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Vanadium Haloperoxidase-Catalyzed Bromination and Cyclization of Terpenes

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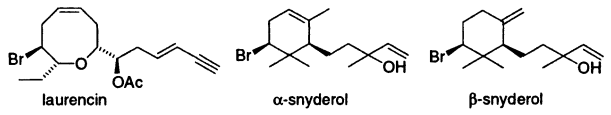
Marine red algae (Rhodophyta) are a rich source of bioactive halogenated natural products, including cyclic terpenes.[1−3] Initial studies on the biogenesis of halogenated terpenes, carried out at a time when the existence of marine haloperoxidase enzymes had only been hypothesized,[2,4] demonstrated that reactions of terpenes with 2,4,4,6-tetrabromocyclohexa-2,5-dienone (TBCO), or Br_2/$AgBF_4$ (i.e., reagents that generate bromonium ion), in nitromethane or dichloromethane (i.e., nonnucleophilic solvents), can induce bromination and cyclization of terpenes.[4,6,7] When the same reactions were carried out in water, however, bromohydrin formation resulted without cyclization.[6,8] The subsequent discovery of marine algal haloperoxidase enzymes[9,10] coupled to recent results on the selectivity of these enzymes[11,12] provides an attractive basis from which to begin to elucidate the biosynthesis of halogenated marine natural products. Vanadium bromoperoxidase (V−BrPO) is particularly prevalent, having been found in all classes of marine algae.[12,13] The active site is comprised of a vanadate ion coordinated to the protein by a single histidine residue which resides at the bottom of a broad active-site channel.[14,15] These enzymes function by coordination of hydrogen peroxide to V(V), subsequent oxidation of halide (Cl^-, Br^-, I^-) producing a two-electron oxidized halogen species (e.g., "Br^+" in the case of Br^- oxidation), followed by electrophilic halogenation of the organic substrate.[11−13] We report, for the first time, evidence that V−BrPO isolated and cloned from marine red algae that produce halogenated compounds (e.g., *Plocamium cartilagineum*, *Laurencia pacifica*, *Corallina officinalis*) can catalyze the bromination and cyclization of terpenes and terpene analogues, producing cyclic structures similar to laurencin, a brominated C_{15} acetogenin, from *Laurencia glandulifera*,[16,17] and α and *β* snyderols, brominated sesquiterpenes from *Laurencia*, spp.[5]



Reaction of 0.5 mM nerol, **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 (Figure 1), along with the terminal bromohydrin, dibrominated, and epoxide products.[18] 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, 1H, $J = 4.8$ and 12 Hz), characteristic of bromocyclic structures of *Laurencia* metabolites (Figure 1).[4,5,19] 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**.[20] The reaction between nerol and the Br^+-generating species TBCO in nitromethane also resulted in the formation of **2** in 25% yield after

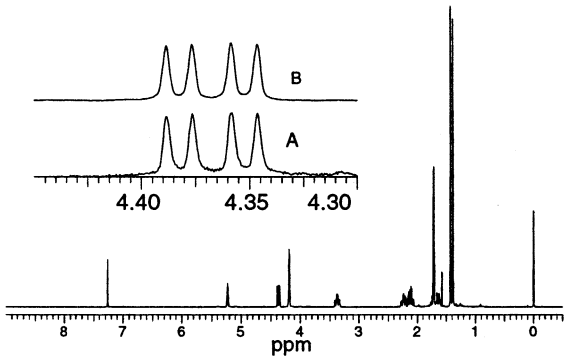
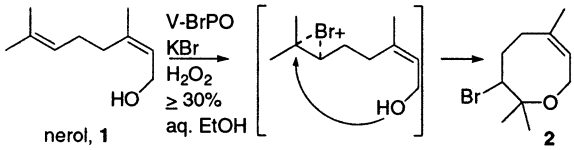


Figure 1. ^{1}H NMR spectra of **2**, 3-bromo-2,2,6-trimethyl-3,4,5,8-tetrahydro-2*H*-oxocine, isolated from the V−BrPO-catalyzed reaction with nerol. Inset: Expanded region around the double of doublet resonances at 4.37 ppm. (A) V−BrPO-catalyzed reaction; (B) TBCO reaction in nitromethane.

