Title
Molecular Approaches in Marine Pharmacology

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MOLECULAR APPROACHES IN MARINE PHARMACOLOGY

Original Goals and Objectives

The overall objectives of this proposal are to clone, express and study the reactivity of the halogenating enzymes in the biosynthetic pathways of the important halogenated and oxidized natural products in marine algae (particularly Rhodophyta, including California species of *Corallina, Laurencia* and *Plocamium*),

Introduction

Many marine natural products and enzymes have important medical applications in the pharmaceutical, diagnostic and biotechnological industries. These compounds and enzymes are at various stages of development, ranging from evaluation of their biological activities, to testing in clinical trials, and to their incorporation into commercial products. Notwithstanding this success, the rise in drug-resistant infections demands new drugs and strategies for eradication or control of invading pathogenic organisms. In addition, early disease detection requires new or more sensitive biochemical diagnostic tools. Solutions to these problems can be provided through discovery of new bioactive compounds, studies of their biogenesis, including endogenous biosynthetic enzyme investigations, and elucidation of their molecular mechanisms of action. Such strategies will allow chemists and pharmacologists, working in a collaborative effort, to pursue the development of novel drugs and diagnostics.

Many halogenated marine natural products show important biological activities of interest to the pharmaceutical industry. These compounds include halogenated and cyclic terpenes. Thus, the hypothesis to be investigated is that haloperoxidases in marine algae producing chiral halogenated terpene natural products (e.g., particularly Rhodophyta, such as species of *Corallina, Plocamium* and *Laurencia*) have unique substrate selectivities that confer stereospecific or regiospecific reactivities with terpene substrates.

Results and Accomplishments

1. V-BrPO Isolation and Cloning

We have isolated and cloned vanadium haloperoxidase enzymes from cDNA libraries that we have constructed to several species of algae (*Laurencia, Delisea, Corallina, etc*). We have expressed some of these clones in *E. coli*and we have compared the enzyme reactivities of recombinant V-BrPO to isolated V-BrPO [Carter et al, 2002].

2. Reactivity of V-BrPO with monoterpenes, nerol and geraniol.

Reaction of nerol, **1** (Scheme 1**)**, with V-BrPO (*C. officinalis, P. cartilagineum, L. pacifica*) in the presence of bromide ion and hydrogen peroxide produces the monobromo eight-membered cyclic ether **2** in 5% yield, as identified by mass spectrometry, m/z 232, 234, and NMR, along with the terminal bromohydrin, dibrominated and epoxide products. The chemical shifts of the *gem*-dimethyl singlets at δ 1.39 and 1.43 indicate that these methyl groups are no longer attached to an olefinic carbon. In addition a signal for the proton α to the bromine was observed at δ 4.37 ppm (dd, 1 H, J = 4.8 and 12 Hz), characteristic of bromocyclic structures of *Laurencia* metabolites previously isolated and reported [Wolinsky and Faulkner 1976; Howard and Fenical 1976; Faulkner 1976]. Cyclic ether **2** was produced without enantioselection. In contrast, the reaction of nerol with aqueous bromine produces only a mixture of bromohydrin, epoxide and dibrominated products, without formation of **2**. The reaction between nerol and the Br^+-generating species TBCO in nitromethane also resulted in the formation of **2** in 25% yield after chromatography, consistent with previous reports on TBCO bromoetherification reactions [Kato et al 1976, 1980]. Therefore, the eight-membered cyclic bromo-ether **2** formed in the V-BrPO-catalyzed brominative cyclization of **1** occurs within the active site of the enzyme without equilibration of the oxidized bromine intermediate with the surrounding aqueous medium. Formation of **2** likely results from an initial V-BrPO-catalyzed bromination reaction at the terminal olefin followed by intramolecular nucleophilic attack by the pendant alcohol (Scheme 1). While this 8-*endo* cyclization reaction is entropically unfavored, the eight-membered ring product is nevertheless the expected Markovnikov addition product and the ring is similar to that in the marine natural product, laurencin, which has been proposed to be derived from a straight-chain C_{15} acetogenin precursor [Ishihara et al 1995].

When the V-BrPO-catalyzed reaction is carried out with geraniol, **3**, two singly brominated cyclic products are isolated along with non cyclic bromohydrin, epoxide and dibromoproducts (Scheme 2). The α and β isomers of the cyclic products from the V-BrPO reaction were distinguished by their characteristic NMR spectra: α isomer **4** contains gem-dimethyl signals at δ 1.01 (s, 3 H) and 1.20 (s, 3 H), a CHBr signal at 4.17 (dd, 1 H, J = 9, 7 Hz), and the olefinic signal at δ 5.38 ppm; β isomer 5 contains gem-dimethyl signals at δ 0.91 (s, 3 H) and 1.20 (s, 3 H), a CHBr signal at 4.15 (dd, 1 H, J = 10.3, 4 Hz, CHBr), and exocyclic methylene signals at δ 4.79 and 5.04. Products **4** and **5** are each isolated as a single diastereomer without enantioselectivity; the nOe observed between H-2 and H-6 (geraniol numbering) indicates the bromine is in the equatorial position. The mechanism likely involves bromonium-ion-initiated cyclization at the terminal alkene generating the singly brominated monocyclic terpenes (Scheme 2). No cyclized monobrominated species were observed in control reactions with aqueous bromine, analogous to the reactivity with nerol. The internal olefin geometry prohibits nucleophilic trapping by the alcohol, leading to the alternative reaction pathway.

