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Suicide Enzyme Inactivators

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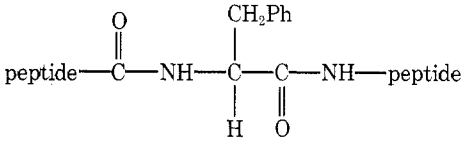
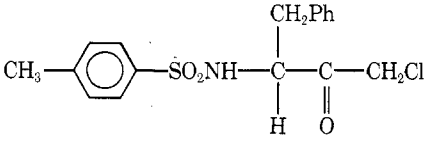
Active-site-directed inhibitors have been used extensively in biochemistry and have been valuable in identifying functional groups at enzyme active sites.[1] The design of these inhibitors is based upon the attachment of a chemically reactive group, generally an electrophile, e.g., an α-halocarbonyl group, to a molecule which has a structural feature resembling that of the enzyme's normal substrate.

This structural similarity enables the molecule to become bound to the active site of the enzyme and the labile functional group allows it to react with any suitably located nucleophile of the enzyme's active site. The resulting covalent adduct is catalytically inactive and stable. The best known example is TPCK, an inhibitor of chymotrypsin.

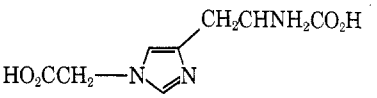


a "normal" chymotrypsin substrate



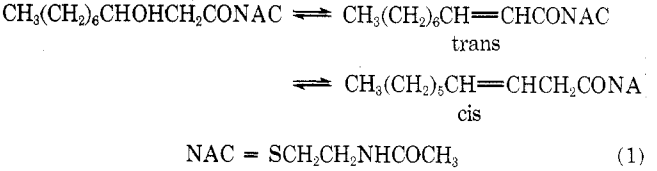
TPCK

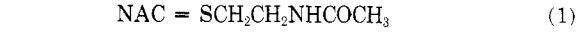
Inhibitors of this type have provided important information about the active sites of a large number of enzymes. For instance, the presence of a histidine residue at the active site of chymotrypsin was first inferred from the fact that enzyme inhibited by TPCK gave upon hydrolysis and oxidation a modified histidine fragment of the following structure:[2]



A disadvantage to the use of these inhibitors is that, because they are intrinsically reactive molecules, they might react nonspecifically (i.e., at a site other than a binding site) with a given enzyme or react with molecules other than the target enzymes. The latter event could be particularly undesirable when one uses these inhibitors *in vivo*.

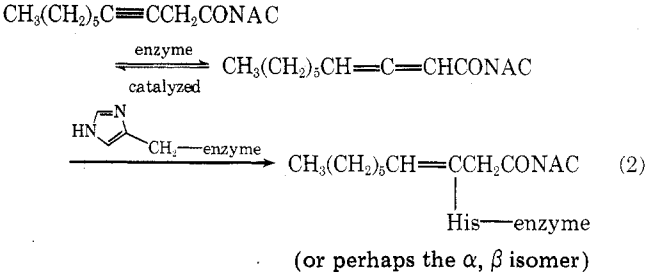
Work by K. Bloch and his associates brought to our attention a principle by which more selective active-site-directed inhibitors could be designed. Bloch has studied the mechanism of action of β-hydroxydecanoyl thioester dehydrase, an enzyme which catalyzes the following reactions:





(1)

It was found that $H_3C(CH_2)_5C{\equiv}CCH_2COSCH_2CH_2NHCOCH_3$ effectively inactivates the enzyme and becomes covalently attached to a histidine residue at the active site.[3] The following mechanism was proposed for the inactivation:



(2)

(or perhaps the α, β isomer)

(1) E. Shaw, *Enzymes, 3rd Ed.*, **1**, 91 (1970).
(2) K. J. Stevenson and L. B. Smillie, *J. Mol. Biol.* **12**, 937 (1965).
(3) K. Bloch, *Enzymes, 3rd Ed.*, **5**, 441 (1971); M. Morisaki and K. Bloch, *Biochemistry*, **11**, 309 (1972).

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The inactivation of β-hydroxydecanoyl thioester dehydrase is probably somewhat more complex than it appears initially. It is likely that the ability of the enzyme to convert the acetylene to the allene is not only dependent upon the removal of the α proton but probably also involves the addition of a proton to the γ position. The inhibitor functions, therefore, because the enzyme can interact with both the α and the γ positions, a function which is essential for the α,β → β,γ isomerization of the double bond (eq 1). Crotonase, a mammalian enzyme which carries out a very similar dehydration reaction but does not catalyze the α,β → β,γ isomerization of the double bond, is not inactivated by this inhibitor, possibly because there is no base present to protonate the γ position. Very severe structural requirements must, therefore, be met in order to achieve inactivation.