Scheme 1. Proposed Reaction Sequence for the V−BrPO-Catalyzed Reaction with Nerol



chromatography, consistent with previous reports on TBCO bromoetherification reactions.[6,21] Therefore, the eight-membered cyclic bromoether **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.[22]

When the V−BrPO-catalyzed reaction is carried out with geraniol, **3**, two singly brominated cyclic products (Scheme 2) are isolated along with noncyclic bromohydrin, epoxide, and dibromoproducts.[18] 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, 3H) and 1.20 (s, 3H), a C*H*Br signal at 4.17 (dd, 1H, $J = 9$, 7 Hz), and the olefinic signal at *δ* 5.38 ppm; *β* isomer **5** contains *gem*-dimethyl signals at *δ* 0.91 (s, 3H) and 1.20 (s, 3H), a C*H*Br signal at 4.15 (dd, 1H, $J = 10.3$, 4 Hz, C*H*Br), 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

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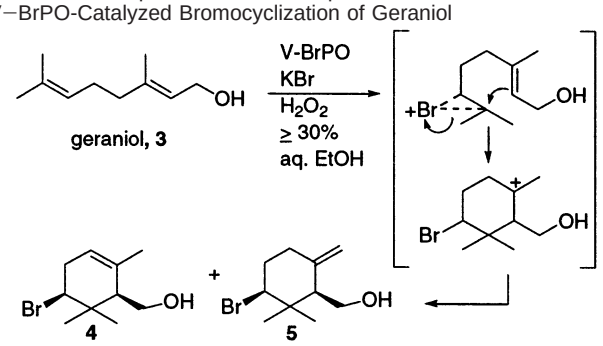
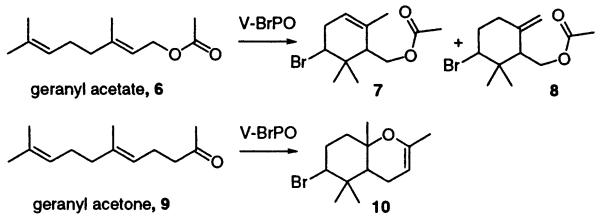
Scheme 2. Proposed Reaction Sequence for the V−BrPO-Catalyzed Bromocyclization of Geraniol



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 analogues geranyl acetate, **6**, and geranyl acetone, **9**, produce cyclic bromoacetate products, **7** and **8**, and a brominated bicyclic vinyl ether, **10**, respectively.[23]



The yields of the V−BrPO-catalyzed reactions leading to **7** and **8** (i.e., 10−20%) are higher than those observed with nerol and geraniol, which may reflect a role for the terminal acetyl oxygen atom in the stabilization[24] of the proposed bromocarbenium ion intermediate. In the case of geranyl acetate, the carbocation is quenched by elimination reactions leading to **7** and **8**. With geranyl acetone, the cation is quenched by reaction with the terminal ketone oxygen atom, leading to the bicyclic product, **10**. The stabilization suggests that terpenyl pyrophosphates may also be able to benefit from a similar stabilization leading to cyclic brominated species. We are currently examining this possibility.

In summary, we report the first V−BrPO-catalyzed brominative cyclization reactions of monoterpene substrates. The cyclic portion of products **4** and **5** is similar to the brominated α and β snyderol sesquiterpenes, which are thought to be derived from the sesquiterpene nerolidol;[6] the cyclic portion of structure **2** is related to laurencin. Terpene binding in the active site channel of V−BrPO may direct the brominative cyclization reactions in aqueous solution. The hydrophobic nature of the active site channel could promote the bromonium ion induced cyclization by limiting the reaction of the initial bromonium ion-terpene complex with nucleophilic solvents such as H_2O. This mechanism of bromonium-ion-induced cyclization is consistent with previous biosynthetic studies on the formation of α- and β-snyderols from nerolidol employing TBCO in nonnucleophilic solvents.[6] Continuing investigations are focused on elucidating the reactivity with other terpene substrates such as sesquiterpenes and diterpenes, as well as the reactivity of vanadium haloperoxidases from different marine algae that produce different halogenated cyclic terpene natural products.

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Supporting Information Available: Experimental details and spectroscopic data for compounds **2**, **4**, and **5** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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