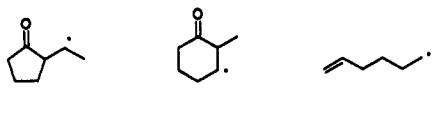

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Thus, we have demonstrated a convergent synthesis of β,γ-unsaturated ketones by free-radical-mediated three-component coupling of alkyl iodides, CO, and allylstannanes. The results above show that *free-radical carbonylation of an alkyl radical under low CO pressure is possible when the competing reaction of the alkyl radical is sluggish.* The success of low-pressure carbonylation now encourages us to move on the second stage of this work, other multicomponent coupling processes, double CO trapping with cyclizations, etc. The full scope of this methodology is under active study in our laboratory.

Supplementary Material Available: Detailed experimental procedures and characterization of products (4 pages). Ordering information is given on any current masthead page.

Mechanism-Based Inactivation of a Bacterial Phosphotriesterase by an Alkynyl Phosphate Ester

Jeffrey N. Blankenship,† Husam Abu-Soud, Wilson A. Francisco, and Frank M. Raushel*

Departments of Chemistry and Biochemistry Texas A&M University, College Station, Texas 77843

David R. Fischer and Peter J. Stang*

Department of Chemistry, University of Utah Salt Lake City, Utah 84112

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Enzyme-catalyzed reactions involving the transfer of phosphoryl groups make up a large class of group-transfer reactions that are central to metabolism. These enzymes catalyze reactions that involve nucleophilic attack at an electrophilic phosphorus center, with subsequent cleavage of a phosphorus–oxygen or phosphorus–nitrogen bond. Although phosphoryl-transfer enzymes utilize a variety of mechanistic alternatives, relatively few suicide substrates have been designed or discovered that react with this class of enzymes. Recently, a series of alkynyl phosphate esters have been synthesized,[1] and these compounds have the potential to form a highly reactive ketene intermediate upon cleavage of the phosphorus–oxygen bond as illustrated in Scheme I.

The inherent reactivity of the ketene intermediate is expected to lead to the rapid and irreversible inactivation of enzyme activity if a nucleophilic group of an amino acid side chain is appropriately situated within the active site. On the basis of the previously determined substrate specificity for the phosphotriesterase from *Pseudomonas diminuta*, the alkynyl phosphate esters would be expected to be efficiently hydrolyzed by this enzyme.[2] In this report we demonstrate that this novel class of enzyme inhibitor rapidly inactivates the bacterial phosphotriesterase via a mechanism-based process.

The bacterial phosphotriesterase used in this study was purified to apparent homogeneity using the method of Dumas et al.[2] The diethyl 1-hexynyl phosphate was synthesized according to the method of Stang et al.[1] The inhibition experiments with the alkynyl phosphate ester were conducted in 3% acetonitrile containing 100 mM PIPES,[3] pH 7.0, at 25 °C.

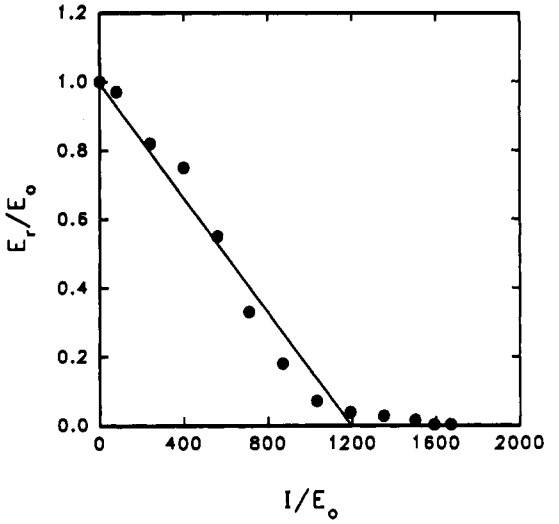
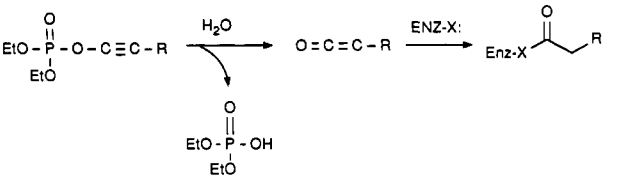
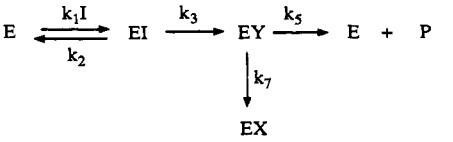


Figure 1. Inactivation of phosphotriesterase (125 pM) by variable amounts of diethyl 1-hexynyl phosphate. Enzyme and inhibitor were mixed at pH 7.0, and the remaining enzyme activity was measured after 15 min of incubation. Additional details are given in the text.