The inhibition discussed above has the following important properties which distinguish it from the classical active-site-directed reagent. (1) The inhibitor itself is a relatively unreactive compound and becomes reactive only after interaction with the enzyme's active site. (2) Conversion to the reactive form depends upon the specific catalytic capabilities of the active site; i.e., some part of the enzyme's normal catalytic process is required. This feature adds a dimension to the specificity of an active-site-directed inhibitor in that the inactivation process depends upon both binding of the molecule and catalytic conversion to a reactive species.[4] Nonspecific interactions might still occur, however, if enzymes other than the target catalyze the production of a reactive species[5] or if a reactive species escapes from an enzyme before reacting with it.

We shall refer to inactivators of this type as suicide inactivators. The purpose of this Account is to summarize our experimental efforts to design and utilize suicide inactivators. The examples which we cite will illustrate how we have used inactivators of this type to probe enzyme structure, enzyme mechanism, and enzyme physiological function. Previously others have described examples of suicide inactivation. The chemistry of suicide inhibition as well as examples of suicide inhibitors have been reviewed by Rando and by Walsh.[6]

Identifying Suicide Inactivation

Before discussing additional examples of suicide inhibitors, we will briefly consider the experimental criteria by which these inhibitors can be identified. The most definitive way requires the complete characterization of the enzyme–inhibitor adduct, i.e., identification of the functional group on the enzyme which is labeled and identification of the structural changes which the inhibitor has undergone. Such an investigation is time consuming and frequently very difficult due to the limited amounts of enzyme which are generally available.

However, relatively simple kinetic experiments can give reasonable assurance that an inhibitor is acting as a suicide inhibitor before an extensive investigation is undertaken. In examining a potential suicide inhibitor, one must show whether loss of enzyme activity is a time-dependent, first-order process. Time dependence provides good but not definitive evidence that covalent modification has taken place. Demonstration that the loss of enzyme activity at constant inactivator concentration is first order provides evidence that inactivation occurs before the inactivator is released from the enzyme, a fundamental purpose of suicide inhibition. This distinguishes the inactivation process from cases in which the enzyme converts a substrate to a reactive species which is released and then later reacts with the enzyme from solution.

This latter possibility can be further tested by making a second addition of enzyme to the reaction after the enzyme originally reacted has become inactivated. If inactivation again occurs at the same rate as that originally observed, it is highly improbable that a product which has accumulated in the reaction mixture is responsible for the inactivation. For instance, a recent report of the inactivation of D-amino acid oxidase by propargylglycine may well be a case of this latter type.[7] First-order loss of activity has not been observed for the inactivation. Furthermore, addition of nucleophiles reduces the rate of inactivation.[8] It is reasonable to suppose that the process involves oxidation of propargylglycine to a reactive species which is released into solution and later reacts with the enzyme in some manner, not necessarily involving the active site, to cause inactivation.

The rate of inactivation should be proportional to the inhibitor concentration at low concentrations but independent of it at high concentrations. Also, the rate of inactivation at a given inhibitor concentration should diminish as the substrate concentration is raised. These two kinetic phenomena, saturation kinetics and substrate protection against inactivation, are necessary consequences of the involvement of the enzyme's active site in the inactivation process.

It is also important to show that enzyme inactivation is irreversible. This can be done by showing that extensive dialysis or passage through Sephadex columns does not lead to reactivation of the enzyme. Additional and very convincing evidence for covalent labeling of the enzyme can be obtained by using radioactively labeled inactivators to show that radioactivity becomes irreversibly associated with protein. The use of these inactivators allows the stoichiometry of labeling to be established. One generally expects 1 mol of inactivator to be incorporated per mol of enzyme active site inactivated. In the discussion that follows, the inactivators that we have studied in our laboratory generally meet all of the criteria outlined above, with the exception that in some cases studies with isotopically labeled inactivators have not, as yet, been carried out.

In some special cases, e.g., the flavin-dependent oxidases and plasma amine oxidase discussed below, we were able to use an additional criterion to demonstrate

(4) It is important to realize that even when a compound meets the above two requirements there is no assurance that it will cause inactivation. Bloch and Miesowicz have recently presented a particularly good example of such a situation (F. M. Miesowicz and K. E. Bloch, *Biochem. Biophys. Res. Commun.*, **65**, 331 (1975)). They have purified an enzyme which catalyzes the conversion of 3-acetylenic fatty acyl thioesters to 2,3-dienoyl fatty acyl thioesters without being inactivated.

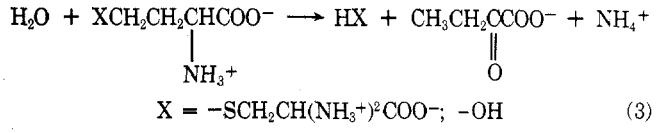
(5) For instance, vinyl glycolate is oxidized by D-lactate dehydrogenase of *E. coli* membrane vesicles to the 2-keto-3-butenoate. This keto acid inactivates enzyme I of the phosphotransferase system and abolishes active transport (C. T. Walsh and H. R. Kaback, *J. Biol. Chem.*, 248, 5456 (1973)).

(6) R. R. Rando, *Science*, **185**, 320 (1974); R. R. Rando, *Acc. Chem. Res.*, **8**, 281 (1975); R. R. Rando, *Biochem. Pharmacol.*, **24**, 1153 (1975); C. T. Walsh, "Horizons in Biochemistry and Biophysics", in press.