The V-BrPO-catalyzed reactions with the terpene analogs geranyl acetate, **6** (Scheme 3), and geranyl acetone, **9**, produce cyclic bromoacetate products, **7** and **8**, and a brominated bicyclic vinyl ether, **10**, respectively. The NMR spectra of **7, 8**, and **10** are identical to that previously reported [Wolinsky and Faulkner 1976].

3. Reactivity of V-BrPO with the sesquiterpene, nerolidol.

Vanadium bromoperoxidase catalyzes the asymmetric bromination and cyclization of (*E*)-(+)- nerolidol (**11**) to produce single diastereomers of the marine natural products □-, and □-snyderol (**13** and **14**), and a mixture of diastereomers of (+)-3□-bromo-8-epicaparrapi oxide (**15**) (Scheme 4; [Carter-Franklin and Butler 2004]). In contrast, reaction of **11** with aqueous bromine produced only minimal quantities of these brominated cyclized products. Reaction of **11** with TBCO in nitromethane produced an equal mixture of each diastereomeric product of **13, 14,** and **15**. Diastereomers of **12** could not be resolved by chiral GC. The observed diastereoselectivity is the first report of V-BrPO-catalyzed enantiospecific bromination and cyclization of sesquiterpenes forming chiral brominated marine natural products and establishes a role for V-BrPO in the biogenesis of halogenated metabolites in marine algae [Carter-Franklin and Butler 2004]. The high specificity of these V-BrPO-catalyzed reactions suggests that **11** docks within the active-site channel of V-BrPO in a specific orientation and is not randomly binding within the active-site cavity. In the case of random substrate binding, one would expect symmetric bromination.

Summary of accomplishments

We have tested our hypothesis that vanadium bromoperoxidase can catalyze bromo-cyclization reactions of terpenes. We have found that V-BrPO (Rhodophyta) catalyzes the asymmetric bromination and cyclization of certain terpenes, forming some identified natural products; thus clearly V-BrPO is important to the biogenesis of important halogenated marine natural products. However, many natural products have multiple sites of halogenation and different ring structures. Thus we are pursuing our studies of the biosynthesis of many other halogenated terpene natural products.

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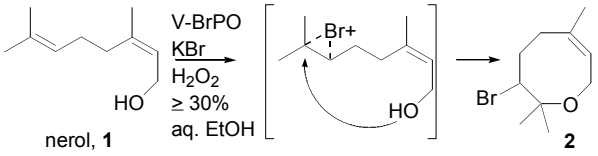
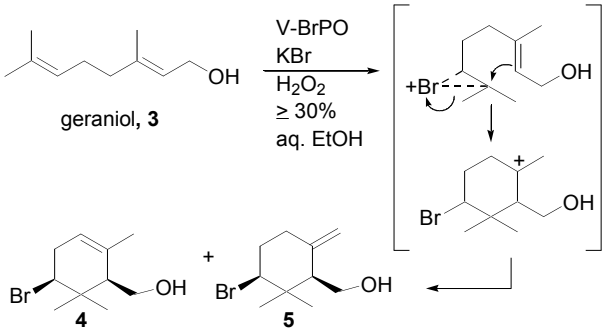
Figures and Schemes

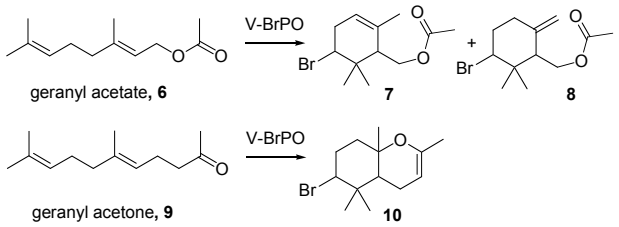
Scheme 1. Proposed reaction sequence for the V-BrPO-catalyzed reaction with nerol [Carter et al 2003, Butler and Carter-Franklin, 2004].



Scheme 2. Proposed reaction sequence for the V-BrPO-catalyzed bromocyclization of geraniol [Carter et al 2003, Butler and Carter-Franklin, 2004].



Scheme 3 [Carter et al 2003, Butler and Carter-Franklin, 2004]



Scheme 4 [Carter-Franklin and Butler 2004].