Scheme I



Scheme II



The initial inhibition experiments with diethyl 1-hexynyl phosphate and the phosphotriesterase were conducted at a constant enzyme concentration of 50 pM. Incubation of the inhibitor (1–1000 μM) and enzyme for 2 min at pH 7 resulted in >99% loss of all enzyme activity. Dialysis of the inhibited enzyme solution against 100 mM PIPES, pH 7.0, for up to 72 h gave no detectable increase in enzyme activity. In a control experiment, uninhibited enzyme, dialyzed against 100 mM PIPES, pH 7.0, for 72 h lost only 5% of the original activity. The failure of extensive dialysis to reactivate the enzyme is consistent with the formation of a covalent bond between enzyme and inhibitor. Incubation of the inhibited enzyme with 50–100 mM hydroxylamine, at pH 7.0, for up to 24 h also failed to regenerate any enzyme activity.

The efficiency of enzyme inactivation by the alkynyl phosphate ester was determined by incubation of a fixed enzyme concentration (125 pM) with variable concentrations of inhibitor (0–0.21 μM) as described by Knight and Waley.[4] Shown in Figure 1 is the plot of the fraction of enzyme activity remaining ($[E]_r/[E]_0$) versus the initial ratio of inhibitor to enzyme ($[I]/[E]_0$). The intercept on the horizontal axis is 1200, and thus 1200 ester molecules are hydrolyzed for every enzyme molecule inactivated. The inactivation of the phosphotriesterase by the alkynyl phosphate ester is therefore consistent with the model illustrated in Scheme II, where E is the enzyme, I is the inhibitor, EI is the initial noncovalent enzyme–inhibitor complex, and EY is the activated species which can either react within the complex to produce the

† This paper is dedicated to the memory of Jeffrey N. Blankenship, who died on June 16, 1991.

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(3) PIPES is an abbreviation for piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

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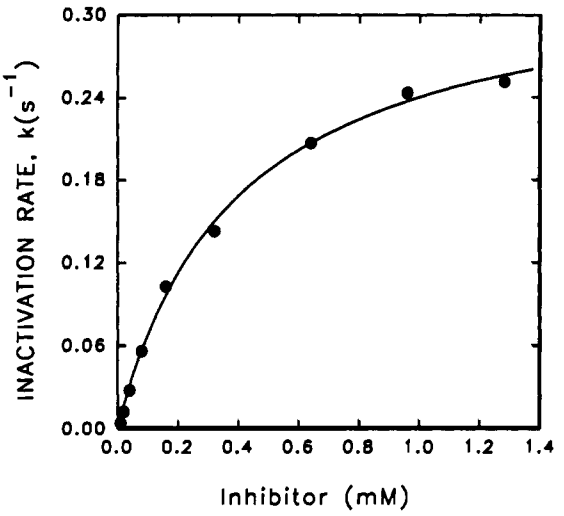

Figure 2. Variation of inactivation rate versus the initial concentration of diethyl 1-hexynyl phosphate. The solid line represents a fit of the data to eq 1. Additional details are given in the text.

irreversibly inhibited enzyme (EX) or dissociate to yield free enzyme (E) and product (P). The value of k_5/k_7 is the partition ratio of 1200. The products of the hydrolysis reaction were identified by ^1H and ^{31}P NMR spectroscopy to be diethyl phosphate and hexanoic acid.