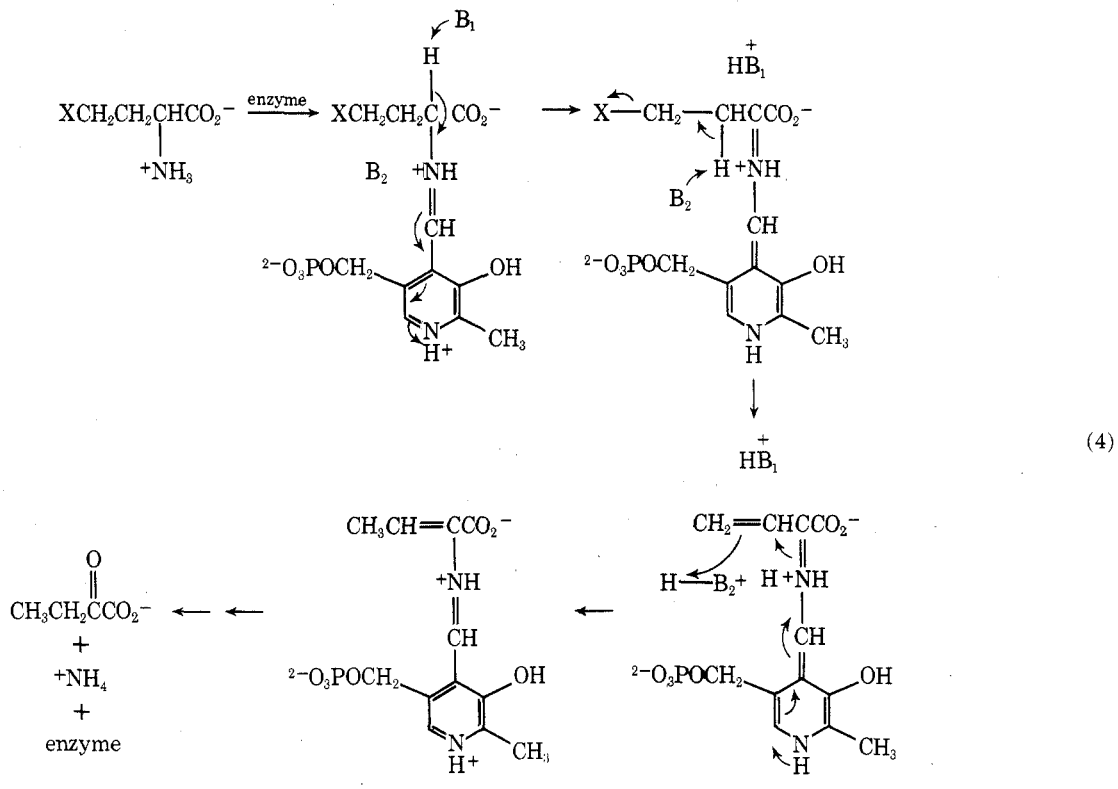
(7) H. Horikke, Y. Nishina, Y. Miyake and T. Yamano, *J. Biochem. (Tokyo)*, 78, 57 (1975).

(8) C. T. Walsh, personal communication.

suicide inhibition. Many of the compounds tested were not only inhibitors but also substrates for these enzymes; i.e., they were oxidized. It was then possible to determine the number of catalytic events which occurred before the enzymes were inactivated. It was found that this number was independent of substrate concentration. This indicates that inhibition and catalysis have the same K_m and indicates that both events occur from the same binding site and further suggests that inactivation and the catalytic process may have additional stages in common. This type of approach has been discussed by Meloche.[9]



(3)



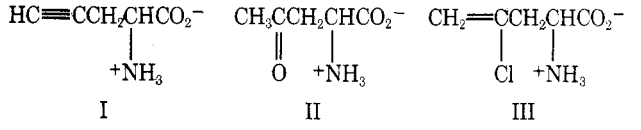
(4)

(B_1 and B_2 are nucleophilic groups of the enzyme)

