet and F. Chatanger, *Biochem. Biophys. Acta*, **230**, 434 (1971).

an active site residue. The exact nature of the linkage is now under investigation.

Studies were conducted with propargylglycine in intact mice. Groups of three mice were injected intraperitoneally with 2 and 5 μmol of D,L-propargylglycine, respectively, while three control mice received the same volume of saline solution. After 24 hr, the mice were sacrificed, and the livers were removed and assayed for γ-cystathionase. The livers from the treated mice had 10–20 and 0%, respectively, of the enzyme levels from control livers. Propargylglycine inactivation of the enzyme *in vivo* indicates induction of a condition similar to that found in the genetic defect cystathionuria[10] in which liver γ-cystathionase is absent or defective.

An essential feature of the mechanism of action of γ-cystathionase is the abstraction of a proton from the β position.[6] We, therefore, tentatively propose the mechanism in Scheme I for the inactivation by propargylglycine. The allene formed after proton abstraction is in conjugation with the ketimine and should be capable of ready Michael addition by an enzyme active site nucleophile to produce covalent labeling of the active site (III). (B_1 and B_2 are active site basic groups and PyCHO is pyridoxal-P.)

Scheme I



In the proposed mechanism, the acetylenic linkage is essential. This is consistent with finding that allylglycine (Sigma Chemical Co.) (2-amino-4-pentenoic acid) does not inactivate γ-cystathionase. Also, only those pyridoxal-dependent enzymes which abstract substrate β-hydrogens should catalyze their own destruction by propargylglycine. Preliminary experiments[11] with threonine deaminase and transaminase show no inactivation by propargylglycine.

In summary, then, we have shown for the first time that an acetylenic amino acid will irreversibly inactivate a pyridoxal-P dependent enzyme, rat liver γ-cystathionase, *in vitro* and *in vivo*, presumably *via* a reactive allene intermediate. This extends our recent observations that several flavine coenzyme-dependent enzymes[3,12] are irreversibly inactivated by acetylenic substrates. It confirms the expectation that, in general, acetylenic substrate analogs should be potent and specific irreversible inactivators in enzymatic catalyses where the carbon-bound hydrogen adjacent to the acetylenic moiety is abstracted as a proton.

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Highly Specific Enzyme Inhibitors. Inhibition of Plasma Amine Oxidase

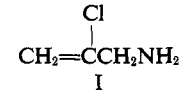
Sir:

We have recently suggested[1] that substrate analogs containing an acetylenic group adjacent to a carbon-bound hydrogen should be generally useful as enzyme inactivators when this hydrogen is abstracted as a proton during catalysis.

Based upon earlier work[2] from the laboratory of Bloch, it was suggested that this inactivation proceeds through rearrangement of the acetylene to the allene which then reacts with a nucleophile at the active site. Results obtained in our studies with beef plasma amine oxidase[3] suggest that an early step in the oxidation involves proton abstraction[4] from the carbon atom which is oxidized. Therefore, this enzyme might be susceptible to inactivation by appropriate acetylenic substrates.

As reported below, we have found that 1-amino-2-alkynes are capable of inactivating plasma amine oxidase. Furthermore, if the proposed mechanism of inactivation is correct, other substrates which can form allenes during the catalytic process should also inactivate the enzyme.

2-Chloroallylamine (I), which could undergo allene



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formation by expulsion of chloride after the C-1 hydrogen is abstracted as a proton, is found to inactivate the enzyme irreversibly.

(1) R. H. Abeles and C. T. Walsh, *J. Amer. Chem. Soc.*, **95**, 6124 (1973).
(2) M. Morisaki and K. Bloch, *Biochemistry*, **11**, 309 (1972).
(3) Plasma amine oxidase is a nonflavoprotein oxidase, which catalyzes the oxidation of certain primary amines according to the equation $RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$. The enzyme is believed to contain Cu^{II} and pyridoxal phosphate: H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **237**, 3077 (1962); **238**, 2669 (1963). We feel, however, that the presence of pyridoxal phosphate is not firmly established. The enzyme used in these studies was purified and assayed according to the procedure of H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **237**, 1511 (1962).
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(10) G. W. Frimpter, *Science*, **149**, 1095 (1965); F. C. Brown and P. H. Gordon, *Biochim. Biophys. Acta*, **230**, 434 (1971).
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