Attempts to directly measure the rate of inactivation by the alkynyl phosphate ester failed because incubation of the enzyme and inhibitor eliminated all catalytic activity within 1 min and thus no kinetic data were obtainable. This problem was circumvented by the rapid mixing of enzyme and inhibitor with a High-Tech stopped-flow spectrophotometer (Model SF-51). The chromogenic substrate paraoxon (diethyl *p*-nitrophenyl phosphate) was added as a reporter substrate to monitor the activity of the enzyme as a function of time. For these experiments the ratio of the initial inhibitor concentration ([I]) to the initial enzyme concentration ($[E]_0$) was kept constant at 1600. Incubation of enzyme, inhibitor, and 0.25 mM paraoxon resulted in time-dependent loss of catalytic activity when the reaction was continuously monitored at 400 nm. Shown in Figure 2 is the plot of the apparent first-order rate constant for enzyme inactivation versus the initial concentration of inhibitor at constant $[I]/[E]_0$. These data were fitted to eq 1, where k_{obsd} is the observed first-order

$$k_{obsd} = (k_{in}[I])/(K_i(1 + A/K_a) + [I]) \qquad (1)$$

rate constant for inactivation, k_{in} is the maximal rate of inactivation at saturating inhibitor, [I] is the initial inhibitor concentration, K_i is the Michaelis constant for [I], A is the paraoxon concentration, and K_a is the Michaelis constant for A. The maximal rate of inactivation at saturating inhibitor is 0.33 ± 0.01 s^{-1} and $K_i = 58 \pm 4$ μM (A = 0.25 mM and K_a = 42 μM).

Using the minimal model for mechanism-based inhibition which appears in Scheme II, the rate expression for the inactivation rate is $k_{in} = (k_3k_7)/(k_3 + k_5 + k_7)$. Since $k_5 \gg k_7$, the minimum value of the rate constant (k_7) for the reaction of the putative ketene intermediate with the protein is 0.33 s^{-1}. The minimum value of the rate constant for P–O bond cleavage and product release (k_3 and k_5) is 410 s^{-1}. This value compares very favorably with the k_{cat} for paraoxon of 2100 s^{-1}, and thus the alkynyl phosphate esters are quite respectable substrates for the bacterial phosphotriesterase.

Although irreversible inhibition of many enzymes by alkynes and alkenes has been previously reported,[5] diethyl 1-hexynyl phosphate is the first alkynyl phosphate ester to irreversibly inactivate an enzyme. This compound is the first mechanism-based inhibitor reported for the bacterial phosphotriesterase. The alkynyl phosphate esters may prove to be useful for the active-site labeling of the bacterial phosphotriesterase.

Acknowledgment. This work was supported at Texas A&M University by the Army Research Office (DAAL03-90-G-0045) and the Naval Research Laboratory and at the University of Utah by the National Cancer Institute of the NIH (2 ROCA 16903) and the Dow Chemical Company.

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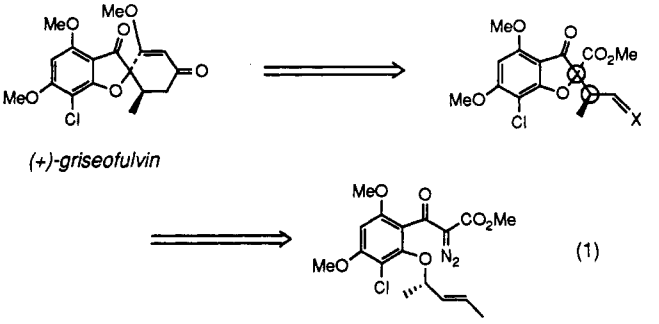
Total Synthesis of (+)-Griseofulvin

Michael C. Pirrung,* William L. Brown, Sushil Rege, and Pierre Laughton

Department of Chemistry, Duke University
P. M. Gross Chemical Laboratory
Durham, North Carolina 27706
Received July 18, 1991

Griseofulvin is a classical antifungal agent still used in the treatment of dermatomycoses in animals and humans[1] and in plant protection. It acts via binding specifically to fungal tubulin at submillimolar concentrations[2] and has been used in cell biological studies to induce the formation of large (up to 100 nm diameter), hollow tubes called megatubules.[3] It has been prepared by several routes, as part of structure–activity studies[4] and in the development of modern organic synthesis.[5] However, all of the syntheses reported thus far yield the racemate. The spirocyclic structure and vicinal stereogenic centers (eq 1) of griseofulvin form a challenging proving ground for asymmetric synthesis methods. This communication reports the first synthesis of the natural enantiomer of griseofulvin and demonstrates the power of the sigmatropic rearrangement of oxonium ylides[6] in diastereoselective and enantioselective synthesis.



(1)

Commercially available dimethoxyphenol is chlorinated by a known method[7] and acetylated to provide **2** (Scheme I). Fries rearrangement gives an *o*-hydroxyacetophenone, which is used directly in a Mitsunobu coupling[8] with (*R*)-pent-3-en-2-ol of 97%

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