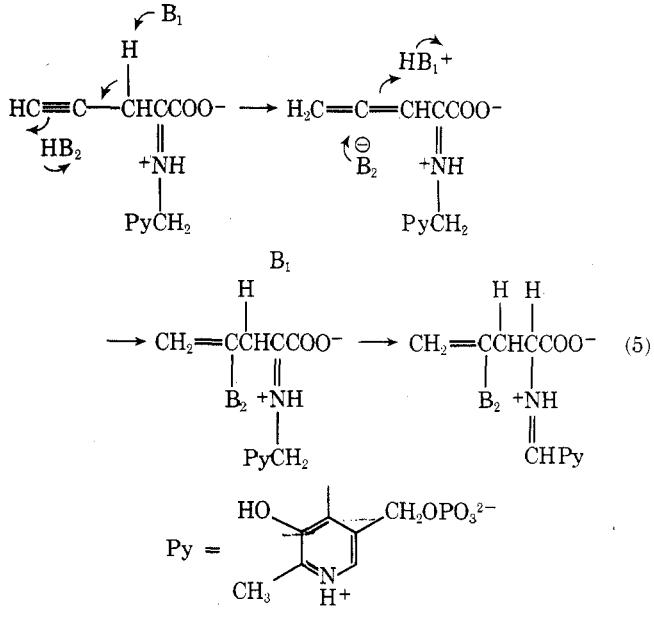
Pyridoxal-Dependent Enzymes

The ability of certain enzymes to abstract a proton is a property which can be utilized in the design of suicide inhibitors. Enzymes which utilize pyridoxal phosphate as a coenzyme have become masters in the art of proton abstraction and are, therefore, obvious potential victims of suicide inhibitors.[10] A particularly interesting enzyme is γ-cystathionase, which carries out the reaction shown in eq 3.[11] The mechanism by which this enzyme probably operates is illustrated in eq 4. The enzyme has the ability to catalyze the abstraction of both the α and the β hydrogens of the substrate, and probably also facilitates the leaving of group X by protonation.

Propargylglycine (I) is a suicide inhibitor for this enzyme.[12] A probable mechanism is shown by eq 5. When this inactivation was examined with [1-^{14}C]propargylglycine, 1 mol of inactivator was incorporated per mol of enzyme. After acid hydrolysis of the inactivated enzyme 2-amino-4-ketopentanoic acid (II) was isolated.[13] Formation of this compound is consistent with the enzyme–inhibitor adduct proposed in eq 5.



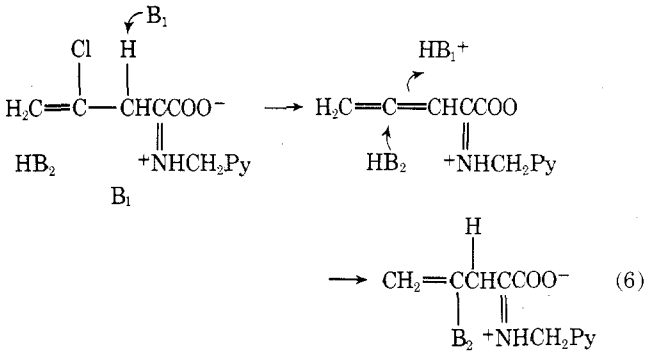
I II III



(5)

Compound III[14] also inactivates the enzyme, probably through the mechanism shown in eq 6. Here the

(9) H. P. Meloche, M. A. Luczak, and J. M. Wurster, *J. Biol. Chem.*, **247**, 4186 (1972).
(10) E. E. Snell and S. J. DiMari, *Enzymes, 3rd Ed.*, **2**, 335 (1970).
(11) L. Davis and D. E. Metzler, *Enzymes, 3rd Ed.*, **7**, 33 (1972).
(12) C. T. Walsh and R. H. Abeles, *J. Am. Chem. Soc.*, **95**, 6124 (1973).
(13) W. Washtien and R. H. Abeles, unpublished observations.



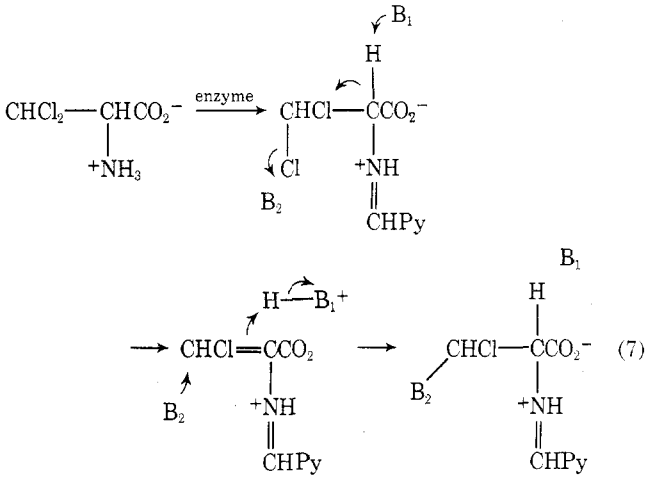
(6)

intermediate allene can be formed by the elimination of HCl, rather than through the rearrangement of an acetylenic bond. Both of these inactivations take advantage of the fact that the enzyme can catalyze abstraction of substrate β protons.

Compounds IV and V inactivate γ-cystathionase and

IV V

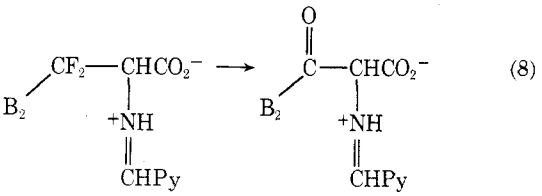
meet the criteria for suicide inactivators outlined above.[15] (Isotopically labeled compounds have not yet been prepared.) Although we have not yet determined the modes of action, we consider the mechanism of eq 7 to be likely for the inactivation by IV. The enzyme



(7)

catalyzes the elimination of HCl, and the resulting unsaturated imine undergoes a Michael-type addition. Such an addition would be facilitated by the electrophilic substituent chlorine or fluorine.

That this enzyme can catalyze elimination reactions as proposed in eq 7 is established by its ability to catalyze the conversion of β-chloroalanine and β-fluoroalanine to pyruvate.[15,16] However, in these cases a nonactivated Michael acceptor is formed which is not sufficiently electrophilic to react with a nucleophile at the active site, and consequently no inactivation occurs. In the case of trifluoroalanine (V) the initial adduct might hydrolyze to afford a stable acyl derivative (eq 8). This process would be analogous to that proposed by Santi to account for the inactivation of thymidylate synthetase by trifluorothymidylic acid.[17]



(8)

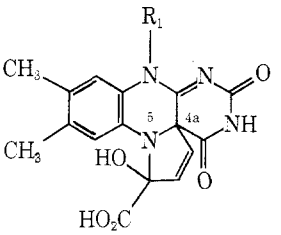
Trifluoroalanine (V) acts as a suicide inhibitor for a number of other pyridoxal enzymes: alanine racemase, tryptophanase, tryptophane synthetase, β-cystathionase, and pyruvate–glutamate transaminase. Since all of these enzymes can, in addition to their normal catalytic process, carry out elimination reactions, mechanisms similar to those shown in eq 7 and 8 may well account for their inactivation.

Flavin-Dependent Enzymes

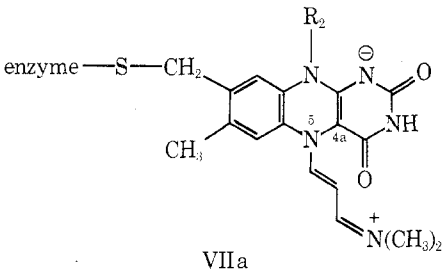
Suicide inactivators have also been used with other enzymes which catalyze proton abstraction. Mechanistic studies have suggested that an early step in the reactions catalyzed by several flavoproteins may involve proton abstraction.[18] Lactic acid oxidase has been inactivated with 2-hydroxy-3-butynoic acid (VI)[19] and mitochondrial amine oxidase with *N,N*-dimethylpropargylamine (VII).[20] At least three other flavin-dependent enzymes have been inactivated with this compound.[21]

These inactivators react with the enzyme-bound flavin to form the adducts VIa and VIIa, respectively.

VI VII



VIa



VIIa

R_1 = side chain of FMN
R_2 = side chain of FAD

In these cases we think it is unlikely that the isomeric allene is the reactive species because it would not be particularly prone to nucleophilic addition. However,

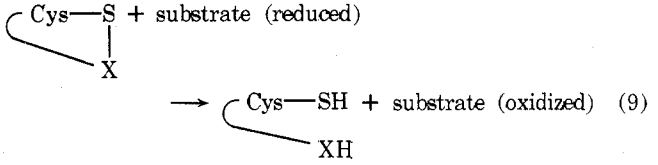
(14) L. Brodsky, A. L. Maycock, and R. H. Abeles, unpublished observations.
(15) R. Silverman and R. H. Abeles, unpublished observations.
(16) W. Washtien, unpublished observations.
(17) D. V. Santi and T. T. Sakai, *Biochemistry*, **10**, 3598 (1971).
(18) C. T. Walsh, O. Lockridge, V. Massey, and R. H. Abeles, *J. Biol. Chem.*, **248**, 7049 (1973); C. T. Walsh, E. Krodel, V. Massey, and R. H. Abeles, *ibid.*, **248**, 1946 (1973); C. T. Walsh, A. Schonbrunn and R. H. Abeles, *ibid.*, **246**, 6855 (1971).
(19) C. T. Walsh, A. Schronbrunn, O. Lockridge, V. Massey, and R. H. Abeles, *J. Biol. Chem.* **247**, 6004 (1972); A. Schonbrunn, R. H. Abeles, C. T. Walsh, S. Ghisla, H. Ogata, and V. Massey, submitted for publication.
(20) A. L. Maycock, R. H. Abeles, J. I. Salach, and T. P. Singer, *Biochemistry*, **15**, 114 (1976).
(21) T. H. Cromartie and C. T. Walsh, *Biochemistry*, **14**, 2588 (1975); F. Lederer, *Eur. J. Biochem.*, **46**, 393 (1974); C. T. Walsh, R. H. Abeles, and H. R. Kaback, *J. Biol. Chem.*, **247**, 7858 (1972).

the anion ($HC{\equiv}C{-}C^{-}XY \leftrightarrow H^{-}C{=}C{=}CXY$) derived from loss of an α proton from inactivators VI or VII could add to the flavin at C-4a or at N-5 to form the observed adducts. Alternatively, the acetylenes could first be oxidized to corresponding keto or imino compounds, which could react with the reduced flavin through Michael addition. If the latter mechanism applies, the key step in the formation of the active inhibitor is not simply proton abstraction, but completely normal enzyme-catalyzed oxidation. This process activates the triple bond toward nucleophilic addition. A discussion of the implication of these reactions with respect to the mechanism of action of flavin-containing enzymes is beyond the scope of this review, but has been presented elsewhere.[20,22]

Plasma Amine Oxidase

Another example in which activation of an inhibitor likely occurs through oxidation is the inactivation of plasma amine oxidase (a copper-containing,[23] nonflavin enzyme) by β-bromoethylamine.[24] This compound meets all of the criteria of a suicide inactivator. When the inactivation is carried out with β-bromo[U-^{14}C]-ethylamine, the enzyme becomes covalently labeled. We suggest that the inactivator is oxidized at the active site to bromoacetaldehyde. Bromine is now α to a carbonyl group, and thus is considerably more susceptible to displacement reaction. β-Bromoethylamine, therefore, as a substrate for enzymic oxidation, is converted to a much more effective alkylating agent. It should be noted that, since the compound meets the criteria discussed above, this inactivation is not due to reaction with free bromoacetaldehyde.

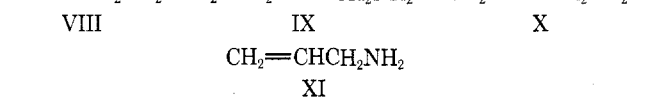
We have also shown that in the enzyme, reduced by addition of substrate under anaerobic conditions, an SH group can be alkylated. A tentative hypothesis for the structure of the active site and its interaction with the substrate is shown in eq 9. The nature of X is unknown.



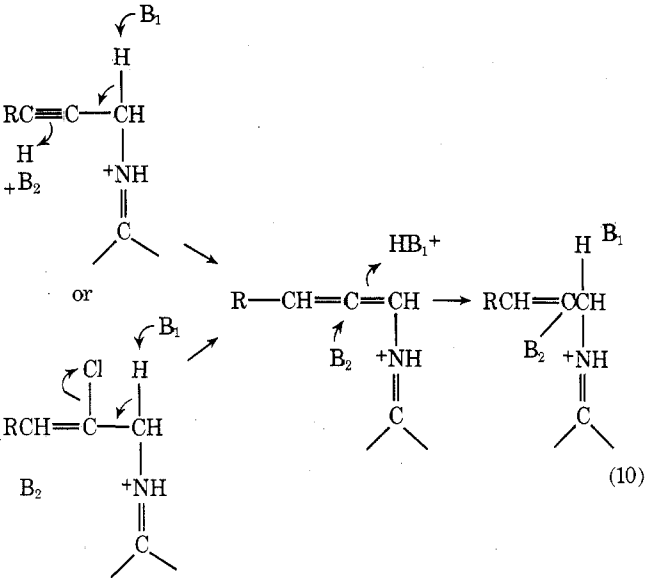
(9)

Results available to date indicate that it is not –S. β-Bromoethylamine may well prove useful in elucidating the second component of the active site.

Plasma amine oxidase is capable of catalyzing proton abstraction as demonstrated by its ability to catalyze elimination reactions.[24] We have used this fact to design several other suicide inhibitors for the enzyme.[25] Thus we found that plasma amine oxidase is inactivated by compounds VIII, IX and X. We suggested that the

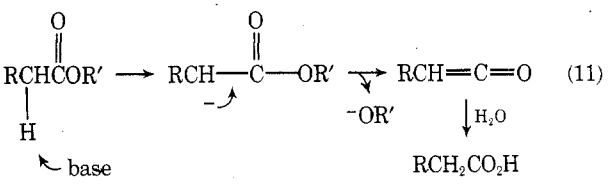


mechanism of inactivation involves α-proton abstraction from an enzyme-bound (Schiff base?) intermediate and reprotonation (or chloride loss) to form the isomeric (bound) allene, a very electrophilic species, which could then undergo addition of a nucleophilic group of the active site to form a stable covalent adduct (eq 10).



Preliminary investigations have shown that inactivation by X is accompanied by release of chloride ion. Although it could be argued that compounds VIII, IX, and X inactivate the enzyme through an oxidative pathway similar to that of β-bromoethylamine (eq 9) rather than through an allene intermediate (eq 10), we feel the latter mechanism is preferable for the following reasons. Allylamine (XI), which cannot form an allene but can be oxidized to a highly electrophilic species, does not inactivate the enzyme but is a very good substrate. The observed chloride ion release from X is required by the mechanism of eq 10, but it is not clear why it would occur at all in the oxidative inactivation pathway (eq 9). Presumably these two mechanisms can be distinguished by establishing the identity of the active-site residue labeled with compounds VIII or IX, and at what carbon of the inactivator molecule nucleophilic addition has taken place. If the allene mechanism is operative, the nucleophilic group will most probably interact with the β carbon; if the oxidative pathway is involved, it would probably interact with the γ position.

We have designed other suicide inhibitors based upon the ability of plasma amine oxidase to abstract substrate α protons. Bruice and collaborators observed that esters with relatively acidic α protons and good leaving groups can "hydrolyze" via intermediate formation of a ketene (eq 11).[26] Generation of a ketene at an enzyme active



(11)

site could readily lead to acylation of the enzyme and inactivation.

To explore this possibility, we examined the action of plasma amine oxidase upon the ethyl, phenyl, and p-nitrophenyl esters of glycine.[27] All three compounds

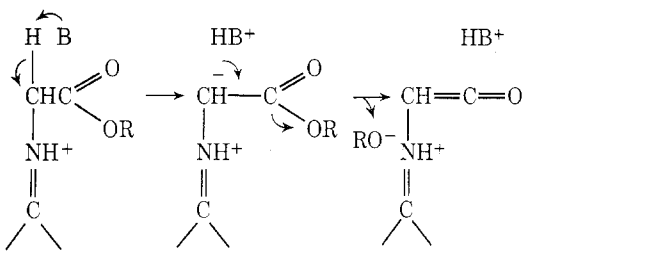
(22) A. Schonbrunn, R. H. Abeles, C. T. Walsh, S. Ghisla, H. Ogata, and V. Massey, submitted for publication.
(23) H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **237**, 1511 (1962).
(24) R. Neumann, R. Hevey, and R. H. Abeles, *J. Biol. Chem.*, **250**, 6362 (1975).
(25) R. C. Hevey, J. Babson, A. L. Maycock, and R. H. Abeles, *J. Am. Chem. Soc.*, **95**, 6125 (1973).
(26) R. F. Pratt and T. C. Bruice, *J. Am. Chem. Soc.*, **92**, 5956 (1970).
(27) A. L. Maycock, R. H. Suva, and R. H. Abeles, *J. Am. Chem. Soc.*, **97**, 5613 (1975).

Table I
The Effectiveness of Some Suicide Inactivators

Enzyme	Inactivator	Concentration (M)	$t_{1/2}$ for inactivation (min), 25 °C
γ-Cystathionase	$HC{\equiv}CCH_2CH(NH_3^+)CO_2^-$ (I)	6.6×10^{-5}	2
	$H_2C{=}CClCH_2CH(NH_3^+)CO_2^-$ (III)	2×10^{-3}	9
	$HCCl_2CH(NH_3^+)CO_2^-$ (IV)	2×10^{-3}	19
	$CF_3CH(NH_3^+)CO_2^-$ (V)	4×10^{-3}	2
Alanine racemase	V	4×10^{-2}	5
Tryptophanase	V	8×10^{-2}	9[a]
	IV	8×10^{-2}	47[a]
Tryptophan synthase (β_2)	V	4×10^{-2}	9[a]
Tryptophan synthase ($\alpha_2\beta_2$)	V	4×10^{-2}	12[a]
	IV	4×10^{-2}	10[a]
β-Cystathionase	V	1×10^{-4}	7
Lactate oxidase	$HC{\equiv}CCHOHCO_2H$ (VI)	8×10^{-4}	1.5
Monoamine oxidase	$HC{\equiv}CCH_2N(CH_3)_2$ (VII)	3.3×10^{-4}	10
Plasma amine oxidase	$BrCH_2CH_2NH_2$	4×10^{-3}	0.5
	$HC{\equiv}CCH_2NH_2$ (VIII)	4×10^{-4}	<1
	$H_2C{=}CClCH_2NH_2$ (X)	2.4×10^{-3}	2
	$NH_2CH_2C{\equiv}CCH_2NH_2$ (IX)	2.5×10^{-3}	0.23
	NH_2CH_2COOPh	6×10^{-5}	1.4
	$NH_2CH_2COO\text{-}C_6H_4\text{-}p\text{-}NO_2$	4×10^{-5}	0.3
	NH_2CH_2CN	2.2×10^{-5}	0.8
	$H_2NCH_2C{\equiv}CCH_2CHNH_2COOMe$	2.5×10^{-4}	1
Diamine oxidase	IX	2.5×10^{-3}	2.5

[a] At 37 °C.

are excellent substrates for the oxidase. The phenyl and *p*-nitrophenyl esters inactivate the enzyme rapidly, the phenyl ester in 1 out of 80 turnovers and the *p*-nitrophenyl ester in 1 out of 10 turnovers. Glycine phenyl ester covalently labels the enzyme. We tentatively suggest the following mechanism for the inactivation:



→ inactive enzyme

The inactivated enzyme slowly regains activity upon standing, which is not unexpected if a group has become acylated. With [1-^{14}C,2-^{3}H]glycine phenyl ester it was shown that one hydrogen atom is lost when the enzyme is inactivated. Furthermore, a good acylating agent, *p*-nitrophenyl acetate, at 200 times the concentration of glycine phenyl ester does not affect the enzyme. β-Alanine phenyl ester is a substrate and does not inactivate the enzyme. Although these results are consistent with the proposed mechanism, they do not establish it. Alternative inactivation mechanisms cannot be excluded at this point.

The results with propargylamine (VIII) and the glycine esters prompted us to explore the effects of aminoacetonitrile (NH_2CH_2CN) on plasma amine oxidase.[27] Enzyme-catalyzed proton abstraction from the carbon adjacent to the cyano group might induce isomerization to the allene analogue (a ketenimine) which could also acylate a nucleophile at the active site. Aminoacetonitrile does effectively inactivate plasma amine oxidase. With [1-^{14}C]aminoacetonitrile it was shown that the enzyme became covalently labeled. The mechanism of inactivation by this compound is unknown.

An in Vivo Application of Selective Suicide Inactivators

A major justification for developing highly selective enzyme inhibitors is their projected utility for in vivo applications where selectivity requirements may be very stringent. Such inhibitors could be of great practical value, for instance, in studies designed to elucidate the physiological roles of specific enzymes, to create animal models of particular human diseases, and to control specific physiological function or malfunction. Administration of propargylglycine (I) to rats leads to inactivation of liver cystathionase. This is accompanied by urinary excretion of cystathionine. An animal model of the genetic disease cystathionineuria can, therefore, be produced.

A few of the amine oxidase inhibitors we have described have been used in vivo to study a specific physiological process, namely the metabolism of mescaline by rabbits.[28] Although it was known that mescaline injected intravenously was rapidly metabolized by rabbits, it was not known which enzymes were responsible. Utilizing selective inhibitors of amine oxidases, to wit, pargyline (methylbenzylpropargylamine) for the flavin-dependent ones, aminoacetonitrile for the copper-dependent ones, and propargylamine to inactivate both classes made it possible to show that the copper-dependent enzymes were primarily responsible for the metabolism of the compound.

Summary

We have described how we have designed and ex-

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perimentally tested a number of suicide enzyme inactivators. Some of our data are collected in Table I to indicate the effectiveness of these compounds. We believe the effectiveness of so-called suicide inactivators depends upon two properties: (1) their ability to bind at the enzyme active site and (2) their ability to undergo enzyme-catalyzed conversion to reactivate species capable of reacting irreversibly with the active site.

That suicide inactivation occurs can best be demonstrated by identifying the structural changes which take place in both the enzyme and the inhibitor. However, the careful application of a number of kinetic criteria can be used to demonstrate with reasonable certainty that suicide inactivation has in fact occurred. All the inhibitors we have described were activated, we believe, by one of the following enzymatically catalyzed processes: proton abstraction leading to carbanion formation, proton abstraction leading to isomerization, proton abstraction leading to elimination, or oxidation.

We hope the examples cited have illustrated the potential usefulness of suicide enzyme inactivators for a wide variety of studies, both in vitro and in vivo. We believe that the design of suicide inactivators may in the future provide one rational approach to the design of pharmacologically useful compounds.

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Total Synthesis of Natural Products by Retro Mass Spectral Synthesis

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The structure of natural compounds can be determined by the systematic use of chemical reactions and spectroscopic methods.[1] In contrast, one can not establish a definite method for synthesis of natural products, although there is a long history in synthetic organic chemistry. Biogenetic-type synthesis[2] is one effective route for the synthesis of natural products, such as morphine, and sometimes photolytic reactions can be applied to the total synthesis of complicated natural products.[3,4] Recently, Corey[5] developed a method of computer-assisted synthetic analysis that allows the automatic processing of a target molecule in the antithetic (retrosynthetic) direction.

The mass spectrum of an organic compound usually provides two types of information: one is a knowledge of the molecular weight and formula based on the molecular ion, and the second is a determination of the molecular structure on the ground of its fragmentation pattern. Since fragmentation is a chemical process that results in bond breaking, fragmentation of a compound in the mass spectrum is sometimes closely similar to chemical degradation reactions. For example, cyclohexene produces butadiene ion radical and ethylene in its fragmentation, a process which is also observed in chemical reaction. On the other hand, cyclohexenes can be obtained from butadienes and ethylene derivatives by a Diels–Alder reaction. These facts indicate that some mass spectral fragmentations parallel chemical degradation processes and therefore also parallel retroprocesses of synthetic reactions of organic compounds. In this Account, we will discuss *retro mass spectral synthesis* as an effective method of analysis for designing synthetic approaches. This analysis is based on fragmentation processes in the mass spectrometer.

Retro Mass Spectral Synthesis

In the mass spectra[6] of a series of 1-monosubstituted 1,2,3,4-tetrahydro-2-methylisoquinolines (**1**), fragment ions (**2** and **3**) formed by loss of the C-1 substituent or C-1 hydrogen are observed, in addition to an $M^+ - 43$ ion (**4**) derived by a retro-Diels–Alder reaction of **1**.

Reduction of 1-substituted 3,4-dihydroisoquinolines (**2**) is the most common method for the synthesis[7,8] of 1-monosubstituted 1,2,3,4-tetrahydroisoquinoline derivatives (**1**). Another method is an alkylation of 1-unsubstituted 3,4-dihydroisoquinolines (**3**) with alkyl anions, derived from Grignard reagents.

On comparison of these syntheses with the mass spectra of isoquinolines, the reduction method corresponds to a retrograde of the formation of the 3,4-

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